Exploration of bacterial communities of economically important *Rhipicephalus* tick species in South Africa

A Matshotshi

orcid.org/ 0000-0003-0510-2762

Thesis accepted in fulfilment of the requirements for the degree *Doctor of Philosophy in Science with Zoology* at the North-West University

Promoter: Prof MMO Thekisoe

Co-promoter: Dr MO Taioe

Graduation: October 2025

DEDICATION

This thesis is dedicated to my husband Brayne Khwathelani Matshotshi, daughter- Rokunda Matshotshi and son- Rotondwa Saul Matshotshi. For their love, support, encourangement and understanding throughout my study.



Philippians 1:6*; "*being confident of this, that He who began a good work in you will carry it on to completion until the day of Christ Jesus*."*

ACKNOWLEDGEMENTS

I thank my promoter, Prof Oriel. M.M. Thekisoe for his support and patience. I truly appreciate the guidance and encouragement you gave to me throughout this study. All teachings received will not end with me, I will ensure that I transfer the knowledge to others.

I also thank my co-promoter Dr Moeti O. Taioe for his patience and contribution to the study.

My appreciation goes out to Dr Tawana Maguvu for assisting with 16S metabarcoding data analysis.

I thank Dr Lehlohonolo “Sanchez” Mofokeng and Dr. Tsepo Ramatla for being my go-to people each time I faced challenges in the laboratory. Thank you for availing yourselves.

A special thank you to Dr Clara-Lee Van Wyk, Ms Cleary Simpson, Ms Mucke van Zyl, Ms Rendani Matshotshi and Clinvet Laboratories for sharing some of their tick samples with me.

My greatest appreciation and thanks go to my parents; Mr Ntsengeni Lithole, Ms Lillian Mathagu for love and support during the duration of my studies. Thank you for believing in me and cheering me on. I love you.

I thank Apostle Humphrey and Prophetes Mufunwa Netshifhefhe for their prayer and scriptural declarations.

I thank the National Research Foundation (NRF) for awarding me the Thuthuka Grant (PhD Track) Grant Unique Number: 117914.

A special thanks to University of South Africa, for providing me with the opportunity to study further, awarding me the Academic Qualification Improvement Programme (AQIP) bursary and the Study assistance Busary grant ID: 18605459.

To my God, my fuel, my jump starter. I can never thank you enough. Thank you for your presence and protection. Because of YOU I am.

TABLE OF CONTENTS

DEDICATION ..... **II**

ACKNOWLEDGEMENTS ..... **III**

TABLE OF CONTENTS ..... **V**

LIST OF TABLES ..... **XII**

LIST OF FIGURES ..... **XIII**

LIST OF PLATES ..... **XVII**

ABBREVIATIONS ..... **XVIII**

RESEARCH OUTPUTS ..... **XIX**

ABSTRACT ..... **XX**

CHAPTER 1: INTRODUCTION **1**

1.1 Background **1**

1.2 Problem statement **2**

1.3 Aim **3**

1.4 Objectives **3**

1.5 Thesis outline **5**

CHAPTER 2: LITERATURE REVEIW **7**

2.1 Tick taxonomy **7**

2.2 Genus *Rhipicephalus* **8**

2.3 *Rhipicephalus* species morphology **9**

2.4 Different types of *Rhipicephalus* tick life cycles **17**

2.4.1 One-host tick **17**

2.4.2 Two-host tick **18**

2.4.3 Three-host tick 19

2.5 Pathogens transmitted by ticks 21

2.6 *Rhipicephalus* ticks species of veterinary importance 23

2.7 Economic importance of ticks and tickborne diseases in companion animals 24

2.8 Modern day techniques used for diagnosis of tick-borne pathogens 25

2.8.1 Polymerase chain reaction (PCR) 25

2.8.2 Next generation sequencing techniques 26

CHAPTER 3: MOLECULAR CHARACTERIZATION OF THE ECONOMICALLY IMPORTANNT SOUTH AFRICAN *RHIPICEPHALUS* TICK SPECIES OF DOMESTIC ANIMALS 29

3.1 Introduction 29

3.2 Materials and methods 33

3.2.1 Tick sampling 33

3.2.2 Tick morphological identification 36

3.2.3 Molecular identification of *Rhipicephalus* tick species 42

3.2.3.1 Tick DNA extraction 42

3.2.3.2 Amplification for tick identification 43

3.3. Results 47

3.3.1 Morphological identification of ticks 47

3.3.1.1 Morphological identification of *R. e. evertsi* Neumann (1897) 47

3.3.1.2 Morphological identification of *R. Sanguineus* (1806) 47

3.3.1.3 Morphological identification of *R. appendiculatus*, Neumann (1901) 48

3.3.1.4 Morphological identification of *R. (B) decoloratus* koch (1844) 48

3.3.1.5 Morphological identification of *R. (B) microplus* Canestrini (1888) 48

3.3.2 Molecular identification of ticks **52**

3.3.2.1 Amplification of *CO1* gene **52**

3.3.2.2 Phylogenetic analysis of the tick *CO1* gene **54**

3.3.2.3 Amplification of the *16S rRNA* gene **56**

3.3.2.4 Phylogenetic analysis of the tick *16S rRNA* gene **57**

3.3.2.5 Amplification of the *ITS2* gene **60**

3.3.2.6 Phylogenetic analysis of the tick *ITS2* gene **61**

3.3.2.7 Amplification of the *18S rRNA* gene **64**

3.3.2.8 Phylogenetic analysis of the tick *18S rRNA* gene **64**

3.4 Discussion **68**

CHAPTER 4: MICROBIAL COMMUNITIES HARBOURED BY *RHIPICEPHALUS* TICK SPECIES OF DOMESTIC ANIMALS **74**

4.1 Introduction **74**

4.2 Materials and methods **76**

4.2.1 Tick samples and Identification **76**

4.2.2 DNA extraction **76**

4.2.3 The 16S library preparation workflow **77**

4.2.3.1 Initial amplicon PCR **77**

4.2.3.2 Initial PCR clean-up **78**

4.2.3.3 Index PCR **79**

4.2.3.4 PCR clean-up 2 ..... **80**

4.2.3.5 Library quantification, normalization and pooling **81**

4.2.3.6 Library denaturation and MiSeq sample loading **81**

4.2.3.7 Denaturation and dilution of PhiX control 82

4.2.3.8 Combined amplicon library and PhiX control 82

4.2.4 Sequencing data analysis 83

4.2.5 Core microbiome determination 83

4.2.6 Alpha diversity analysis 84

4.2.7 Beta diversity analysis 84

4.2.8 Sequencing depth assessment and Rarefaction analysis 85

4.2.9 Data normalization and differential abundance testing 85

4.3 Results 86

4.3.1 Overall bacteria detected from ticks collected from different hosts in four provinces of South Africa 87

4.3.2 Bacteria detected from *Rhipicephalus* tick species collected from cattle 91

4.3.3 Bacteria detected from *Rhipicephalus* ticks collected from goats 94

4.3.4 Bacteria detected from *Rhipicephalus* ticks collected from sheep 97

4.3.5 Bacteria detected from *Rhipicephalus* ticks collected from horses 100

4.3.6 Bacteria detected from *Rhipicephalus* ticks collected from dogs 103

4.3.7 Bacteria detected from *Rhipicephalus* ticks collected from vegetation 106

4.3.8 Bacteria detected from colony *Rhipicephalus* ticks 109

4.3.9 Seasonal classification of bacteria detected from *Rhipicephalus* ticks collected . 112

4.3.10 Core microbiota 115

4.3.11 Diversity measures 120

4.3.12 Univariate statistical comparisons 125

4.4 Discussion 135

4.4.1 Phylum Proteobacteria **140**

4.4.2 Phylum Firmicutes **143**

4.4.3 Phylum Actinobacteria **144**

4.4.4 Phylum Bacteroidetes **145**

4.4.5 Other detected significant phyla **146**

CHAPTER 5: MOLECULAR DETECTION OF ZOONOTIC PATHOGENS FROM *RHIPICEPHALUS* TICKS COLLECTED FROM DOMESTIC ANIMALS **148**

5.1 Introduction **148**

5.2 Materials and methods **150**

5.2.1 Sampling of *Rhipicephalus* tick species **150**

5.2.2 DNA extraction of *Rhipicephalus* tick species **150**

5.2.3 Detection of zoonotic pathogens **150**

5.2.3.1 Detection of *Coxiella* species **150**

5.2.3.2 Detection of *Rickettsia* species **152**

5.2.3.3 Detection of *Escherichia-Shigella* **153**

5.2.3.4 Detection of *Anaplasma* species **153**

5.2.3.5 Detection of *Ehrlichia* species **154**

5.2.3.6 Detection of *Francisella* species **155**

5.2.3.7 Detection of *Staphylococcus* species **156**

5.2.3.8 Detection of *Clostridium* species **156**

5.2.3.9 Detection of *Pseudomonas* species **157**

5.2.3.10 Detection of *Campylobacter* species **158**

5.2.3.11 Detection of *Streptococcus* species **158**

5.2.3.12 Sequence Alignment and GenBank Submission 159

5.3 Results 162

5.3.1 *Coxiella* species 164

5.3.2 *Rickettsia* species 165

5.3.3 *Escherichia-Shigella* species complex 166

5.3.4 *Ehrlichia* species 167

5.3.5 *Francisella* species 168

5.3.6 *Anaplasma* species 169

5.3.7 *Staphylococcus* species 170

5.3.8 *Pseudomonas* species 170

5.3.9 *Clostridium* species 171

5.3.10 *Campylobacter* species 172

5.3.11 *Streptococcus species* 173

5.4 Discussion 174

CHAPTER 6: GENERAL CONCLUSION AND RECOMMENDATIONS 188

6.1 Introduction 188

6.2.1 Characterization of *Rhipichepalus* ticks collected from domestic animals and/or vegetation in four provinces in South Africa 188

6.2.2 Recommendations 190

6.3.1 Microbiota in *Rhipicephalus* ticks from four provinces in South Africa 190

6.3.2 Recommendations 191

6.4.1 Molecular detection of zoonotic pathogens from collected *Rhipicephalus* ticks 192

6.4.2 Recommendations 193

7. REFERENCES **195**

8. ANNEXURES **252**

8.1 BLASTn results for tick identification samples targeting the small subunit *ribosomal RNA* (*16S rRNA*) gene **252**

8.2 BLASTn results for tick identification samples targeting the *Cytochrome c oxidase I* (*CO1*) gene **259**

8.3 BLASTn results for tick identification samples targeting the *internal transcribed spacer 2* (*ITS2*) gene **266**

8.4 Blast search results for tick identification samples targeting the *small subunit ribosomal RNA* (*18S rRNA*) gene **271**

8.5 BLASTn results for pathogen identification **278**

8.6 ASVs produced from South African *Rhipicephalus* Ticks **285**

8.7 Tick identification: *Cytochrome oxidase subunit 1* (*CO1*) sequences **312**

8.8 Tick identification: *16S ribosomal RNA* gene sequences **318**

8.9 Tick identification: *Internal transcribed spacer 2* (*ITS2*) **323**

8.10 Tick identification: *18S ribosomal RNA* gene sequences **333**

LIST OF TABLES

Table 2.1: *Rhipicephalus* species discovered to date [Adopted from Guglielmone *et al.*, (2010)] 11

Table 3.1: Morphological characteristics used to identify collected ticks to species level (Walker *et al* 2003; Coetzer and Tusting 2004; Latif 2013; Barker and Walker 2014; Walker *et al*., 2014) 38

Table 3.2: Oligonucleotide primers used for molecular identification of ticks 46

Table 3.3: *Rhipicephalus* ticks species identified from four provinces in South Africa and the total number of ticks collected from each host type. 49

Table 3.4: Laboratory reared ticks. 49

Table 3.5: A summary of samples that where successfully sequenced targeting four different gene regions used in this study for tick characterization. 67

Table 4.1: Samples used for final metabarcoding analysis. 86

Table 4.2: A summary of the most dominant bacterial phylum, family and genus for ticks collected from the various host's species used in the present study 90

Table 4.3: Potential pathogenic genera found in the microbiota of 105 *Rhipicephalus* ticks obtained from 5 different hosts, vegetation and laboratory reared ticks 133

Table 4.4: Co-infections with various pathogens found in *Rhipicephalus* ticks taken from 5 different hosts, vegetation and laboratory reared ticks 134

Table 5.1: PCR Primers used for detection of tick-borne zoonotic pathogens 160

Table 5.2: PCR confirmation of zoonotic pathogens detected using metabarcoding 163

LIST OF FIGURES

Figure 2.1: General anatomy of male and female hard ticks (Walker *et al*., 2003) 8

Figure 2.2: Life cycle of one-host tick. Example is *R. (B.) annalutus* (Walker *et al*., 2003)18

Figure 2.3: Life cycle of two-host tick. Example is *R. e. evertsi* (Walker *et al*., 2003). 19

Figure 2.4: Life cycle of three-host tick). Example is *R. sanguineus* (Brown dog tick) feeding on dogs (Walker *et al*., 2003). 20

Figure 3.1: Map showing four sampled provinces in South Africa 35

Figure 3.2: Phylogenetic tree of the tick *CO1* gene using the Maximum Likelihood (ML) method with 1000 bootsrap percentage replicates. 55

Figure 3.3: Phylogenetic analysis of the tick *16S rRNA* gene using the Maximum Likelihood (ML) method with 1000 bootsrap percentage replicates. 59

Figure 3.4: Phylogenetic analysis of the *ITS2* gene using the Maximum Likelihood (ML) method with 1000 bootsrap percentage replicates. 63

Figure 3.5: Phylogenetic analysis of the tick *18S rRNA* gene using the Maximum Likelihood (ML) method with 1000 bootsrap percentage replicates 66

Figure 4.1: Bacterial phyla (**A**), Family (**B**), and genera (**C**) detected from from *R. e. evertsi* ticks collected from cattle 93

Figure 4.2: Bacterial phyla (**A**), Family (**B**), and genera (**C**) detected from from *R. e. evertsi* ticks collected from goats 96

Figure 4.3: Bacterial phyla (**A**), Family (**B**), and genera (**C**) detected from *R. e. evertsi* ticks collected from sheep 99

Figure 4.4: Bacterial phyla (**A**), Family (**B**), and genera (**C**) detected from from *R. e. evertsi* ticks collected from horses 102

Figure 4.5: Bacterial phyla (**A**), Family (**B**), and genera (**C**) detected from from *R. sanguineus* ticks collected from dogs 105

Figure 4.6: Bacterial phyla (**A**), Family (**B**), and genera (**C**) detected from from *R. appendiculatus* ticks collected from vegetation 108

Figure 4.7: Bacterial phyla (**A**), Family (**B**), and genera (**C**) detected from *R. e. evertsi* and *R. appendiculatus* ticks collected from Clinvet 111

Figure 4.8: Bacterial phyla detected in *Rhipicephalus* species collected in autumn (**A**), Summer (**B**), spring (**C**) and winter (**D**) seasons 114

Figure 4.9: Core-microbiome of bacteria genera from *Rhipicephalus* tick species collected in autumn (**A**), summer (**B**), spring (**C**) and winter **(D**) seasons 119

Figure 4.10: Alpha-diversity of *Rhipicephalu*s tick species collected in different seasons (**A**) and from different hosts (**B**) using the Chao1 index..... 120

Figure 4.11: Principal coordinate analysis (PCoA) plots based on the Bray-Curtis index distance method; i) using ANOSIM test on the ticks collected in different seasons (**A**) and tick samples collected from different hosts (**B**); ii) using PERMDISP test for ticks collected in different seasons (**C**) and tick samples collected different hosts (**D**); lastly iii) using Permanaova test for ticks collected in different seasons (**E**) and samples collected from different hosts (**F**). 123

Figure 4.12: Rarefaction curves showing level plateau in all sampling seasons (winter, summer, spring and autumn). 124

Figure 4.13: Seasonal differential abundance analysis done by a nonparametric test (Mann–Whitney/the Kruskal–Wallis test) as it is implemented in Microbiome Analyst (Dhariwal *et al.* 2017). Differential abundance analysis of the genus (**A**) genus Family XIII AD3011 group, (**B**) *Sphingobium*, (**C**) *Staphylococcus*, (**D**) Brevundimonas, (**E**) *Corynebacterium*, (**F**) *Ehrlichia* and (**G**) unnamed genera from the phylum Fusobacteria..... 129

Figure 4.14: Differential abundance analysis done by a nonparametric test (Mann–Whitney/the Kruskal–Wallis test) as it is implemented in Microbiome Analyst (Dhariwal *et al*. 2017). Differential abundance analysis of the genus (**A**) *Corynebacterium*, (**B**) *Tissierella*, (**C**) *Hafnia-Obesumbacterium*, (**D**) *Globicatella*, (**E**) *Staphylococcus* 132

Figure 5.1: PCR amplicons for the 16S *Coxiella* gene with an expected product size of 719–826 bp and 23S *Coxiella* gene with an expected product size of 583–867 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane H3-4 and Lane R1-5 show samples positive for presence of *Coxiella* spp DNA from *Rhipicephalu*s tick samples.. 164

Figure 5.2: PCR amplicons for the *Coxiella burnetti* gene with an expected product size of 632 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane 1, 2 & 4 show samples positive for presence of *C. burnett*i DNA from *Rhipicephalus* tick samples.. 165

Figure 5.3: PCR amplicons for the *Rickettsia* gene (*OmpA*) with an expected product size of 632 bp. M is the molecular marker (1k bp), -ve is for the no template control. Lane v1-4 show samples positive for presence of *Rickettsia* DNA from *Rhipicephalus* tick samples. 166

Figure 5.4: PCR amplicons for the *Rickettsia* (*gltA*) gene with an expected product size of 382 bp. M is the molecular marker (1kb bp), -ve is for the no template control. Lane s2 and s4 show samples positive for presence of *Rickettsia* DNA from *Rhipicephalus* tick samples.. 166

Figure 5.5: PCR amplicons for the *Escherichia coli* gene with an expected product size of 148 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane E1-E6 show samples positive for presence of *E. coli* DNA from *Rhipicephalus* tick samples. 167

Figure 5.6: PCR amplicons for the *Anaplasma phagocytophilum* (*EHR-521/EHR-747*) gene with an expected product size of 250 bp and *Ehlichia/Anaplasma* (*Ehr-F/Ehr-R biotin*) gene with an expected product size of 460-520bp. M is the molecular marker (100 bp), -ve is for the template control. Lane E1-3 show samples positive for presence of *Ehlichia* DNA from *Rhipicephalus* tick samples.. 168

Figure 5.7: PCR amplicons for the *Ehrlichia canis* gene with an expected product size of 154 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane J1-3 show samples positive for presence of *Ehlichia* DNA from *Rhipicephalus* tick samples.. 168

Figure 5.8: PCR amplicons for the *Francisella* gene with an expected product size of 1151 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane F1-5 show samples positive for presence of *Francisella like endosymbiont* DNA from *Rhipicephalus* tick samples.. 169

Figure 5.9: PCR amplicons for the *Anaplasma ovis* (MSP43/MSP45) gene with an expected product size of 869 bp and *Anaplasma phagocytophilum* (SSAP2f/SSAP2r) gene with an expected product size of 641-642 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane A1-3 show samples positive for presence of *A. ovis* DNA from *Rhipicephalus* tick samples.. 169

Figure 5.10: : PCR amplicons for the *Staphylococcus* (16Sup-1/16Sup-2) gene with an expected product size of 886 bp and *Staphylococcus (*SSU-bact-27f / SSU-bact-907r) gene with an expected product size of 450 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane T1-3, T5 and S1-5 show samples with positive amplification... 170

Figure 5.11: PCR amplicons for the *Pseudomonas* gene with an expected product size of +-550bp bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane P1-5 show samples with positive amplification.. 171

Figure 5.12: PCR amplicons for the *Clostridium* gene with an expected product size of 800 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane C1-5 show samples with positive amplification.. 171

Figure 5.13: PCR amplicons for the *Camphylobacter* (C412F/C1288R) gene with an expected product size of 817 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane C5 and C6 show samples with positive amplification... 172

Figure 5.14: PCR amplicons for the *Camphylobacter* (MD16S1 N/MD16S2) gene with an expected product size of 857 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane T1-6 and T8 show samples with positive amplification.. 172

Figure 5.15: PCR amplicons for the *Streptococcus* (*Strep-F/strep-R*) gene with an expected product size of 912 bp. M (left) is the molecular marker (1Kb), M (right) is the molecular marker (100 bp), -ve is for the no template control. Lane 10 show positive amplification.. 173

Figure 5.16: PCR amplicons for the *Streptococcus* (StrepDysF/strepDysR) gene with an expected product size of 401 bp. M (left) is the molecular marker (1Kb), M (right) is the molecular marker (100 bp), -ve is for the no template control. Lane12 show samples positive for presence of *Streptococcus dysgalactiae subspecies* DNA from *Rhipicephalus* tick samples.. 173

LIST OF PLATES

Plate I: *Rhipicephalus e.evertsi* A-dorsal & B- ventral female; C-dorsal and D-ventral male 50

Plate II: *Rhipicephalus appendiculatus* A-dorsal & B- ventral female; C-dorsal and D-ventral male..... 50

Plate III: *Rhipicephalus sanguineus* A-dorsal & B- ventral female; C-dorsal and D-ventral male 51

Plate IV: *Rhipicephalus decoloratus nympth*: A-Dorsal & B-Ventral 51

Plate V: *Rhipicephalus microplus* nympth:A-Dorsal & B-Ventral. 52

ABBREVIATIONS

RMRD SA	Red Meat Research and Development South Africa
NGS	Next Generation Sequencing
PCR	Polymerase chain reaction
glta	Citrate Synthase Encoding Gene
OTU	Operational Taxonomic Unit
NCBI	National Center for Biotechnology Information
ASV	Amplicon Sequence Variant
QIIME	Quantitative Insights into Microbial Ecology
BLAST	Basic Local Alignment Search Tool
ML	Maximum Likelihood
ITS2	Internal transcribed spacer 2
DNA	Deoxyribonucleic acid
CO1	Cytochrome Oxidase subunit 1
rRNA	Ribosomal Ribonucleic acid
GTR + G	General Time-Reversible model using discrete Gama distribution
CDC	Center for Disease Control
mPCR	multiple Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
PANDAseq	Paired-end Assembler for Illumina Sequences
T92 model	Tamura 3-parameter model
K2+G	Kimura 2-parameter using discrete Gama distribution

RESEARCH OUTPUTS

Conference papers

Matshotshi A., Taioe M.O. & Thekisoe O.M.M., Molecular characterization of the economically important South African *Rhipicephalu*s tick species of domestic animals and their bacterial communities. Oral presentation. 14-15 September 2021**.** 49th Parasitological Society of Southern Africa (PARSA) Conference. Virtual. Pg 25

ABSTRACT

Tick infestation and tick-borne diseases are a major concern for domestic animal health. Accurate identification and characterization of ticks and their associated microbial communities may lead to the development of improved control strategies targeting known tick-bacterial interactions. Current technologies enable simultaneous detection of tick microbiota. These include microbial communities of ecological, commensal, symbiotic or parasitic importance in nature. The aim of this study was to characterize tick microbial populations from field derived *Rhipicephalus* tick species in terms of their symbiotic, medical, veterinary and environmental importance in comparison to colony reared controls. To achieve this, field-derived ticks collected from domestic animals including cattle, sheep, goats, horses, dogs and vegetation were morphologically identified as *Rhipicephalus evertsi evertsi*, *R. appendiculatus* and *R. sanguineus* using published taxonomic keys; whilst colony reared ticks were identified as *R. e. evertsi*, *R. appendiculatus*, *R. decoloratus* and *R. microplus*. To supplement morphological identification, the present study used mitochodrial DNA [*cytochrome oxidase I* (*CO1)* and *16S ribosomal ribonucleic acid* (*16S rRNA*)] and nuclear DNA markers [(*internal transcribed spacer 2* (*ITS2)* and *18S ribosomal RNA*]. The performance of three DNA markers (*CO1*, *16S rRNA* and *ITS2*) in identifying *Rhipicephalus* tick species was a success using PCR, sequencing, BLASTn and phylogenetic tree corroborating morphological identification. The phylogenetic analysis of the above-mentioned ticks indicated that the collected species were accurately identified as they clustered with their respective reference species from the GenBank database. There was no clear separation of species using the *18S rRNA* marker, this marker is mostly significant in resolving relationships among higher taxa.

The 16S metabarcoding of the whole tick was conducted on the Illumina MiSeq platform to characterize the microbiota harboured by *Rhipicephalus* ticks. A total of 221 tick DNA samples were extracted and sequenced from field derived and laboratory reared ticks. However, only 105 FASTQ sequences passed the quality check to generate a total of 689 ASV amplicon sequence variants (ASVs). These were composed of 27 bacterial phyla; 64 classes; 145 bacterial order, 234 bacterial family and 580 bacterial genera.

The dominant phyla detected from ticks collected from the different domestic animals, vegetation and laboratory reared tick included Proteobacteria (30.8%) Firmicutes (18.3%), Actinobacteria (14.9%) and Bacteroidetes (13.8%). The remaining 23 phyla in this study contributed to 22.2% of the overall ASV sequences and each were in low abundance. Detected bacterial genera of veterinary, medical and environmental importance included *Bacillus, Staphylococcus, Streptococcus, Neisseria, Campylobacter, Proteus, Serratia, Escherichia-Shigella, Providencia, Acinetobacter, Comamonas, Pseudomonas* and *Enterococcus, Achromobacter, Aerococcus, Caulobacter, Corynebacterium, Corynebacterium_1, Fusobacterium, Haemophilus, Lactobacillus, Lawsonella, Legionella, Micrococcus, Moraxella, Micobacterium, Paracoccus, Porphyromonas, Ralstonia, Rhodococcus, Streptomyces. Cloacibacterium, Clostridium sensu stricto 1,13,18, and 8.* Potential genera of tick-borne zoonotic pathogens detected include *Rickettsiae, Coxiella, Francisella, Ehrlichia and Anaplasma.*

Conventional PCR was conducted to detect bacterial zoonotic pathogens and endosymbionts from *Rhipicephalus* tick DNA samples which showed presence of their respective genera using metabarcoding. Conventional PCR and sequencing resolved the following species, *Ehrlichia canis*, *Escherichia coli*, *Ricketssia aeschlimanni, Coxiella burnetii, Streptococcus dysgalactiae subspecies, Anaplasma ovis,* and *A.*

phagocytophilum. This study further identified symbionts such as *Coxiella*-like endosymbiont and *Francisella*- like endosymbiont. The livestock tick microbiota data generated in this study provides baseline knowledge of the microbiome of *Rhipicephalus* tick species harboured by domestic animals in South Africa.

Keywords: *Rhipicephalus appendiculatus, R. decoloratus*, *R. evertsi evertsi, R. microplus* and *R. sanguineus*, Microbial communities, Zoonotic pathogens

CHAPTER 1

Introduction

1.1 Background

South Africa is known as a developing country with many people that still depend on subsistence farming and live in close contact with domestic animals such as dogs, cats, sheep, cattle, goats, horses and chickens, which they keep as companion animals or as livestock. This lifestyle involving frequent encounters with domestic animals, which are common hosts for ticks, subsequently increases the risk of accidental tick bites and consequently, tick-borne illnesses (Skotarczak, 2018; Nimo-Paintsil *et al*., 2022). Multiple pathogenic agents may be carried by an individual tick, which could be transmitted accidentally to human hosts during a bite (Carmichael and Fuerst, 2006).

Ticks are haematophagous arthropods infesting on four vertebrate classes (Amphibia, Aves, Mammalia, and Reptilia) (Brites-Neto *et al*., 2015). They are second to mosquitoes regarding their ability to act as vectors and reservoirs for many non-infectious and infectious microbial agents (Steinbrik *et al*., 2022). Some of the world's most destructive diseases are vector-borne, contributing to society's burden of morbidity and mortality (Spellman and Stoudt, 2013). These infectious diseases are not easily controlled or prevented if the vector's biology, population dynamics and transmission routes are not well-understood. An example of one major vector-borne disease caused by ticks is Lyme disease caused by *Borrelia burgdorferi*, transmitted to humans through an infected tick bite (Steere *et al.,* 2004; Mysterud, *et al*., 2017). To control this disease, identification of the responsible tick species (*Ixodes scapularis*,

I. pacificus, I. ricinus, I. persulcatus), understanding of the tick life cycle (including life stages); habitat preferences (distribution and seasonality), behavior (questing behavior) are factors influencing transmission that needs to be considered (Bush and Vazquez-Pertejo 2018). The veterinary significance of tick infestations and tick-borne diseases in a region depends not only on the local tick species and the diseases they transmit, but largely on the composition of the livestock population. Most pathogens which are transmitted by ticks to livestock cause diseases such as anaplasmosis (ruminants), babesiosis (ruminants, horses, dogs), theileriosis (ruminants, horses) and cowdriosis (ruminants) as well as ehrlichiosis in ruminants and dogs (Uilenberg, 1992). Investigating microbial communities in ticks is crucial for understanding and to reduce the prevalences of tick-borne infections.

The microbial diversity of different *Rhipicephalus* tick species of economic importance in South Africa has not been explored in detail. Most studies in South Africa have focused on parasites transmitted by ticks in livestock and there is lack of information on bacterial communities harbored by ticks in South Africa. As a result, the current study sought to pioneer the exploration of bacterial communities of various *Rhipicephalus* tick species of economic importance in South Africa using *16S rRNA* metabarcoding profiles.

1.2 Problem statement

A large portion of South Africa's land is available for farming, with most of it being suitable for livestock production, which plays an important role in the economy. Livestock farming significantly contributes to agricultural income, supporting rural households by providing essential resources like meat, milk, and hides. However,

livestock health is important for maintaining productivity and sustainability. Ticks pose a major challenge by causing weight loss, anemia, skin damage, and reduced milk and hide quality, while also transmitting diseases that lower productivity. Although chemical treatments are commonly used for tick control, resistance development and contamination risks make them less effective over time. A more integrated approach that considers tick life stages and the pathogens they carry is necessary for improving control strategies and ensuring the sustainability of livestock farming.

Most studies in South Africa have focused on parasites transmitted by ticks in livestock. However, there is lack of information on bacterial communities harboured by these ticks in South Africa. As a result, the current study was formulated to determine bacterial communites associated with *Rhipicephalus* tick species in efforts to better understand the role played by these microbes as either simbiotic or parasitic to ticks or potential hosts. The focus was on *R. e. evertsi*, *R. (B) microplus*, *R. (B) decoloratus*, *R. appendiculatus* and *R. sanguineus*.

1.3 Aim

To characterize tick microbial populations of field derived *Rhipicephalus* tick species in terms of their symbiotic, medical, veterinary and environmental importance.

1.4 Objectives

1. To identify *Rhipicephalus* tick species morphologically using microscopy and by amplification and sequencing of *CO1*, *16S rRNA*, *ITS2* and *18S rRNA* genes and phylogenetic analysis.

2. To characterize the bacterial communities associated with *Rhipicephalus* ticks (*R. sanguineus, R. appendiculatus, R. e. evertsi, R. (B) decoloratus,* and *R. (B) microplus*) through *16S rRNA* V3-V4 hypervariable region amplicon sequencing using the Illumina next-generation sequencing platform. Additionally, the study seeks to assess the impact of seasonal variation on the composition of these bacterial communities.
3. To conduct species identification of selected bacterial pathogens of zoonotic importance from *Rhipicephalus* ticks collected from the environment (vegetation), livestock and domestic animals using conventional PCR and Sanger sequencing.

1.5 Thesis outline

Chapter 1 – Introduction

This chapter contains background leading to statement of the problem, hypothesis, aim and objectives.

Chapter 2 – Literature review

Literature review includes an overview of Ixodid ticks, with special focus on *Rhipicephalus* ticks infesting domestic animals in South Africa. The life cycles and role of these ticks in disease transmission of tick-borne pathogens is also described. Emphasis on the importance of ticks and tick-borne disease in livestock and companion animals is reviewed. Different diagnostic tools such as microscopy, PCR, metagenomics and NGS technologies are also highlighted.

Chapter 3 – Morphological and molecular identification of *Rhipicephalus* ticks

This chapter gives a description of the study area, sample collection and identification of ticks. Ticks were morphologically identified, further confirmed using molecular identification targeting four genes and lastly, phylogenetics was also used to confirm the species identification.

Chapter 4 – Characterization of *Rhipicephalus* tick bacteria micobiota using *16S rRNA* metabarcoding

This chapter explores microbial communities harboured by *Rhipicephalus* tick species collected from domestic animals. The next-generation sequencing technology (Illumina Miseq) was used to amplify *16S rRNA* for bacteria haboured by different tick species collected in this study.

Chapter 5 – Molecular detection of tick-borne zoonotic pathogens as well as their endosymbionts

This chapter uses conventional PCR to detect zoonotic pathogens and endosymbionts from *Rhipicephalus* tick DNA samples which showed presence of their respective genera in Chapter 4.

Chapter 6 – Conclusion and recommendations

This chapter contains drawn conclusions based on the findings from this study. Furthermore, the chapter includes recommendations for future studies.

CHAPTER 2

Literature Review

2.1 Tick taxonomy

Ticks are said to have originated during the pre-med Cretaceous period (146 to 66 million years ago) (Brites-Neto *et al.,* 2015). Ticks belong to the phylum Arthropoda, subphylum Chelicerata, class Arachnida, subclass Acari, order Parasitiformes and suborder Ixodides (Soneshina, 1991; Horak *et al.*, 2002; Anderson and Magnarelli 2008; Walker *et al.*, 2014). There are three known tick families; Argasidae also known as soft ticks (Jongejan and Uilenberg 2004); the family Nuttalliellidae, which consists of one species called *Nuttalliella namaqua* Bedford, 1931 (Latif *et al.,* 2012), and the lastly the family Ixodidae, representing hard ticks, (Noh *et al.*, 2017). Members of Ixodidae are commonly referred to as ixodid ticks and are known to have evolved from parasites of reptiles in the Paleozoic and Mesozoic era (Brites-Neto *et al.*, 2015).

Ixodid ticks (hard ticks) possess a large antero-dorsal sclerite and a dorsal plate (scutum) with a remarkable sexual dimorphism. They also possess a posterior border of opistome that can be divided into sclerotized structures called fastoons (Figure 2.1) (Brites-Neto *et al.*, 2015). Hard ticks feed once before moulting and may attach to host for a long period even days before transmission of pathogen can occur, whereas soft ticks are fast feeders needing only minutes to fully engorge and can feed on a host as many times as required (Schorderet-Weber *et al.*, 2017). About 896 tick species have been described in the world, with more than 702 tick species falling under the family Ixodidae consisting of 14 genera (Guglielmone *et al.,* 2010). The family of hard ticks (Ixodidae) include ticks from the genera *Amblyomma*, *Dermacenter*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Margaropus*, *Rhipicentor, Rhipicephalus Anomalohimalaya*,

Bothriocroton, *Cosmiomma*, *Cornupalpatum*, *Compluriscutula* and *Nosomma* (Guglielmone *et al.,* 2010). This study focused on hard tick species from the genus *Rhipicephalus,* which are economically significant parasites of livestock, wildlife, and people (Murrel and Barker, 2003).

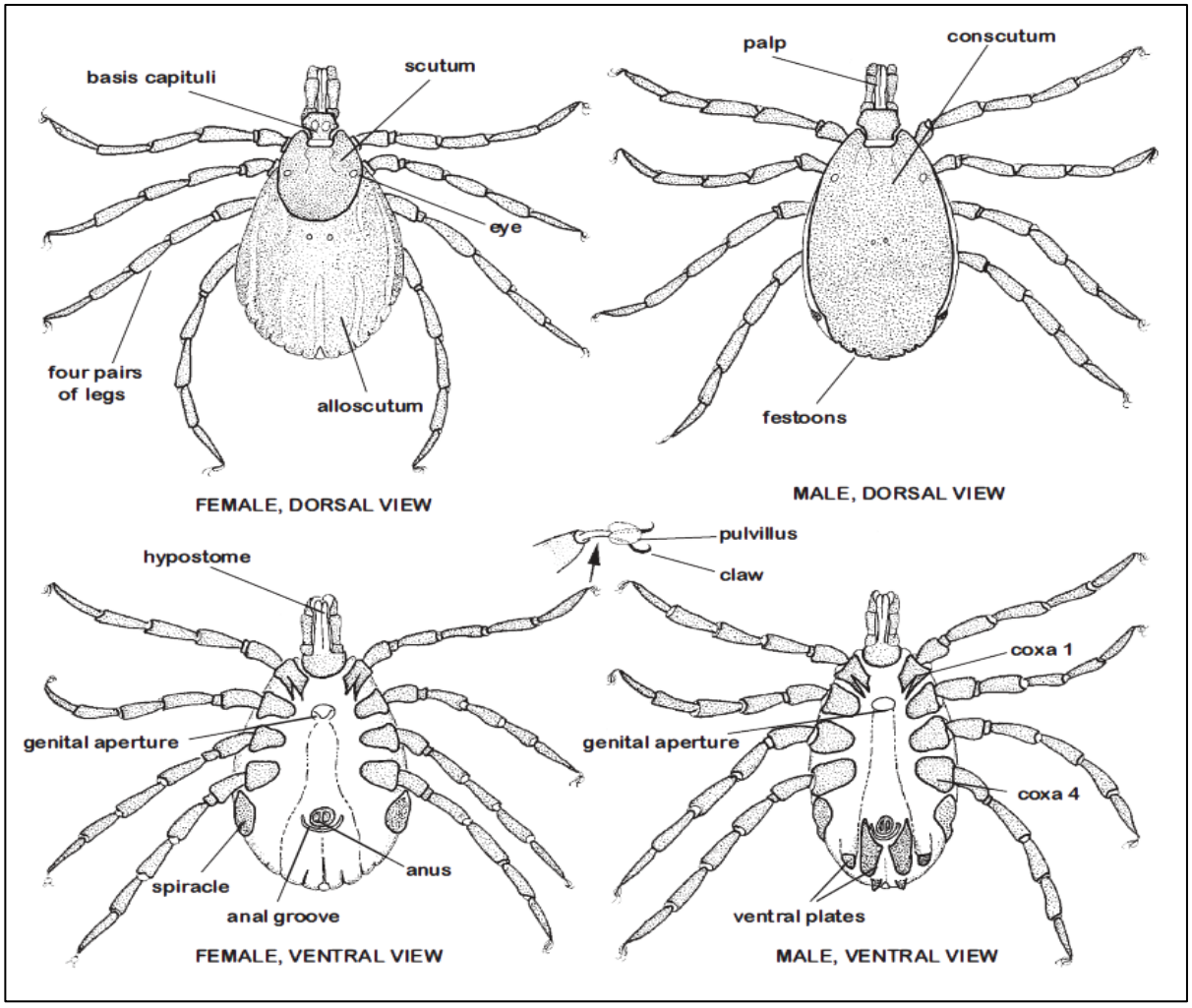


Figure 2.1: General anatomy of male and female hard ticks from "Ticks of Domestic Animals in Africa" (Walker *et al*., 2003).

2.2 Genus *Rhipicephalus*

The genus *Rhipicephalus* originated from Africa with 84 described species (Guglielmone *et al*., 2010; Brites-Neto *et al*., 2015). Five members of this genus were previously considered to be a separate genus called *Boophilus* Curtice (1891) (Gothe, 1967). Members of this genus included, *Boophilus annulatus* (Say, 1821), *B.*

decolaratus (Koch, 1844), *B. microplus* (Canestrinis, 1887), *B. kohlsi* (Hoogstraal & Kaiser, 1960) and lastly *B. geigyi* (Aeschlimann and Morell, 1965) (Murrel and Barker 2003). There has been a lot of controversies around the taxonomic status of the aforementioned *Boophilus* species.

Murrel and Barker (2003) proposed that all *Boophilus* become members of the *Rhiphicephalus* by updating the nomenclature of *Boophilus* Curtice (1891) ticks as junior synonym and subgenus of *Rhipicephalus* Koch (1844). These authors believed that there was sufficient evidence from morphology and nucleotide sequences suggesting that *Rhipicephalus* is paraphyletic with respect to *Boophilus* and that even though *Boophilus* species form a monophyletic group, this group is embedded within the genus *Rhipicephalus* (Murrel, and Barker 2003). Recent studies have relegated this group of five species to a subgenus of *Rhipicephalus*; *Rhipicephalus (Boophilus) annulatus, Rhipicephalus (Boophilus) decolaratus*, *Rhipicephalus (Boophilus) microplus*, *Rhipicephalus (Boophilus) kohsli* and *Rhipicephalus (Boophilus) geigyi* a (Guglielmone, 2010; Spickett *et al*., 2011; Wang *et al*., 2017; Wang *et al*., 2020; Tan *et al*., 2021; Dzemo *et al*., 2022).

2.3 *Rhipicephalus* species morphology

Rhipicephalus ticks are known to have a hexagonal shaped basis capituli with mouthparts that are short to medium in length (Dantas-Torress *et al*., 2013). Ticks from this genus have festoons, eyes and the male species of this genus usually possess accessory adanal plates (Walker *et al*., 2000). The coxae on the first pair of legs usually has a long prominent posteriorly directed spurs and the scutum is usually uniformly brown, except in four species (*R. dux, R. humeralis, R. maculatus and R.*

pulchellus) that have ivory coloured ornamentation (Walker *et al.,* 2003). The majority of *Rhipicephalus* ticks are three-host ticks that moult on the host, a few are two-host ticks that moult from larvae to nymph on one host. All five *Boophilus* ticks which are said to fall under the *Rhipicephalus* genus are one-host ticks that do not leave their host to moult to different tick stages on the same host animal (Murrel and Barker, 2003). All species are brown or reddish in colour (Dantas-Torress *et al.,* 2013).

In Africa, 19 *Rhipicephalus* tick species have been identified from domestic animals; *R.* (*B.*) *annulatus*, *R. (B.) decoloratus*, *R. (B.) geigyi*, *R. (B.) microplus*, *R. appendiculatus*, *R. bursa*, *R. camicusi, R. evertsi, R. guilhoni*, *R. lanulatus*, *R. mahsamae*, *R. praetextatus*, *R. pravus*, *R. pulchellus*, *R. sanguineus*, *R. senegalensis*, *R. simus, R. turanicus* and lastly *R. zambeziensis* (Walker *et al.*, 2003). Table 2.1 shows detailed information on the discovery of different *Rhipicephalus* tick species globally.

Table 2.1: *Rhipicephalus* species discovered to date [Adopted from Guglielmone *et al.,* (2010)]

Species names	Authors	Notes
1. Rhipicephalus annulatus	Say, 1821	This is the type species of the genus *Boophilus.* This species was original *Ixodes annulatus*.
2. *Rhipicephalus appendiculatus*	Neumann, 1901	
3. Rhipicephalus aquatilis	Walker, Keirans and Pegram, 1993	
4. Rhipicephalus armatus	Pocock, 1900	
5. Rhipicephalus arnoldi	Theiler and Zumpt, 1949	
6. Rhipicephalus aurantiacus	Neumann, 1907.	Guglielmone *et al.* (2009) note that this tick is considered a synonym of *R. ziemanni* by some authors, but because there has been no comparison of types, we provisionally regard *R. aurantiacus* as valid. Kolonin (2009) does not include *R. aurantiacus* in his list of worlds Ixodidae
7. *Rhipicephalus bequaerti*	Zumpt, 1949	
8. *Rhipicephalus bergeoni*	Morel and Balis, 1976	
9. Rhipicephalus boueti	Morel, 1957	
10. *Rhipicephalus bursa*	Canestrini and Fanzago, 1878	
11. *Rhipicephalus camicasi*	Morel, Mouchet and Rodhain, 1976	
12. *Rhipicephalus capensis*	Koch, 1844	
13. *Rhipicephalus carnivoralis*	Walker, 1966	

14. Rhipicephalus cliffordi	Morel, 1965	Guglielmone *et al.* (2009) noted that this tick has been considered a synonym of *R. pseudolongus*, but because there has been no comparison of types, was provisionally regard *R. cliffordi* as valid. Kolonin (2009) does not share this opinion.
15. *Rhipicephalus complanatus*	Neumann, 1911	
16. *Rhipicephalus compositus*	Neumann, 1897	
17. *Rhipicephalus cuspidatus*	Neumann, 1906	
18. *Rhipicephalus decoloratus*	Koch, 1844 (*Boophilus*)	
19. *Rhipicephalus deltoideus*	Neumann, 1910	
20. *Rhipicephalus distinctus*	Bedford, 1932	
21. *Rhipicephalus duttoni*	Neumann, 1907	
22. *Rhipicephalus dux*	Dönitz, 1910	
23. *Rhipicephalus evertsi*	Neumann, 1897	
24. *Rhipicephalus exophthalmos*	Keirans and Walker, 1993	
25. *Rhipicephalus follis*	Dönitz, 1910	
26. *Rhipicephalus fulvus*	Neumann, 1913	
27. *Rhipicephalus geigyi*	Aeschlimann and Morel, 1965 (*Boophilus)*	
28. *Rhipicephalus gertrudae*	Feldman-Muhsam, 1960	
29. *Rhipicephalus glabroscutatum*	Du Toit, 1941	

30. *Rhipicephalus guilhoni*	Morel and Vassiliades, 1963	
31. *Rhipicephalus haemaphysaloides*	Supino, 1897	
32. *Rhipicephalus humeralis*	Tonelli-Rondelli, 1926	
. *Rhipicephalus hurti*	Wilson, 1954	
34. *Rhipicephalus interventus*	Walker, Pegram and Keirans, 1995	
35. *Rhipicephalus jeanneli*	Neumann, 1913	
36. *Rhipicephalus kochi*	Dönitz, 1905	
37. *Rhipicephalus kohlsi*	Hoogstraal and Kaiser, 1960 (*Boophilus*)	
38. *Rhipicephalus leporis*	Pomerantzev, 1946	
39. *Rhipicephalus longiceps*	Warburton, 1912	
40. *Rhipicephalus longicoxatus*	Neumann, 1905	
41. *Rhipicephalus longus*	Neumann, 1907	See *R. pseudolongus.*
42. *Rhipicephalus lounsburyi*	Walker, 1990	
43. *Rhipicephalus lunulatus*	Neumann, 1907	
44. *Rhipicephalus maculatus*	Neumann, 1901	
45. *Rhipicephalus masseyi*	Nuttall and Warburton, 1908	
46. *Rhipicephalus microplus*	Canestrini, 1888) (*Boophilus*).	Some authors have listed the year 1887 for the original description of *Rhipicephalus microplus* (the species was described as *Haemophysalis* [sic] *micropla*), while others have used the year 1888. An inquiry to the U.S. Library of Congress has shown that Canestrini (1887) was actually published in Padova by Stabilimento Prosperini in 1888; therefore, we

		recognize the latter year for the description of *R. microplus*. A recent study by Labruna *et al*. (2009b) presents evidence that populations of *R. microplus* from Australia are not conspecific with Afrotropical and Neotropical populations, although Afrotropical and Neotropical populations are conspecific.
47. *Rhipicephalus moucheti*	Morel, 1965	
48. *Rhipicephalus muehlensi*	Zumpt, 1943	
49. *Rhipicephalus muhsamae*	Morel and Vassiliades, 1965	
50. *Rhipicephalus neumanni*	Walker, 1990	
51. *Rhipicephalus nitens*	Neumann, 1904	
52. *Rhipicephalus oculatus*	Neumann, 1901	
53. *Rhipicephalus oreotragi*	Walker and Horak, 2000	
54. *Rhipicephalus pilans*	Schulze, 1935	
55. *Rhipicephalus planus*	Neumann, 1907	
56. *Rhipicephalus praetextatus*	Gerstäcker, 1873	
57. *Rhipicephalus pravus*	Dönitz, 1910	
58. *Rhipicephalus pseudolongus*	Santos Dias, 1953	Guglielmone *et al.* (2009) note that, this tick is considered a synonym of *R. longus* by some authors, but no evidence has been forthcoming to support such a view; therefore, we regard *R. pseudolongus* as tentatively valid. See also *R. cliffordi.*
59. *Rhipicephalus pulchellus*	Gerstäcker, 1873	
60. *Rhipicephalus pumilio*	Schulze, 1935	

61. *Rhipicephalus punctatus*	Warburton, 1912	See *R. serranoi.*
62. *Rhipicephalus pusillus*	Gil Collado, 1936	
63. *Rhipicephalus ramachandrai*	Dhanda, 1966	
64. *Rhipicephalus rossicus*	Yakimov and Kol-Yakimova, 1911	
65. *Rhipicephalus sanguineus*	Latreille, 1806	This is the type species of the genus *Rhipicephalus*; it was originally named *Ixodes sanguineus*.
. 66. *Rhipicephalus scalpturatus*	Santos Dias, 1959	
67. *Rhipicephalus schulzei*	Olenev, 1929	
68. *Rhipicephalus sculptus*	Warburton, 1912	
69. *Rhipicephalus senegalensis*	Koch, 1844	
70. *Rhipicephalus serranoi*	Santos Dias, 1950	Guglielmone *et al.,* (2009) note that this species is considered a synonym of *R. punctatus* by some authors, but characters useful for separating the two species have been published.
71. *Rhipicephalus simpsoni*	Nuttall, 1910	
72. *Rhipicephalus simus*	Koch, 1844	
73. *Rhipicephalus sulcatus*	Neumann, 1908	
74. *Rhipicephalus supertritus*	Neumann, 1907	
75. *Rhipicephalus tetracornus*	Kitaoka and Suzuki, 1983	Kolonin (2009) excludes *R. tetracornus* from his list of worlds Ixodidae, but the holotype nymph exists and no evidence has been proffered to support synonymization (Guglielmone *et al.,* 2009).
76. *Rhipicephalus theileri*	Bedford and Hewitt, 1925	

77. *Rhipicephalus tricuspis*	Dönitz, 1906	
78. *Rhipicephalus turanicus*	Pomerantzev, 1940.	While most Western workers list the year 1936 for the original description of *R. turanicus*, we follow Filippova (1997), who considers 1940 to be the correct year.
79. *Rhipicephalus warburtoni*	Walker and Horak, 2000	
80. *Rhipicephalus zambeziensis*	Walker, Norval and Corwin, 1981	
81. *Rhipicephalus ziemanni*	Neumann, 1904	
82. *Rhipicephalus zumpti*	Santos Dias, 1950	

2.4 Different types of *Rhipicephalus* tick life cycles

The *Rhipicephalus* ticks have one, two or three host life cycles associated with different life stages (eggs, larvae, nymph and adult) living in physical environment (soil, vegetation) or on their host (Walker *et al.*, 2003).

2.4.1 One-host tick

Figure 2.2 shows the sequence of feeding and moulting during the different developmental stages (eggs, larvae, nymph and adult) of a one-host species tick *R. (B.) annulatus*. This tick infests mainly cattle but can also be found in some domestic animals such as horses, sheep and goats (Hoogstraal *et al.,* 1981; Abdel-Shafy, 2018). The cattle tick *R. annulatus* remains active year-round with larvae and nymphs peaking in summer and autumn, and adults predominetly active in autumn, a pattern supported by the humid climate (Estrada-Peña and Salman, 2013; Bedouhene *et al*., 2022). Eggs are laid on the ground until larvae develop, hatch and then crawl up onto vegetation to quest for a host (Coetzer and Tustin, 2004, Barker and Walker, 2014). Once the larvae are attached to the host, then all life stages occur on the same host (cattle) until an adult female tick mates with a male tick (Figure 2.2). After mating, the female tick will engorge fully and then fall off the host to lay eggs (Walker, 2003; Barker and Walker, 2014).

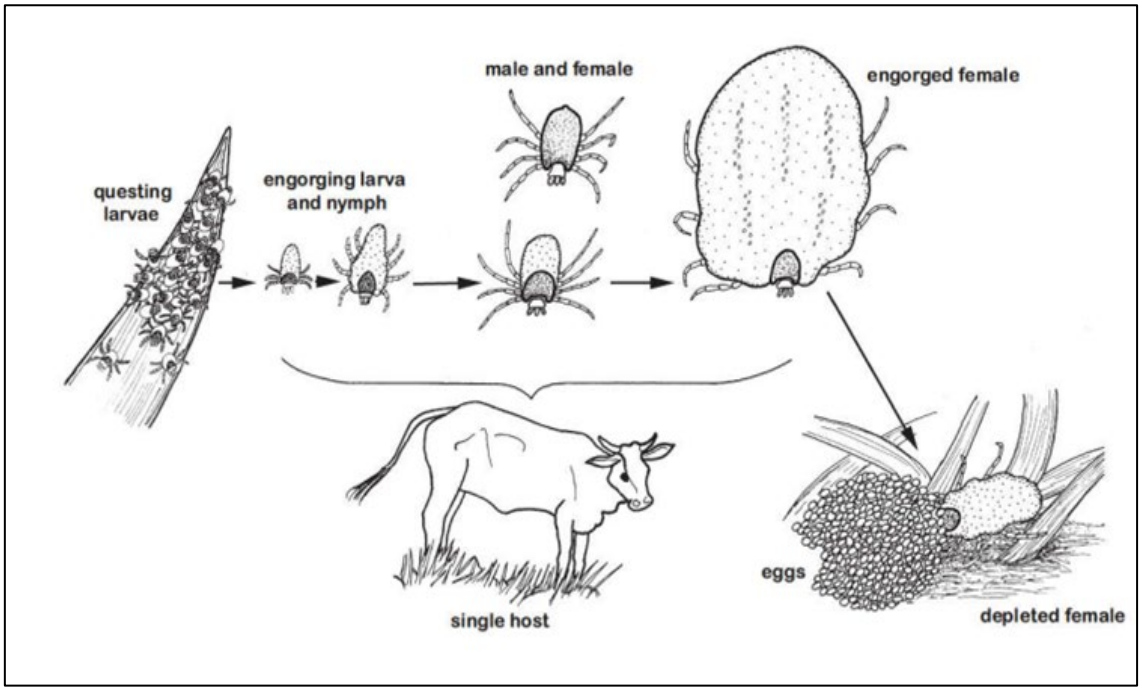


Figure 2.2: Life cycle of a one-host tick. Example is *R. (B.) annalutus (*Walker *et al.,* 2003).

2.4.2 Two-host tick

The two-host life cycle is similar to that of the one host-cycle. The only difference is that the larvae and the nymph stages of the tick occur on the same host (Figure 2.3). The *R. evertsi evertsi* is present year-round with peak activity during the warmer, wetter months (Reilly and Schroder, 2013; Makwarela *et al*., 2023). The larvae and the nymphs of *R. evertsi evertsi* prefer to feed deep in the external ear canals of ungulates and on hares, while the adult ticks prefer feeding in the peri-anal and inner thigh regions of ungulates (Figure 2.3; Coetzer and Tustin, 2004). Once the larvae attach to the host, it remains on the host feeding until it develops into a nymph, feeds and then falls off (Coetzer and Tustin, 2004; Barker and Walker 2014). The nymph will then develop into an adult tick, crawls to the vegetation to quest for a second host of the same species. Once attached to the second host, the adult male and female tick

will feed and mate. Once the female tick is fully engorged, the female tick will fall off the host to lay eggs (Barker and Walker, 2014; Walker *et al.,* 2014).

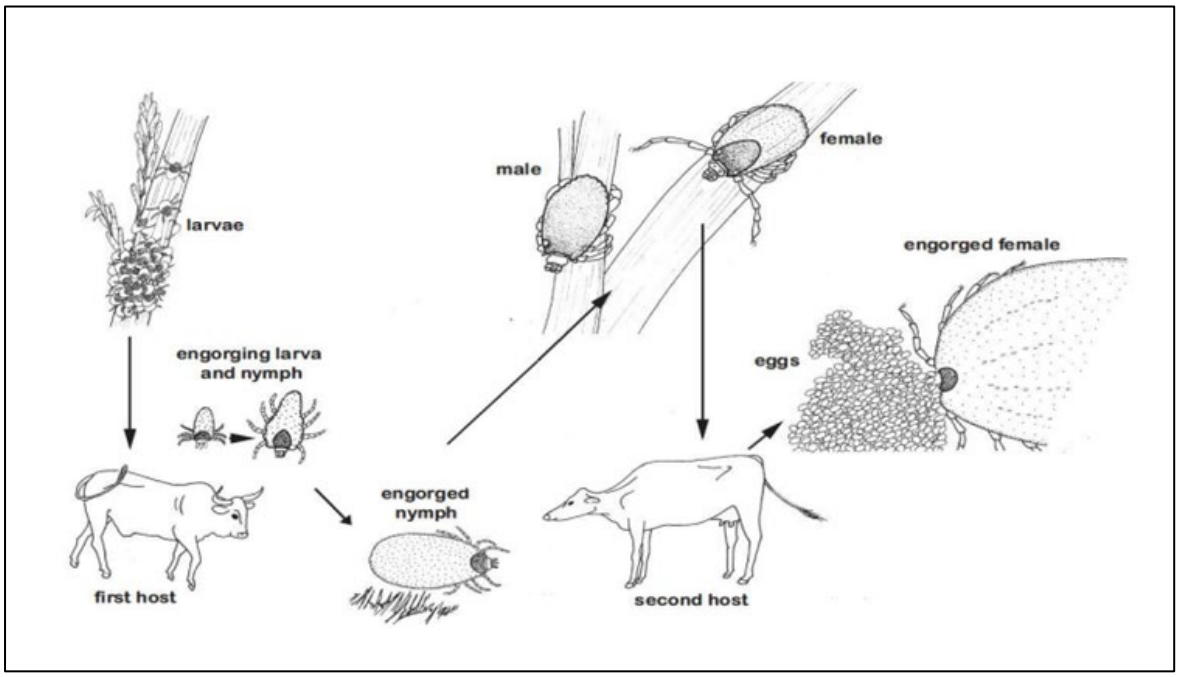


Figure 2.3: Life cycle of a two-host tick. Example is *R. e. evertsi* (Walker *et al*., 2003).

2.4.3 Three-host tick

The most common type of life cycle is the three-host tick shown in figure 2.4. *Rhipicephalus sanguineus* is a highly adaptable tick found on dogs in both urban and rural settings, capable of thriving indoors and remaining active year-round across tropical, subtropical, and some temperate regions, with up to four generations annually depending on climate and host availability (Dantas-Torres, 2010). Eggs are laid on the physical environment, the larvae then develop inside the egg until hatching. Once the larvae hatches from the eggs, it will crawl up on vegetation to wait for the first host for feeding (Walker *et al.,* 2014). The larvae will then attach to the host, feeds and will eventually fall off the host. The larvae will then moult into a nymph; the nymph will crawl on vegetation to quest. The nymph will then attach to the second host and feed.

Once the nymph has fully engorged, it will fall off the host to moult into an adult tick (Barker and Walker, 2014). The adult tick will repeat the same procedure by questing on vegetation to wait for the third host (Walker *et al.,* 2014). The adult ticks will then mate and feed on the third host until fully engorged and ready to lay eggs. The female tick will fall off the host lay eggs and then die. In summary, the *R. sanguineus* larvae, nymph, adult life stages feed on the same host (dog), adult ticks prefer to attach on the ears, neck and shoulders; nymphs are also found on the ears and shoulders, and larvae prefer to attach on the belly and flanks of dogs (Barker and Walker, 2014).

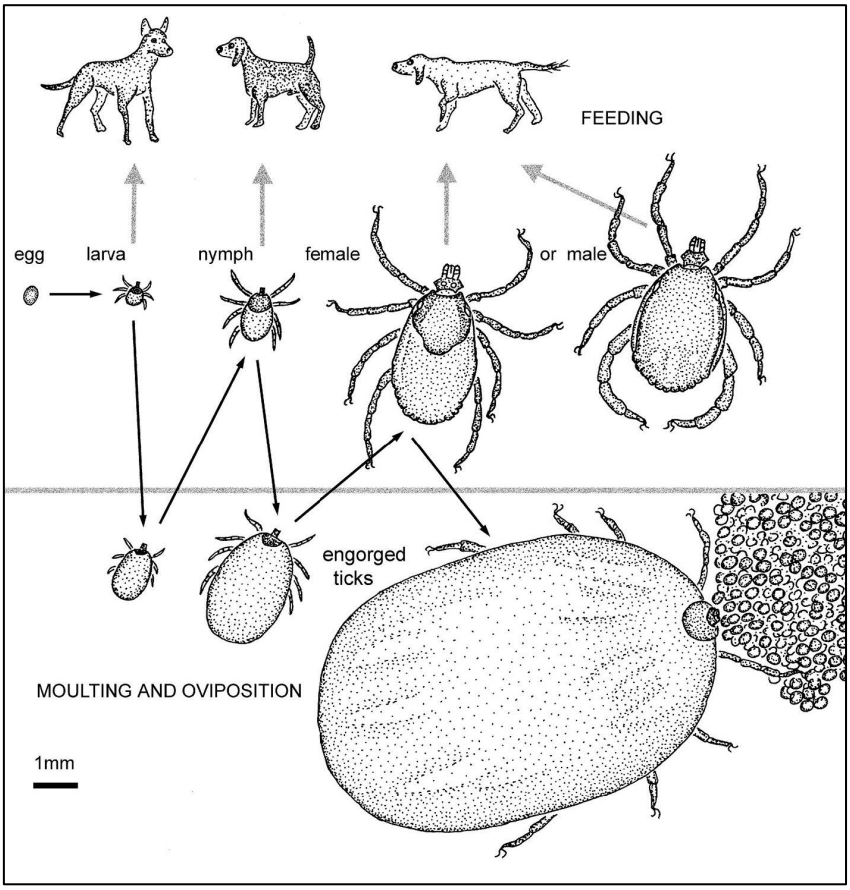


Figure 2.4: Life cycle of a three-host tick. Example is *R. sanguineus* (brown dog tick) feeding on dogs (Walker *et al.,* 2003).

2.5 Pathogens transmitted by ticks

Ticks transmit a wide variety of pathogens ranging from bacteria, protozoa, viruses, helminths and fungi (Yin *et al.,* 2002; Gulia-Nuss *et al.,* 2016; Noh *et al.,* 2017). Bacterial pathogens transmitted by ticks can range from *Coxiella burnetti*, which is an intracellular, gram-negative bacterium that causes chronic endocarditis, febrile illness, pneumonia or hepatitis spread by *Ixodes holocyclus*, *Haemaphysalis bispinosa* and *Rhipicephalus sanguineus sensu lato* ticks (Brites-Neto *et al.*, 2015) although it was first identified in *Ixodes ricinus* (Sassera *et al.,* 2006). This bacterium has a unique ability to invade the mitochondria within female tick ovarian cells and is transovarially transmitted to the next progeny with 100% efficiency (Mukhacheva and Kovalev, 2017).

Borrelia burgdorferi sensu lato bacterium detected in *Ixodid scapularis* ticks collected from dogs, cats and humans in the Grand River valley, Ontarrio, Canada cause Lyme disease in the mammalian host (Burgdorfer *et al.,* 1982; Scott *et al.,* 2017). *Rickettsia* gram-negative obligate intracellular bacteria are maintained within tick populations by vertical, transovarial and horizontal transmission and cause spotted fever (Harris *et al.,* 2017). The gulf coast tick *Amblyomma maculatum* is the primary vector of *Rickettsia parkeri* bacteria causing an emerging, eschar-associated spotted fever group of rickettsioses (Paddock *et al.,* 2004; Paddock and Goddard, 2015). *Rickettsia parkeri* has evolved mechanisms to exploit the actin polymerization machinery of the host to induce actin-based motility, enabling this pathogen to spread between host cells without exposing the bacteria to the extracellular milieu (Carlsson and Brown, 2006). The Rocky Mountain spotted fever is caused by *Rickettsia rickettsii* bacterium which is spread by several tick vectors such as *Demacentor variablilis*, *D. andersoni*,

Rhipicephalus sanguineus, *Haemaphysalis leporispalustris*. (Parker *et al.,* 1951; Shirai *et al.,* 1961; Philip *et al.,* 1978; Anacker *et al*., 1986; Demma *et al.,* 2005).

Protozoa transmitted by ticks cause emerging zoonosis such as babesiosis*,* a vector–borne disease transmitted by ixodid ticks (Brites-Neto *et al.,* 2015). Bovine babesiosis known as cattle tick fever in Brazil and Taxas fever in USA is caused by *Babesia bovis* and *B. bigemina,* this disease can be particularly severe for animals introduced in endemic areas that have not had previous exposure to the protozoan (Pérez de León *et al.,* 2010). Haemoprotozoans *Theileria ovis* and *Babesia ovis* protozoan are also reported in ticks collected from sheep and goats in Northern Algeria (Aouadi *et al*., 2017).

Examples of tick-borne viruses may include Kyasanur forest disease virus transmitted by *Haemaphysalis spinigera* and Omsk hemorrhagic fever virus transmitted by *Dermacentor* spp. causing hemorrhagic fever and encephalitis (Neelakanta and Sultana, 2016). *Ixodes cookie* and *I. persulcatus* ticks transmit Powassan virus and tick-borne encephalitis virus causing encephalitis (Neelakanta and Sultana, 2016). A study conducted in Japan suggested that *H. flava* and *H. japonica* ticks are possible intermediate hosts of *Cercopithfilaria* spp. (Nematoda: Onchocercidae) from Japanese serows (Uni *et al.*, 2013).

Another interesting fact is that ticks can also transmit different fungi. Tick-borne fungal disease in animals is known as mycotic proliferative dermatitis, being characterized by extensive inflammation with hyperemia and swelling of the affected area and formation of thick scabs of the skin in bovines, sheep, goats and horses (Dalis *et al*., 2014).

Furthermore, ticks are implicated in rare cases of Helminthes larvae transmissions associated with veterinary infections (Brianti *et al*., 2012; Otranto *et al*., 2012). According to Henning *et al*., (2016) filarial nematode DNA was recovered in *Amblyomma americanum* ticks in Nothern Virginia.

2.6 Rhipicephalus tick species of veterinary importance

The *Rhipicephalus* tick species of veterinary importance includes *R. decoloratus* a one host tick commonly known as the African blue tick transmitting protozoan *Babesia bigemina* causing bovine babesiosis in cattle, the bacteria *Anaplasma marginale* causing bovine anaplasmosis and *Borrelia theileri* causing spirochaetosis in cattle, sheep, goats and horses (Nakayima *et al.*, 2015; Madder *et al.,* 2013). The *R. microplus,* a one-host tick commonly known as the Asian blue tick transmits the protozoan *B. bovis* and *B. bigemina* which causes bovine babesiosis (redwater) (Pérez de León *et al.*, 2010)**.** Ticks from this species also transmit *A. marginale* which cause anaplasmosis (gall sickness) and *B. theileri* causing spirochaetosis in cattle (Smith *et al.*, 1978; Kocan *et al.*, 2010; Cutler *et al.*, 2012). The *R. appendiculatus* a three-host tick commonly known as the brown ear tick transmit different strains of *Theileria parva* causing disease complex known as either East coast fever or Corridor disease or Zimbabwean theileriosis, and *T taurotragi* causing benign bovine theileriosis (Latif and Hove 2011; Mbizeni *et al.*, 2013). Furthermore, *R. appendiculatus* transmitts *A. bovis* (*Ehrlichia bovis*) causing bovine ehrlichiosis, *Rickettsia conorri* causing tick typhus in humans and the virus causing Nairobi sheep disease (Ghosh and Nagar, 2014).

The *R. e evertsi* a two-host tick transmit *B. caballi and T. equi causing* equine piroplasmosis in horses (De Waal and Potgieter, 1987; Potgieter *et al.*, 1992; Montes

Cortés *et al.*, 2017; Horak *et al.*, 2017), *A. marginale* causing bovine anaplasmosis in cattle (Teshale *et al.*, 2016), and saliva toxin known as toxicosis causing spring lamb paralysis (Mans *et al.,* 2004). The *R. simus* commonly known as the glossy brown tick is a three-host tick transmitting *A. marginale* causing anaplasmosis (Horak and Knight, 1986; Hove *et al.*, 2008). Lastly, *R. zambeziensis* a three-host tick commonly known as the Lowveld brown ear tick transmit *T. parva* and *T. taurotragi* causing Corridor disease and bovine theileriosis respectively*, A. bovis* causing tropical bovine anaplasmosis (Walker *et al.*, 2003; Madder *et al.*, 2013). An example of Argasid ticks of medical and veterinary importance belong to the genera *Argas*, *Ornithodoros* and *Otobius.*

2.7 Economic importance of ticks and tick-borne diseases in companion animals

The enormous potential role of domestic companion animals, particularly dogs and cats were often not given enough attention as a contributing factor in maintaining tick-borne disease transmissions to humans (Day, 2011). Stray animals (free-roaming cats and dogs) provide a reservoir for infectious diseases, increasing the risk of zoonotic diseases particularly for those that are transmitted by vectors sharing the same micro-environment as pets with humans (Irwin, 2004). Human behaviour such as sitting on logs, gathering wood, sitting against trees and walking in tick infested vegetation increases the risk of exposure to ticks (Lane *et al.*, 2004) and the possibility that tick-borne pathogens may emerge as human pathogen.

2.8 Modern day techniques used for diagnosis of tick-borne pathogens

Many different diagnostic techniques are used to detect pathogenic agents in ticks today. Diagnostic techniques previously used to detect tick-borne pathogens in hosts include serology, cell culture and microscopic techniques (Fard and Khalili, 2011). Below are summarized current modern technologies used for tick identification.

2.8.1 Polymerase chain reaction (PCR)

According to Khehra *et al*. (2023), the polymerase chain reaction (PCR) is a laboratory method for amplifying nucleic acids, where short sequences of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) are denatured and renatured using the Taq DNA polymerase enzyme, which is derived from the bacterium *Thermus aquaticus*. This method was introduced by Mullis and colleagues in the1980's (Ghannam and Varacallo 2023). Key elements of PCR include a DNA template, primers, free nucleotide and the DNA polymerase enzyme (Green and Sambrook 2018; Khehra *et al*., 2023; Ghannam and Varacallo 2023). In traditional or conventional PCR, DNA is amplified and analyzed after the reaction is completed. This method has improved over the years into nested PCR, Real-time PCR, multiples PCR and reverse-transcription PCR (RT-PCR) (Ghannam and Varacallo 2023). Nested PCR involves two successive rounds of PCR using two sets of primers to increase sensitivity and/or specificity of PCR (Green and Sambrook 2019). Scarpulla *et al.* (2016) used nested PCR technique to detect and characterize *Rickettsia* in ticks from Italy. The Real-time PCR, also known as qPCR, is a technique that amplifies and quantifies DNA simultaneously, using fluorescent markers to measure the amount of DNA in real-time during the PCR process (Kubista *et al*., 2006). Lastly, Multiplex PCR is a method that

simultaneously amplify multiple sequences in a single reaction (Markoulatos *et al*., 2002).

These methods have been applied in tick research to assist in identifying different types of ticks and their associated pathogens. For example, LV and colleagues used conventional PCR to assess four DNA fragments' (*CO1*, *16S rDNA*, *ITS2*, and *12S rDNA* ability to identify Ixodida ticks to species level in China (Lv *et al*., 2014). Recently, Mofokeng *et al*. (2021) identified different tick-borne bacteria (*Anaplasma* spp., *Borrelia* spp. *Rickettsia* spp. and *Coxiella* spp.) using PCR from *Amblyomma* ticks collected from South African reptiles. A real-time PCR assay was used in Ethopia to detect *Rickettsia aeschlimannii* and *Rickettsia africae* in ixodid ticks (Tomassone *et al*., 2016). Similarly, Truong *et al*. (2021) used Real-time PCR biochip for on-site detection of *Coxiella burnetii* in ticks collected from two provinces in Korea. A multiplex real-time PCR was recently used for screening tick-borne pathogens in dogs and ticks (Cardenas-Cadena *et al*., 2023).

2.8.2 Next generation sequencing techniques

High-thoroughput detection and high sequencing technologies have been developed and are being used today revealing the high diversity of microorganisms, including pathogenic and non-pathogenic microorganisms (Cabezas-Cruz *et al*., 2018). The tick microbiome consists of communities of microorganisms, including viruses, bacteria and eukaryotes (Greay *et al*., 2018). Further studies on tick microbiota are being conducted using modern technologies like next-generation sequencing (NGS) technologies (Greay *et al*., 2018). The Next-generation sequencing refers to a suite of advanced sequencing technologies that allow for the rapid and high-thorouput

sequencing of DNA and RNA (Duan and Cheng 2016; Cabezas-Cruz *et al*., 2018; Greay *et al*., 2018; Bonnet and Pollet 2021). These tools have made it quicker and more affordable to study these microbial communities and strains. The main sequencing methods used to study these communities include (i) shotgun metagenomics to explore the functions these microbes might have, and (ii) shotgun metatranscriptomics to find out which genes are actively being used by the microbes: (iii) using specific gene markers like the *16S rRNA* gene to identify the types of microbes present, or (SSU rRNA genes) (Cabezas-Cruz *et al*., 2018). The first NGS study of a tick microbiome was in 2011 whilst assessing bacteria diversity in the cattle tick *R. (B) microplus* (Andreotti *et al*., 2011)

Metagenomics is the analysis of the collective genomes of the community (Semenov, 2021). Metagenomics techniques are used to identify microbial communities within environmental samples (Thomas *et al.*, 2012). This method provides an opportunity for researchers to identify and study the effect of arthropod microbiota on vector-borne infectious diseases leading to a better understanding of the vector microbiota in relation to mammalian host immune responses (Finney *et al.*, 2015). Whilst transcriptomes focus on RNA; A whole transcriptome analysis of tick RNA was conducted in Western Europe to generate a global picture of tick-borne bacteria and validate an NGS strategy by identifying bacteria at the RNA level *(*Vayssier-Taussat *et al*., 2013*)*. Metabarcoding on the other hand is an NGS- method that focuses on the identification of the community composition via analysis of the sequences of barcode marker genes (Semenov, 2021, Trzebny *et al*., 2022). A total number of 392 possible bacterial genera was detected from ticks collected in domestic animals found in two Orang Asli Villages in Malaysia using metarbarcoding indicating the diverse bacterial

community profile (Khoo *et al., 2016).* Another study was also conducted to analyze the in vivo microbial community structure and diversity differences of *Haemaphysalis flava* in four developmental stages *(*eggs, larva, nymph and adult) targeting the V3 region of the *16S ribosomal DNA* (*16S rDNA*) to determine which bacterial genera could be propagated through transovarial transmission and transmitted by transstadial during different developmental stages (Duan and Cheng, 2016).

CHAPTER 3

Molecular characterization of the economically important South African *Rhipicephalus* tick species of domestic animals

3.1 Introduction

Ticks are hematophagous, obligate ectoparasites of terrestrial vertebrates including reptiles, birds, amphibians, and mammals (Black and Piesman, 1994). They belong to the suborder Ixodida (=Metastigmata), order Parasitiformes (have stigmatal pores on the podosomal and/or opisthosomal portion of the body, well-developed, freely articulated coxae), and subclass Acari (Walker *et al.,* 2014). Heavy tick burdens result in huge economic losses through blood loss, general stress and irritation, decrease in productivity, depression of immune function, damages to hides and transmission of pathogens (Alim *et al.,* 2012; Abbas *et al.,* 2014). Ticks are commonly known as vectors of various zoonotic diseases such as Lyme disease and animal babesiosis, Q fever, epizootic bovine abortion, and relapsing fever (Iv *et al.,* 2014b). An individual tick can carry multiple pathogenic agents and harbour endosymbionts or commensal bacteria (Carmichael and Fuerst 2006; Khoo *et al.,* 2016). Diseases caused by tick infestation in livestock production causes huge economic losses (Horak *et al.,* 1983; Spickett *et al.,* 2011).

Ticks like *Rhipicephalus (Boophilus) microplus* are highly adaptable, prevalent in tropical and subtropical regions of the world (Yawa *et al.,* 2019). This tick species has successfully expanded its geographical range in Africa in such a way that it is reported to have superseded the native tick, *R. (B) decolaratus* (Nyangiwe *et al*., 2013; Yawa *et al*., 2019). These two groups of tick's species are morphologically very similar, almost identical, making it difficult for unseasoned taxonomist to identify using

taxonomic keys. Nyangiwe *et al*., (2013) also reported that some larvae collected in their studies share morphological features with *R. (B.) microplus* and *R. (B.) decolaratus* and are suspected hybrids. *R. (B.) microplus* has been proven to be a competent vector of *Babesia bovis* (causantive agent of Asiatic redwater) and *B. bigemina* (causative agent of African red water) while *R. (B.) decolaratus* is known to transmit *B. bigemina* (Madder *et al*., 2007). They are also known to transmit *Anaplasma marginale* (causative agent of anaplasmosis or gall sickness in cattle) (Horak *et al*., 2015). These diseases are responsible for great losses in livestock and hence, correct identification of the tick species is very much important to apply correct or improved preventative measures.

Traditionally, morphological characteristics of adult ticks is the conventional method used for identification although the used characteristics could sometimes be ambiguous. Often, appearances of adult ticks are used as discriminating features to distinguish between species (Keirans and Clifford, 1978). Identification of sub adult stages (larvae and nymphs) using morphological features has proven to be challenging because discriminating characteristics used relies on continuously graded features and the systematics of these ticks are frequently uncertain (lack of fully developed characteristic and lack of specific taxonomic keys for some genera) (Clifford *et al*., 1961; Caporale *et al*., 1995; Kleinjan and Lane, 2008). Morphological species identification can also be difficult if the tick specimens collected in the field are damaged or fully engorged (Guglielmone *et al.,* 2009; Lv *et al.,* 2014a; Gou *et al*., 2018; Schulz *et al*., 2020). Closely related tick species share similar morphological features and sometimes even seasoned taxonomist experience challenges to differentiate between these species (Caporale *et al.*, 1995).

To overcome these challenges, molecular techniques are used to confirm morphological identification of ticks. Different molecular techniques have been tried and tested to try and overcome the challenges posed by morphological identification.

Today DNA barcoding is the most used technique for species identification (Schindel and Miller, 2005; Taberlet *et al*., 2007; Feng *et al*., 2011). This technique uses short genetic markers for identification. A reliable DNA barcoding system should show high interspecific variability while remaining conserved within species, use a standardized DNA region across taxa, possess robust and conserved priming sites, and have a marker length not exceeding 800 bp for efficient amplification and sequencing (Miller 2007; Feng *et al.*, 2011).

The *cytochrome c oxidase I* (*CO1*) is known as a genetic marker for a broad range of animal phyla and has since been acknowledged as the standard marker for universal DNA barcoding in Metazoa (Hebert *et al*., 2003, Lv *et al*., 2014a, Rach *et al*., 2017). Brown *et al*. (1982) reported that mitochondrial DNA sequences seem to be well-suited to distinguish closely related species because these genes evolve more rapidly than nuclear genes. Folmer *et al*., (1994) developed universal *CO1* primers that were able to generate informative sequences for phylogenetic analyses at the species and higher taxonomic levels for 11 invertebrates' phyla. Intraspecific genetic variation was detected from two morphologically similar tick species, *Ixodes holocyclus* and *I cornuatus* targeting the *mitochondrial cytochrome oxidase subunit 1* (*CO1*) (Song *et al*., 2011). This variation is significant for species delineation, as it helps distinguish between closely related or cryptic species that are otherwise difficult to differentiate based on morphology (Lin *et al*., 2015).

A few studies have successfully used this gene region to molecularly confirm tick identification (Lv *et al*., 2014a; 2014b; Binetruy *et al*., 2019; Schulz *et al*., 2020; Mofokeng *et al*., 2021; (Mahlobo-Shwabede *et al*., 2022).

Another common barcoding gene used for identification of several invetebrate species is the mitochondrial *16S rDNA* (Olson *et al*., 1991; Xiong and Kocher, 1991; 1993; Chitimia-Dobler *et al.,* 2017; Binetruy *et al.,* 2019, Kamani, 2021). Olson *et al.,* (1991) used the *16S rRNA* gene region to distinguish between morphologically identical and co-occuring larvae of two species of sea cucumber *Psolus fabricii* and *Cucumaria frondosa*. The universal constructed *16S rRNA* gene primers where able to amplify the homologous region of the mitochondrial DNA from seven black fly morphospecies (Xiong and Kocher 1991). This gene region also proved to be the most common and useful in examining the phylogenetics of hard and soft tick taxa at or below family level (Black and Piesman, 1994; Guglielmone *et al*., 2006; Chen *et al*., 2012; Lv *et al*., 2014b; Lv *et al*., 2014a; Muhanguzi *et al*., 2020). The mitochondrial *12S rRNA* of the ribosomal DNA is also known to be reliable in identification of ticks to species level (Chitimia-Dobler *et al*., 2017). The *18S rRNA* is reported to being useful in identification of ticks at higher taxonomic level than species level due to the gene being too conserved (Mans *et al*., 2011; Lv *et al*., 2014b).

The *nuclear marker internal transcribed spacer- 2* (*ITS2*) is also used to determine genetic diversity levels among and between species of ixodes ticks (Barker, 1998; Song *et al*., 2011; Lu *et al*., 2013; Brahma *et al*., 2014; Livanova *et al*., 2015). Most of the *Rhipicephalus* tick species are similar and not easy to identify morphologically. To assess the reliability of molecular DNA markers in identification of *Rhipicephalus* tick species in South Africa, the *CO1*, *16S rRNA*, *18S rRNA* and *ITS2* genes were used to

confirm morphological identification of ticks collected from domestic animals in the current study.

3.2 Materials and methods

3.2.1 Tick sampling

Samples were collected from four different provinces is South Africa (Figure 3.1). Tick samples were collected from sheep and goats in 2019 and 2021 at Matlwang village in North West Province (26°44’13”S, 26°55’52”E). Ticks were again collected from cattle, dog, sheep, goat in Kameeldrift, a farming area in Pretoria west Gauteng Province (25°43’13”S, 28°00’27”E) 2021. Some were collected from cattle in a village called Ha-Mashau in Venda, Limpopo Province (23°09’51”S, 30° 11’59”E) and from cattle in Rooderkop, Ventersdop, farm number Z7 Potchefstroom (26°12’29.9”S, 26°41’30.1”E), North West Province in 2021. More tick samples were collected from dogs in Ikageng, Potchefstroom (26° 42’54.16”S, 27°03’18.76”E) 2021. .More dog tick samples used in the present study were collected in Potchefstroom Animal Welfare Society (PAWS) in 2017 from stray dogs rescued from various suburbs; Mieder park (26°45’11.362”S, 27° 5’17.6428”E), Ikageng (26°43’ 32.3738”S, 27°2’59.9492”E), Baillie Park (26° 42’58.1173”S, 27°6’58.2487”E), Kanonierspark (26°41’43.0631”S, 27° 4’19.3904’E), Boskop (26°33’51.9836”S, 27°7’44.0036”E), Potchindustrie (26° 43’7.37”S, 27°4’14.0952”E) in JB Marks Local Municipality and from Fochville (26°28’37.4059”S, 27°29’27.1457”E) Merafong City Municipality and Boipatong (26°44’48.0476”S, 27°1’51.492”E) Emfuleni local Municipality (Van wyk *et al*., 2022). Also, tick samples collected from horses in the city center of Potchestroom (27º10’0”E and 26º40’0”S) (Simpson 2019) formed part of the present study.

Environmental or ticks found on vegetation were collected in three reserves in north-eastern KwaZulu-Natal Province namely Charters Creek (28°13'37"S; 032° 24'1"E), Hluhluwe-Imfolozi (28°9'50"S; 032°12'15"E) and Phinda Private Game Reserve (27°46'39"S; 032°20'57"E) all situated in the uMkhanyakude District Municipality (Taioe, 2017).

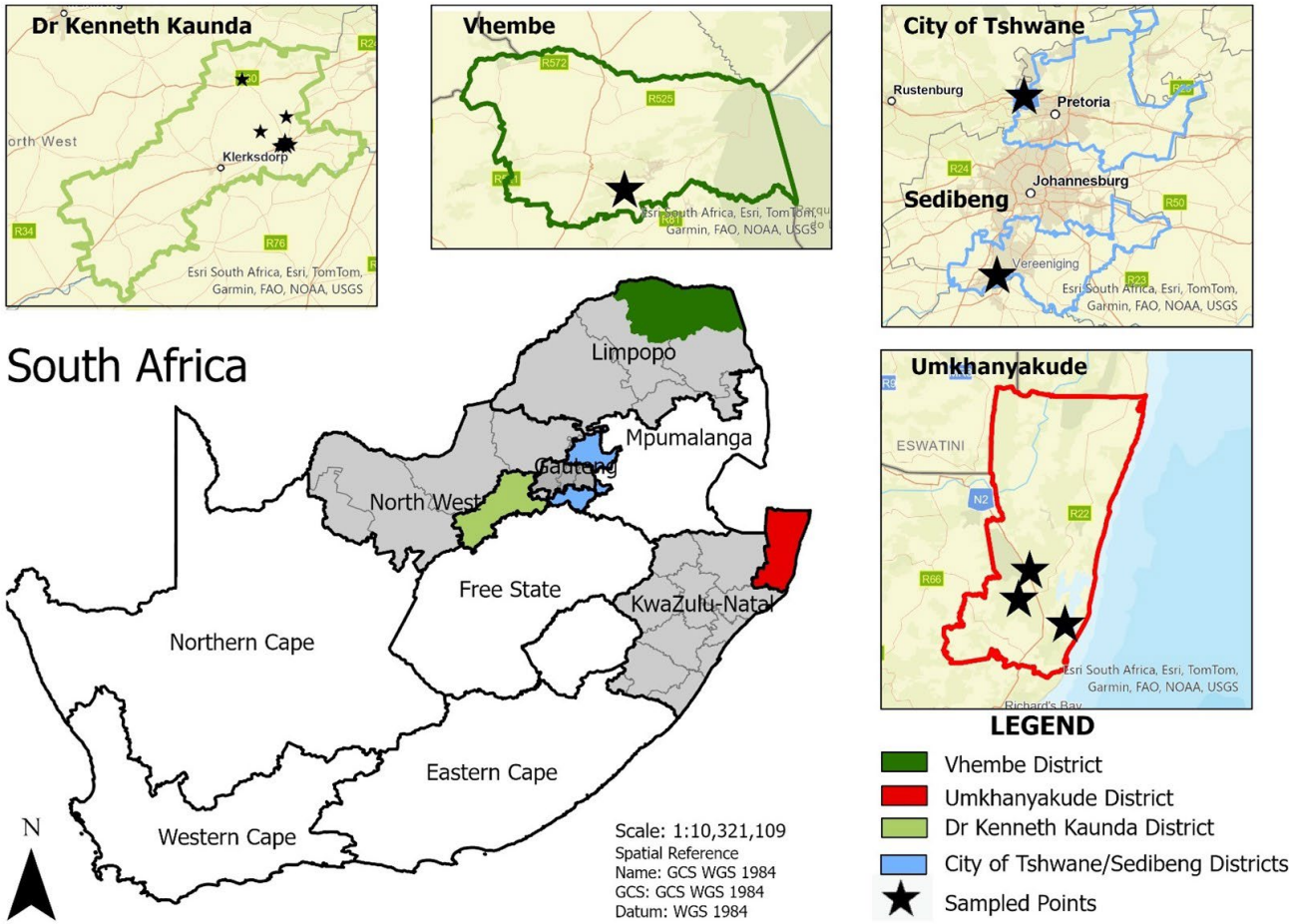


Figure 3.1: Map showing four sampled provinces in South Africa with sampled districts/municipalites. The black star represents the localities where the tick species were sampled.

3.2.2 Tick morphologica identification

A stereo microscope was used for morphological identification of ticks following published tick identification keys (Walker *et al*., 2003; Coetzer and Tusting, 2004; Latif, 2013; Barker and Walker, 2014; Walker *et al*., 2014); characteristics described in Table 2.1. The sex, life stage, host of all collected ticks was noted. Identfied tick samples were stored in 70% ethanol except for female ticks that were alive, fully engorged and in the process of laying eggs. The later were stored separately in individual 5 ml tubes for a couple of weeks to allow the process of laying eggs to take place. After laying eggs, female ticks were stored corresponding to the respective eggs they laid in 1.5 microliter (µl) tubes. Eggs were weighed using analytical balance-(AS 220.R2 PLUS Radway, Lasec (Pty) Ltd), labelled and stored for future use. To confirm the morphological identification done, colony reared ticks bred at ClinVet were compared to a subset of ticks collected from different hosts. Below is a list of morphological characteristics used to identify the genus *Rhipiciphelus* based on the identification key by Walker *et al*., (2014).

- The size of unfed ticks is medium (3 to 5 mm) including mouthparts. Lateral suture is absent. Integument texture has striations
- Mouthparts are anterior with palp articles that are all small
- Basis capituli has distinctly angular lateral margins (making a hexagonal shape of the entire basis capituli).
- Legs are slender, have no pale rings and pulvilli are always present.
- Scutum is present in the female (a conscutum in the male).
- Enamel (= ornamentation) is usually absent from the scutum or conscutum but there are four species with enamel.

- Eyes are present and flat to slightly convex (but in *R. evertsi* the eyes are very convex or highly bulging).
- Festoons are present in males (and in females but unclear when females are fed).
- Spiracular plates are large and posterior to legs 4. Spiracle goblets are scattered over the spiracle plates.
- Ventral plates are present in males only (usually as two pairs of plates)
- Anal groove is posterior to the anus.
- Coxae 4 are of normal size; Coxae 1 have large and equal paired spurs.

Table 3.1: Morphological characteristics used to identify collected ticks to species level (Walker *et al*., 2003; Coetzer and Tusting, 2004; Latif, 2013; Barker and Walker, 2014; Walker *et al.,* 2014).

Tick species	Common name	Morphological characteristics
R. sanguineus	Dog tick, Kennel tick	Three host tick, eyes are distinct (large and slightly convex), sparse punctuation of small medium size, marginal grooves distinct. Posterior grooves poterolateral, posteromedial distinct (deep and wrinkled). Engorged specimens show bulging out of lateral body wall, caudal process present in fed specimen. Festoons are present, columns of teeth on hypostome are 3+3, spiracle plate shape is like a coma and tarsal terminal spurs are absent. interstitial punctuation size is small to medium with a sparse distribution (density of these punctations is highly variable), setiferous punctations are indistinct. Male: Coxa 1 anterior spur is small and not visible dorsally, Punctations on conscutum are conspicuous, lateral grooves are present, male yellowish brown (pale), Setae on conscutum and alloscutum are short and sparse, adanal plate shape is broadly curved at posterior, Caudal appendage is absent in unfed males but broad in fed males, anal groove is distinct. Cervical fields depression is not apparent, and the texture has no wrinkles. Conscutum colour is pale (dark in some populations), Accessory adanal plates are large. The shape of adanal plates is narrow and trapezoid. Spiracle areas have sparse setae and the spiracle plate tails are narrow, half the width of adjacent festoons. Posterior grooves are distinct (deep and wide with wrinkled texture). Lateral grooves type is a distinct groove. Lateral grooves texture is smooth. Female: Punctations on scutum are conspicuous, scutum posterior margin has a double concavity (distinctly sinuous), and each side setae on scutum as well as alloscutum are short and sparse. The scutum colour is pale although in some populations it can be dark. Cervical fields are deep and distinct

		(texture has wrinkled areas) with a large and straight shape. Scapular grooves profile is steep. Genital aperture posterior lips have a broad u shape (but may have a broad v shape). Bases capituli lateral angles are sharp, and the porous areas separation is broad. Palp pedicels are short.
R. evertsi evertsi	Red-legged tick	Two host tick, largest and most distinctive *Rhipicephalus* species. Easy to differentiate by its very convex beady eyes and scutum that is covered with many punctations. Medium sized, dark brown coloured scuta, legs are orange to red. Cervical fields texture has wrinkled areas. Male: Interstitial punctation size is small to medium. Interstitial punctation distribution is dense. Setiferous punctations are indistinct. Coxae 1 anterior spurs are visible, Cervical fields' depression is not apparent. Conscutum colour is dark, Accessory adanal plates are small (they may be absent), Adanal plates shape is broad and curved (in some specimens it may have straighter sides than illustrated), Caudal appendage is absent in fed males. Spiracle plate areas have dense setae, Posterior grooves are indistinct (represented by three flat posterior areas of wrinkled texture). Lateral grooves type is a distinct groove. Lateral grooves texture is wrinkled. Female: Interstitial punctation size is small to medium. Interstitial punctation distribution is dense. Setiferous punctations are indistinct, Basis capituli lateral angles are blunt. Porose areas separation is narrow, Palp pedicels are short. Cervical fields shape is not apparent. Scapular grooves profile is shallow, Spiracle plate areas have dense setae, Scutum posterior margin is slightly sinuous. Scutum colour is dark.

R. (B) microplus	Asian blue tick	One-host tick, adults slightly larger and redder in colour than *R. decolaratus*. Has 4 + 4 columns of teeth on its hypostome and no protuberance bearing setae on the inner margin of palp article 1. Porose areas shape is a broad oval. Male: Cornua are distinct, Coxae 1 spurs length is long (also the anterior spurs of coxae 1 are conspicuous dorsally). Ventral plate spurs are indistinct (3 = accessory adanal plate), ventral plate spurs are indistinct (4 = adanal plate), caudal appendage is narrow, ventral plate spurs are not visible dorsally. Female: Palp articles 1 internal margin has no protuberance and is short and distinctly concave. Coxae 1 spurs are distinct, coxae 2 and 3 spurs are present, Genital aperture posterior lips have a broad U shape.
R. (B) decoloratus	African blue tick	One host tick, small sized inconspicuous, short mouth parts, slender legs. The only species of the subgenus *Boophilus* with 3 + 3 columns of teeth on the hypostome. It also has a protuberance on the internal margin of the first articles of the palps and this protuberance bears two pectinate setae. Caudal process (Appendages) present narrow in fed specimens. Porose areas shape is a narrow oval. Male: Comua are distinct, Coxa 1 spur length is short, ventral plate spurs are distinct (3 = accessory adanal plate), Ventral plate spurs are distinct (4 = adanal plate), caudal appendage is narrow. Ventral plate spurs are visible dorsally. Males are smaller than females. Males are brownish-yellow, dark coloured intestine are visible through the light microscope.

		Female: Palp articles 1 internal margin has a protuberance with pectinate setae, Coxa 1 spurs are distinct and Coxa 2 and 3 spurs are present, genital aperture posterior lips have a narrow U shape. Engorged females are blueish brown.
R. appendiculatus	The brown ear tick	Three host tick, Adult medium sized ticks with short mouthparts. Legs increase markedly in size from the 1st to the 4th pair. Cervical fields texture has wrinkled areas. Eyes are slightly convex. Females have cervical fields similar to the male and fewer punctations. Male: Interstitial punctation size is small to medium. Interstitial punctation is sparse, Setiferous punctations are indistinct, Coxae 1 anterior spurs are visible dorsally, Cervical fields depression is apparent. Accessory adanal plates are small. Adanal plates shape is narrow and trapezoid, Caudal appendage is slender or narrow in fed males, Spiracle areas have sparse setae. Posterior grooves are distinct (shallow and with wrinkled texture). Lateral grooves type is a distinct groove. Lateral grooves, texture is wrinkled. Female: Basis capituli lateral angles are blunt. Porose areas separation is broad, Palp pedicels are short, Cervical fields shape is large and curved, Spiracle areas have sparse setae. Scutum posterior margin is distinctly sinuous and the scutum colour is dark. Interstitial punctation size is small to medium with a sparse distribution. Setiferous punctations are indistinct, Scapular grooves profile is shallow. Genital aperture has posterior lips forming a broad V shape.

3.2.3 Molecular identification of *Rhipicephalus* tick species

3.2.3.1 Tick DNA extraction

Ticks were washed once with 70% ethanol and three times with distilled water as described previously to remove all environmental contaminants (Carpi *et al*., 2011). Ticks were pooled in three's and put into 1.5 ml tube. Ticks in each tube were dissected using a fine forceps to break the tissues and it was washed in ethanol and distilled water after every use to avoid contamination. DNA was extracted following Qiagen DNeasy Blood and Tissue kit (Qiagen Hilden, Germany) extraction protocol. Firstly, 96% ethanol was added to Buffer AW1 and Buffer AW2 concentrates. Then 180 µl Buffer ATL and 20 µl proteinase *K* was added in each 1.5 ml microcentrifuge tube that had cut tick tissue. This was mixed by vortexing and incubated at 56°C on a preheated heat block until completely lysed. The mixture was vortexed occasionally during incubation. Then 200 µl Buffer AL was added and mixed thoroughly by vortexing. Then 200 µl ethanol (96–100%) was added and mixed thoroughly by vortexing. The mixture was pipetted into a DNeasy Mini spin column placed in a 2 ml collection tube. This was centrifuged at 8000 rpm for 1 min. The flow-through and collection tube was then discarded, and the spin column was inserted into a new 2 ml collection tube. Then 500 µl Buffer AW1 was added and centrifuged for 1 min at 8000 rpm The flow-through and collection tube was discarded, and the spin column was placed in a new 2 ml collection tube. Then 500 µl Buffer AW2 was added and centrifuge for 3 min at 14,000 rpm. The flow-through and collection tube was then discarded, and the spin column was transferred into a new 1.5 ml microcentrifuge tube. The DNA was eluted by adding 200 µl Buffer AE to the center of the spin column membrane. The mixture was incubated for 1 min at room temperature (15–25°C) and then centrifuged for 1 min at 8000 rpm. The last step was repeated step to increase the DNA yield. The extracted

DNA was measured using Qubit ® 3.0 Fluorometer (Life Technologies, California, USA) then stored at -4°C freezer until used.

3.2.3.2 Amplification for tick identification

To confirm the morphological identification of *Rhipicephalus* ticks collected in this study, a polymerase chain reaction (PCR) assays targeting the *cytochrome oxidase 1 (CO1); 18S rRNA*, *16S rRNA* and the *internal transcribed spacer 2 (ITS2)* gene fragments were conducted. Each reaction contained 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water and 2 µl of template DNA making a final volume of 25 µl. A PCR assay of a total volume of 50 µl was prepared to amplify the *ribosomal ribonucleic acid 16S rRNA*, each reaction contained 25 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 3 µl of 10 µM forward primer, 3 µl of 10 µM reverse primer, 2 µl of 360 GC Enhancer, 15 µl of double distilled water and 2 µl of template DNA. Finally, for the amplification of *the cytochrome oxidase subunit 1* (*CO1*) gene. The PCR was performed at a total volume of 25 µl. Each reaction contained 12.5 µl of KAPA HiFi HotStart Ready Mix (KAPA Biosystems, Massachusetts, USA), 0.75 µl of 10 µM forward primer, 0.75 µl of 10 µM reverse primer, 9 µl of double distilled water and 2 µl of template DNA. Double distilled water was used as the negative control. For an improved performance, the protocols used was optimized using the “Hot Start” PCR and a touchdown PCR for *18S* and *16S* as described by Lv *et al*., 2014a. The *R. e. eversti* DNA obtained from colony reared ticks at CLINVET Research Inovation, Bloemfontein, South Africa was used as positive control. The fragment size and list of primers are shown in table 3.2. PCR amplification was carried out using the ProFlex PCR system (Thermo Fisher Scientific, US).

The touchdown PCR amplification of *16S rRNA*, primer set *16S-F/16S-R* (Table 3.2) targeting a 455 bp was performed following an initial denaturation step at 94°C for 5 minutes; followed by 5 cycles of 94 °C for 30 seconds, 49 °C for 30 seconds, and 68 °C for 30 seconds; 5 cycles of 94 °C for 30 seconds, 47 °C for 30 seconds, and 68°C for 30 seconds; 5 cycles of 94 °C for 30 seconds, 45 °C for 30 seconds, and 68 °C for 30 seconds; 25 cycles of 94 °C for 30 seconds, 43 °C for 30 seconds, and 68 °C for 30 seconds; followed by a final extension step of 68 °C for 5 minutes with a final hold at 4°C ∞.

Touchdown PCR protocol for *18S rRNA*, primer set *18S-F/18S-R* (Table 3.2), fragment size 780 bp was as follows: an initial denaturation step 94 °C, 5 minutes, followed by 5 cycles of 94 °C for 30 seconds, 52 °C for 30 seconds, and 68 °C for 1 minute; 5 cycles of 94 °C for 30 seconds, 50 °C for 30 seconds, and 68 °C for 1 minute; 5 cycles of 94 °C for 30 seconds, 48 °C for 30 seconds, and 68 °C for 1 minute; 25 cycles of 94 °C for 30 seconds, 46 °C for 30 seconds, and 68 °C for 1 minute; followed by a final extension step of 68 °C for 5 minutes with a final hold at 4°C ∞.

The amplification protocol for *ITS2*, primer set *ITS2-F/ITS2-R* (Table 3.2), fragment size 900-1200 bp started with an initial denaturation step of 95°C for 10 minutes; Followed by 35 cycles denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds and extension at 72°C for 60 seconds, and the final extension at 72°C for 7 minutes with a final hold at 4°C ∞. Finally, the PCR conditions used to amplify the *CO1* gene, primer set *LCO1490/ HCO2198* (Table 3.2), fragment size 680 bp started with an initial denaturation step 95°C for 3 minutes; Followed by 35 cycles denaturation at 98°C for 20 seconds, annealing at 50°C for 15 seconds and extension at 72°C for 60 seconds, and the final extension at 72°C for 1 minutes with a final hold at 4°C ∞. Reactions were undertaken in a Bio-Rad C1000 Touch™ Thermal Cycler PCR

machine (Bio-Rad, Hemel Hempstead, UK). All PCR amplicons were visualized by agarose electrophoresis in 1% (w/v) agarose gel in 1X TAE buffer, stained with ethidium bromide. A gene ruler 100 bp plus DNA ladder (New England Biolabs, US) was used with each gel. The gels were visualized under UV light using the ENDURO GDS Gel Documentation System (Labnet International), Inc., US. Amplification success rate was measured based on the proportion of samples that will produce sequences of the appropriate length. Subset of positive PCR products were sent to Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa for purification and sequencing for forward and reverse sequences. These sequences were further evaluated for their utility in species identification.

Sequence chromatograms were viewed and edited in Chromas program embedded in Molecular Evolutionary Genetics Analysis version 11 (MEGA 11) for bigger datasets (Tamura *et al*., 2021). A Basic Local Alignment Search Tool (BLASTn) for nucleotides was used (http://blast.ncbi.nlm.nih.gov/) to identify *Rhipicephalus* reference species sequences with the highest nucleotide sequence identity (> 98%) in the Genbank data base to confirm retrieved sequences. For phylogenetic analysis, reference sequences of *Rhipicephalus* tick species were downloaded from Genbank database and aligned to the sequences produced in this study. Sequences were aligned using the Clustal W alignment tool under the default settings and implemented in MEGA 11 (Tamura *et al*., 2021). A best-fit model of nucleotide substitution was identified under the Bayesian Information Criterion (BIC) in MEGA 11 (Tamura *et al*., 2021). Maximum Likelihood (ML) analyses was performed using MEGA11 (Tamura *et al*., 2021). Nodal support was assessed through 1000 non-parametric bootstrap replications.

Table 3.2: Oligonucleotide primers used for molecular identification of ticks

GENE REGION	FORWARD	REVERSE	PCR CONDITIONS	FRAGMENT SIZE (BP)	REFERENCES
COI	5'GGT CAA CAA ATC ATA AAG ATA TTG G 3'	5'TAA ACT TCA GGG TGA CC AAA AAT CA3'	50°C	680	Folmer *et al.,* 1994; Lv *et al.,* 2014a, Kumarb *et al*., 2018
18S	5'CATTAAATCAGTTATGGTTCC3'	5'CGCCGCAATACGAATGC3	52°C, 50°C, 48°C, 46°C	780	Lv *et al.,* 2014b
16S	5'TTAAATTGCTGTRGTATT3'	5'CCGGTCTGAACTCASAWC3'	49°C, 47°C, 45°C, 43°C	455	Lv *et al*., 2014a
ITS2	5'YTGCGARACTTGGTGTGAAT3'	5'TATGCTTAARTTYAGSGGGT3'	50°C	900-1200	Muruthi 2015

3.3 Results

3.3.1 Morphological identification of ticks

A total of 768 ticks were collected from four provinces in South Africa as shown in Table 3.3. Morphological identification revealed a total of three different species of ticks from the genus *Rhipicephalus*. These include *R. evertsi evertsi*, *R. appendiculatus*, and *R. sanguineus* (Plate I-IV). The images in the different plate's shows pictures of the dorsal and ventral sides of collected tick species in their male and female form. The dominant species from the sampled hosts were *R. e. evertsi* (63.15%) collected from sheep, goats, cattle, and horses. This was followed by *R. appendiculatus* (27.34%) collected from the vegetation whilst *R. sanguineus* (9.51%) was the least and collected from dogs. Colony reared ticks provided by Clinvet include *R. e. evertsi* (n=10) and *R. appendiculatus* (n=20) adult ticks; *R. (B) decoloratus* (n=500) and *R. (B) microplus* (n=500) nymphs.

3.3.1.1 Morphological identification of *R. e. evertsi,* Neumann (1897)

This species was collected from three provinces (North West, Gaunteng and Limpopo) in South Africa following the description of Walker *et al*., (2003) and Madder *et al*., (2013). The tick is medium sized with a dack brown scuta with reddish orange body wall. The eyes are beady and convex with orange to red legs (Table.3.1). The scutum and conscutum are covered with many punctuations (Mahlobo-Shwabede *et al*., 2022; Plate I).

3.3.1.2 Morphological identification of *R. sanguineus* (1806)

This species was collected from Gauteng and North West Provinces. The tick species was identified based on descriptive taxonomic keys of Walker *et al.,* (2003) and

Madder *et al*., (2013). The adult tick was brown in colour with large, distinct and slightly convex eyes. Festoons were present and sparse punctuation of small medium size marginal grooves were distinct. The basis capitula of females had broad lateral angles (Mahlobo-Shwabede *et al*., 2022; Table 3.1; Plate II).

3.3.1.3 Morphological identification of *R. appendiculatus,* Neumann (1901)

This species was collected only from KwaZulu-Natal Province in South Africa and identified as described by Walker *et al*., (2003) and Madder *et al*., (2013). The adult is medium sized with short mouthparts. The legs increase markedly in size from the 1st to the 4th pair. The eyes are flat and the basis capitula is hexagonal especially in females. Medium-sized and fine punctations present in the middle of the conscutum and scutum (Mahlobo-Shwabede *et al*., 2022; Table 3.1; plate III).

3.3.1.4 Morphological identification of *R. (B) decoloratus* Koch (1844)

This species was obtainedcollected from Clinvet laboratories (Laboratory reared) in the Free State Province of South Africa as nymphs. The morphological identification was done based on the identification of the adult ticks that were used to breed the nypmth used in the present study (Walker *et al*., 2003). Table.3.1 contains more details and Plate IV are images of *R. (B) decoloratus* nympths from this study. Morphological identification of nympth proved difficult, the tick's distinct features were not fully developed.

3.3.1.5 Morphological identification of *R. (B) microplus* Canestrini (1888)

This species was also obtained in the nymphal stage from Clinvet laboratories (Laboratory reared) in the Free State Province South Africa. The morphological

identification was done based on the identification of the adult ticks that were used to breed the nypmth used in the present study (Walker *et al*., 2003). Table 3.1 contains information of the species while Plate V are images of *R (B) microplus* from this study. Morphological identification of nympth proved difficult, the tick's distinct features were not fully developed.

Table 3.3: *Rhipicephalus* ticks species identified from four provinces in South Africa and the total number of ticks collected from each host type

Host	***Rhipicephalus* species**	**No. of collected ticks**	**Province**	**Abundance %**
Dog	*R. sanguineus*	60	NW	9.51
		13	GP	
Sheep	*R. e. evertsi*	78	NW	10.42
		2	GP	
Horse	*R. e. evertsi*	210	NW	27.34
Goat	*R. e. evertsi*	93	NW	12.11
Cattle	*R. e. evertsi*	81	NW	13.28
		16	GP	
		5	L	
Vegetation	*R. appendiculatus*	210	KZN	27.34
Total		**768**		**100**

*NW = North West, GP = Gauteng, KZN = KwaZulu-Natal, L = Limpopo

Table 3.4: Laboratory reared ticks

Rhipicephalus species	**Stages**	**No. of specimens**
R. e. evertsi	Adult	10
R. appendiculatus	Adult	20
R. decoloratus	Nymphs	+-500
R. microplus	Nymphs	+-500

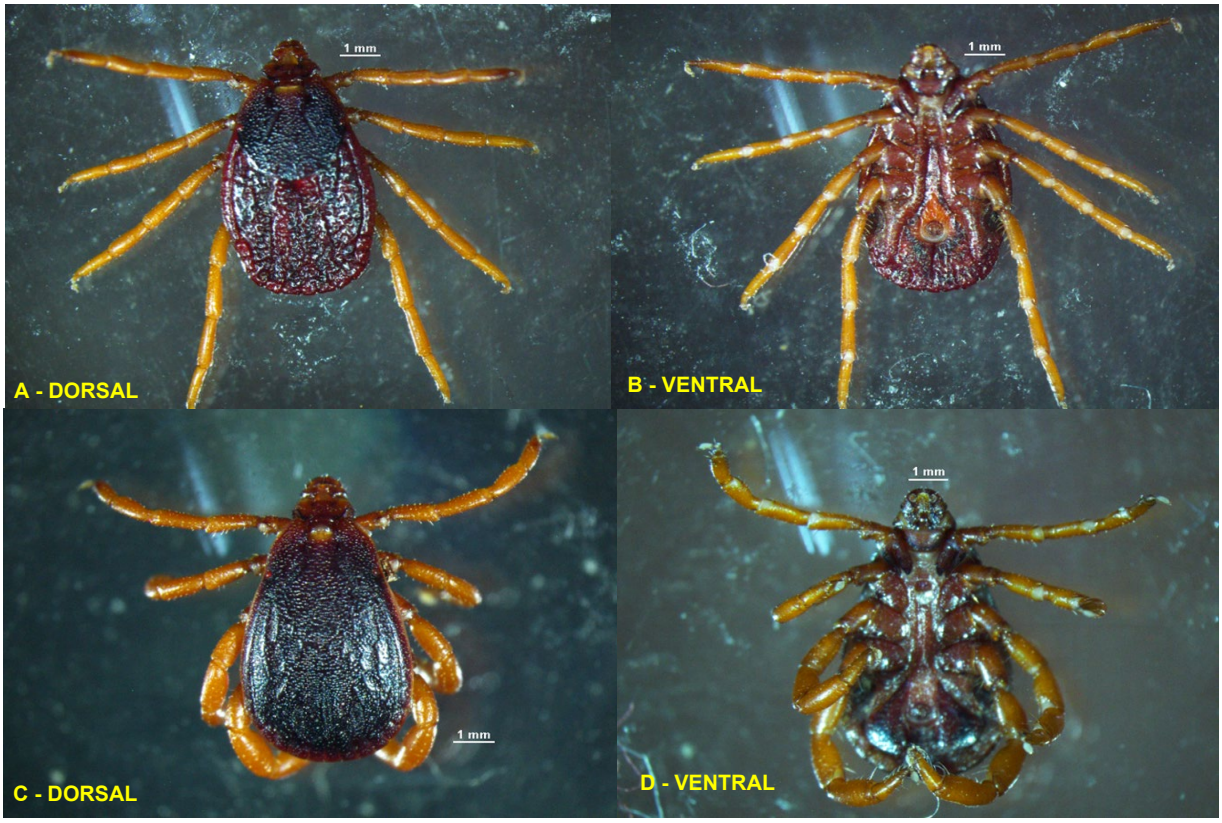


Plate I: *Rhipicephalus evertsi evertsi;* A-dorsal & B-ventral female, C-dorsal & D-ventral male.

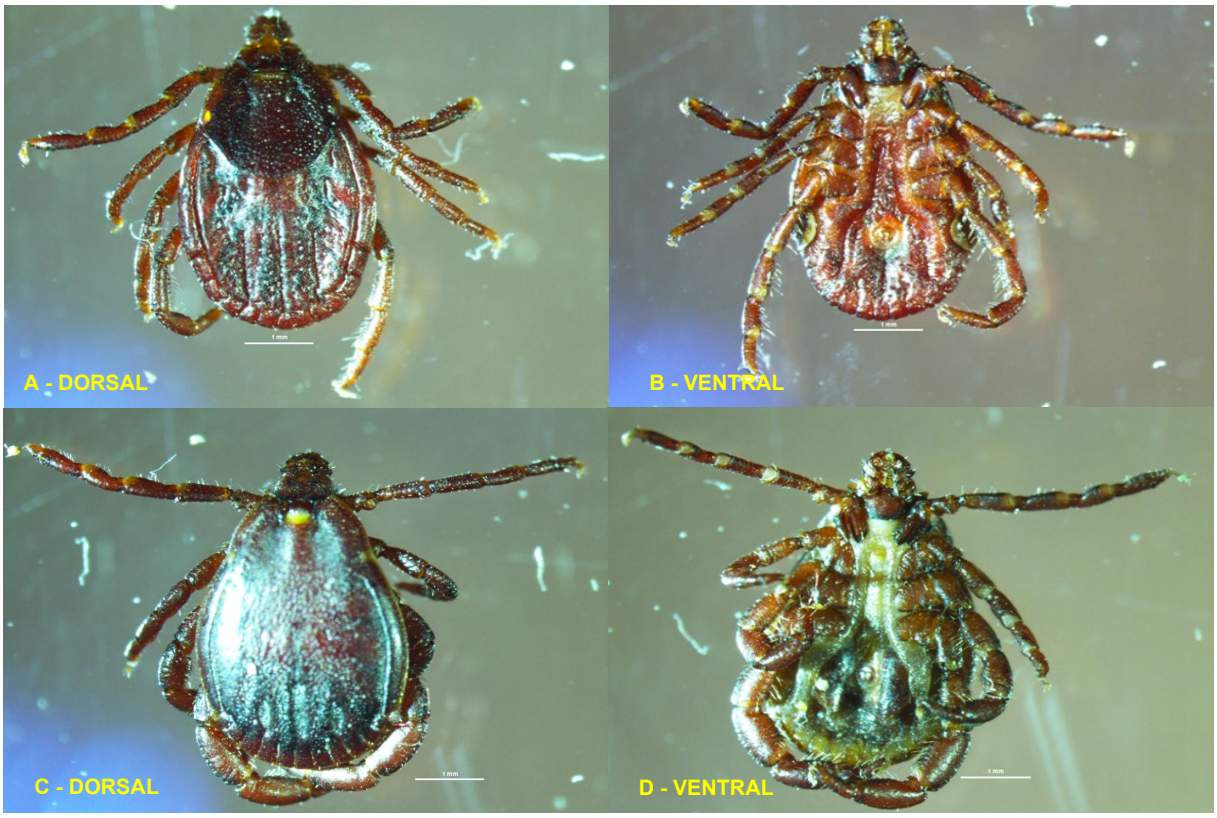


Plate II: *Rhipicephalus appendiculatus*; A-dorsal & B-ventral female, C-dorsal & D-ventral male



Plate III: *Rhipicephalus sanguineus*; A-dorsal & B-ventral female, C-dorsal & D-ventral male

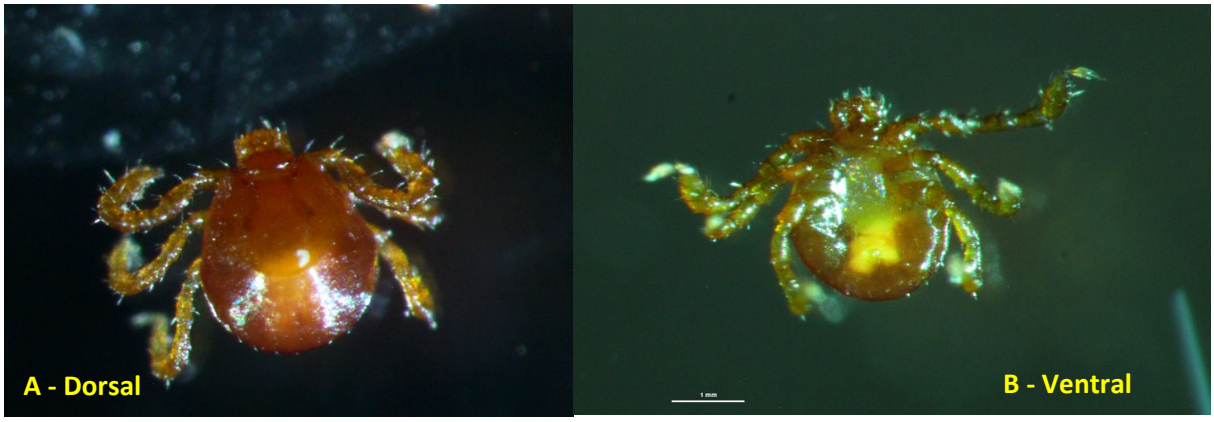


Plate IV: *Rhipicephalus decoloratus* nymph: A-Dorsal & B-Ventral

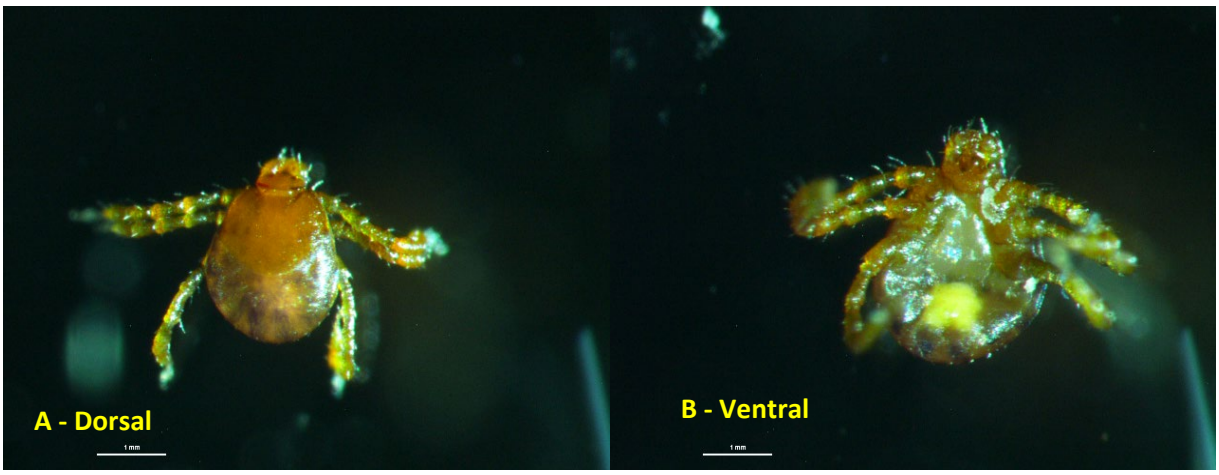


Plate V: *Rhipicephalus microplus* nymph: A-Dorsal & B-Ventral

3.3.2 Molecular identification of ticks

The PCR targeting the *CO1*, *16S rRNA*, *ITS2* and *18S rRNA* gene regions was conducted to confirm the morphological identification of the collected ticks. The molecular identification was successful for all four gene regions. However, there was no successful amplification of tick samples collected from vegetation targeting the 16S gene region using various PCR master mixes (AmpliTaq Gold 360 Master Mix, KAPA HiFi HotStart Ready Mix, and OneTaq Hot Start Quick-Load 2X Master Mix).

3.3.2.1 Amplification of tick *CO1* gene

The partial *CO1* gene with a product size of 680 bp was successfully amplified. An online BLASTn search was performed where sequences of this study were compared to sequences on the NCBI database. All sequences from the current study that had top ranked hits similarities with relevant sequences from the NCBI database were regarded as the identified tick species. BLASTn search for *R. e. evertsi* collected from cattle (sample ID: R1, R2 and R3) in the present study were similar *R. e. evertsi* sequences (GenBank accession numbers MN453671.1 and MN4536691) (Appendix 8.1) on the NCBI database with 100% query covers and 100% matching identity on

for all samples. BLASTn search for *R. e. evertsi* collected from sheep (Re7, Re11, and Re15) were similar to *R. e. evertsi* sequences (GenBank accession numbers MN453671.1 and MN453669.1) (Appendix 8.1) on the NCBI database with 100% query covers and 100% matching identity for sample Re11 and Re15 whilst sample Re7 had 100% query covers and 99.82% matching identity for the same Genbank reference sequences. For the *R. e. eversti* samples collected from horses (sample ID: h6, h7 and h9), the BLASTn search results showed similarities to *R. e. evertsi* sequences (GenBank accession numbers: MN453671.1 and MN453669.1) (Appendix 8.1) on the NCBI database with 100% query covers for all three samples and matching identity of 100% for both h7 and h9 tick samples, whilst for sample h6 the matching identity was 99.82%. For the *R. e. evertsi* ticks collected from goats (Sample ID: gmf1, gmf3 and gmm3), BLASTn search results showed similarities to *R. e. evertsi* sequences (Genbank accession numbers: MZ351136.1 and KY457538.1) (Appendix 8.1) on the NCBI database with 99% query covers for all goat samples and 99.29% matching identity for sample gmf1 and gmm3, 99.01% for sample gmf3. The BLASTn search results for *R. sanguineus* collected from dogs (sample ID: SAN2 and SAN3) showed similarities to *R. sanguineus* sequences (Genbank accession numbers: MG969507.1 and MG969506.1) (Appendix 8.1) on NCBI database with 100% query cover and 98.52% matching identity. To confirm the morphological identification of samples received from clinvet. Amplified tick DNA sequences targeting the *CO1* gene region was successful. The BLASTn search was performed where these samples were compared to other sequences on the NCBI database. The BLASTn search results of *R. e. evertsi* (sample ID: Re1) matched with those *R. e. evertsi* sequences (GenBank accession numbers: MN453671.1 and MN453669.1) (Appendix 8.5) on the NCBI database with 100% query covers and 100% matching identity. The *R.*

decolaratus (sample ID: Rd1) and *R. microplus* (sample ID: Rm1) matched with *R. microplus* sequences (GenBank accession numbers: MT249802.1 and MT249801.1) (Appendix 8.1) on the NCBI database with 100% query covers and 100% matching identity. Lastly *R. appendiculatus* (sample ID: Rp1) was similar to *R. appendiculatus* sequences (GenBank accession numbers: MN756038.1 and MN756037.1) (Appendix 8.1) on the NCBI database with 100% query covers and 100% matching identity.

3.3.2.2 Phylogenetic analysis of the *CO1* gene

The phylogenetic analysis was conducted using the Maximum likelihood (ML) method based on the General Time Reversible model and non-uniform of evolutionary rates among sites modelled using discrete gama distribution (GTR +G) (Nei and Kumar 2000) with sequences from the *Rhipicephalu*s genera. Two sequences from the *Haemaphysalis* genera (Genbank accession number: MK318817 and MK165458) were used as the outgroup. Three tick samples were sequenced from each host species-horses, sheep, dogs, cattle and goats-using the *CO1* gene as a molecular marker. All samples generated high-quality sequences, with the exception of one sample collected from a dog, which failed to produce a usable sequence. Additionally, four samples from laboratory-reared ticks were sequenced and yielded high-quality *CO1* sequences. In total, eighteen sequences were obtained and subsequently used for phylogenetic analysis (see, Table 3.5). These were combined with 14 sequences from NCBI database to create a homologous dataset of 32 sequences used for phylogenetic analysis. (Appendix 8.1). All the ticks from the *CO1* sequences generated from this study clustered with the corresponding congener from Genbank and a monophyletic phylogeny was inferred with four major clades namely, Clade I (*R. e. evertsi*), Clade II (*R. microplus*); Clade III (*R. sanguineus*) and lastely Clade IV (*R.*

appendiculatus) as shown in figure 3.2. Additionally, sequences generated in this study indicated by a black bullet are all submitted to the NCBI database under the accession numbers PQ114275-PQ114292 (Appendix 8.7). The accession numbers for *R. e. evertsi* are PQ114275-PQ114283 and PQ114285-PQ114288. For *R. microplus*, the accesion numbers were PQ114284 and PQ114289; For *R. sanguineus*, PQ114291 and PQ114292; and for *R. appendiculatus* PQ114290.

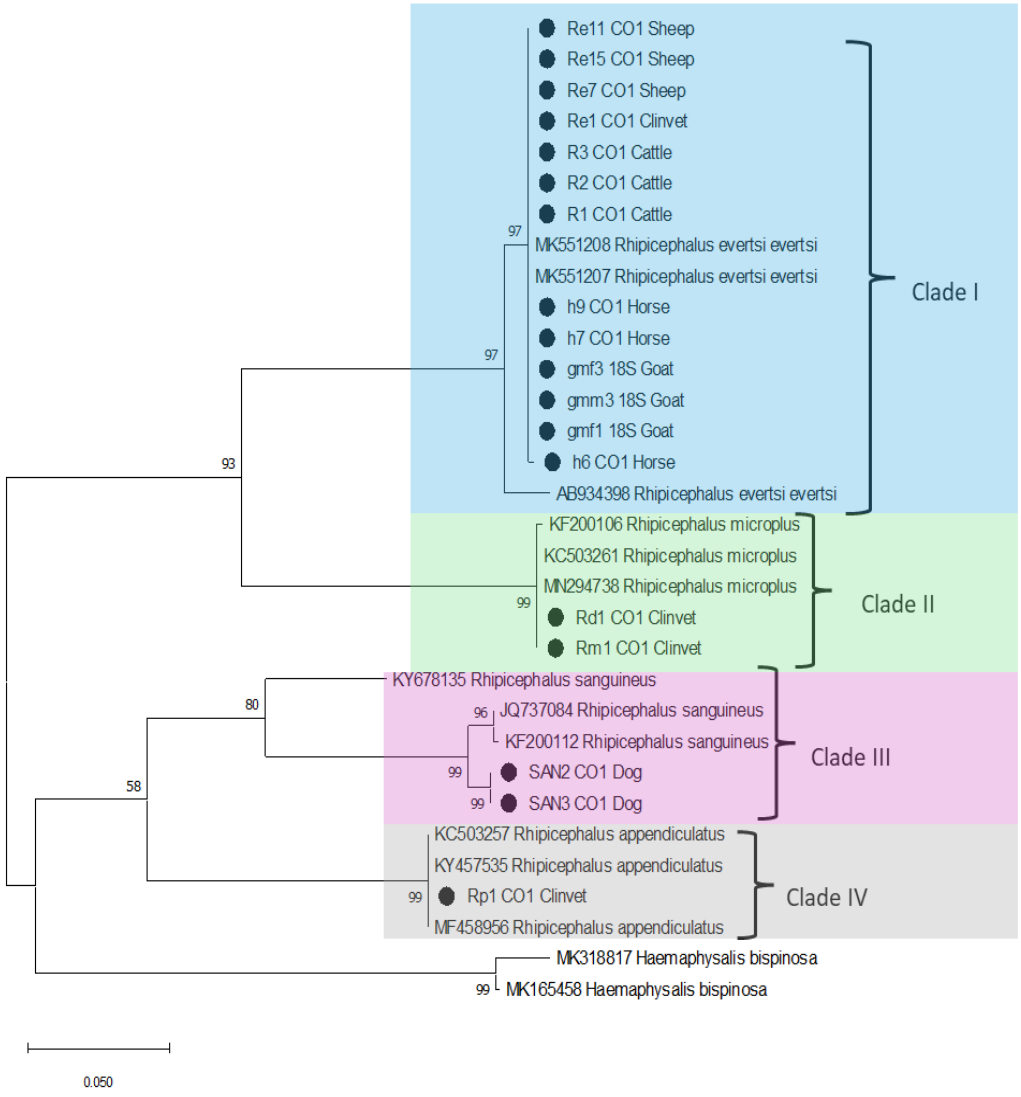


Figure 3.2: Phylogenetic tree of the tick *CO1* gene using the Maximum Likelihood (ML) method with 1000 bootsrap percentage replicates. Sequences of this study are indicated by a black bullet and contain the sample ID The tree was inferred using the aligned 542 nucleotide data set of the *CO1* gene region. Coloured boxes indicate clades where blue = "*R. e. evertsi"*; green = "*R. microplus*"; purple = "*R. sanguineus*" and grey = "*R. appendiculatus*".

3.3.2.3 Amplification of the tick *16S rRNA* gene

Amplification of the tick DNA targeting the tick16S rRNA gene region was successful with a product size of 455 base pair (bp). The average sequence length used for analysis in the present study is 382 bp. BLASTn search was performed where sequences of this study were compared to other sequences on the NCBI database. Similar sequences returned on the NCBI database with the top ranked hits were highly regarded as identification of the tick species. Search results from cattle (sample ID: R1, R2 and R3) on this study were identical to *R. e. evertsi* sequences (GenBank accession numbers: KJ613642.1 and KY457538.1) (Appendix 8.2) on the NCBI database with 100% query covers and 100% matching identity. BLASTn search for *R. e. evertsi* collected from sheep (Re7, Re11 and Re15) matched with *R. e. evertsi* sequences (GenBank accession numbers: KJ613642.1 and KY457538.1) on the NCBI database with 100% query covers and 99.73% matching identity for sample Re11, 99% query covers and 100% matching identity for sample Re15 whilst sample Re7 had 100% query covers and 99.45% matching identity. For the *R. e. eversti* samples collected from horses (sample ID: h6, h7 and h9), the BLASTn search results showed similarities to *R. e. evertsi* sequences (GenBank accession numbers: KJ613642.1 and KY457538.1) on the NCBI database with 100% query covers for all three samples and matching identity of 99.45% for h6, 100% for h7 and 99.72% for h9. For the *R. e. evertsi* ticks collected from goats (Sample ID: gmf1, gmf3 and gmm3), BLASTn search results showed similarities to *R. e evertsi* sequences (Genbank accession numbers: KY457538.1 and KY457537.1) (Appendix 8.2) on the NCBI database with 99% query covers for all goat samples and 98.90% matching identity for sample gmf1 and gmf3, 99.12% for sample gmm3. The BLASTn search results for *R. sanguineus* collected from dogs (sample ID: S3, SAN2 and SAN3) showed similarities to *R. sanguineus*

sequences (Genbank accession numbers: MG651947.1 and LC422405.1) (Appendix 8.2) on NCBI database with 100% query cover for all samples and 100% matching identity for sample S3 and SAN3, whilst SAN2 had 99.73% matching identity. For clinvet samples, BLASTn search results for *R. e. evertsi* sample ID: Re1), matched with *R. e. evertsi* sequences (GenBank accession numbers: KJ613642.1 and KY457538.1) on the NCBI database with 100% query covers and 99.72% matching identity. *Rhipicephalus decolaratus* (sample ID: Rd1) and *R. microplus* (sample ID: Rm1) matched with *R. microplus* sequences (GenBank accession numbers: MN650726.1 and MN650725.1) on the NCBI database with 100% query covers both samples and 100% matching identity for sample Rd1 and 99.73% matching identity for sample Rm1. Lastly *R. appendiculatus* (sample ID: Rp1) was identical to *R. appendiculatus* sequences (GenBank accession numbers: KC503257.1 and LC634572.1) on the NCBI database with 99% query covers and 99.73% matching identity.

3.3.2.4 Phylogenetic analysis of the tick *16S rRNA* gene

Phylogenetic analysis based on Maximum likelihood (ML) method using the Tamura 3-parameter model and non-uniform of evolutionary rates among sites modelled by assuming that certain fractions of sites are evolutionarily invariable (T92 + I) (Nei and Kumar 2000) with sequences from the *Rhipicephalus* genera. Three tick samples from each host species-horses, sheep, dogs, cattle and goats- were sequenced using the *16S rRNA* gene as a molecular marker. All samples produced high-quality sequences. In addition, four laboratory-reared tick samples were sequenced, also yielding high-quality*16S rRNA* sequences. In total, nineteen sequences were obtained and used for subsequent phylogenetic analysis. (Table 3.5). These were combined with 16

sequences from NCBI database to create a homologous dataset of 35 sequences used for phylogenetic analysis. The *Haemaphysalis longicornis* (Genbank accession number: KJ710106) (Appendix 8.2) and *H. japonica douglasi* (Genbank accession number: AB819176.1) (Appendix 8.2) was an outgroup. Additionally, Sequences generated in this study indicated by a black bullet are all submitted to the NCBI database under the accession numbers PQ114090-PQ114108 (Appendix 8.8). The accession numbers for *R. e. evertsi* are PQ114090-PQ114095 and PQ114100-PQ114106. For *R. microplus*, the accesion numbers were PQ114096 and PQ114097; For *R. sanguineus*, PQ114099, PQ114107 and PQ114108 and for *R. appendiculatus* PQ114098.

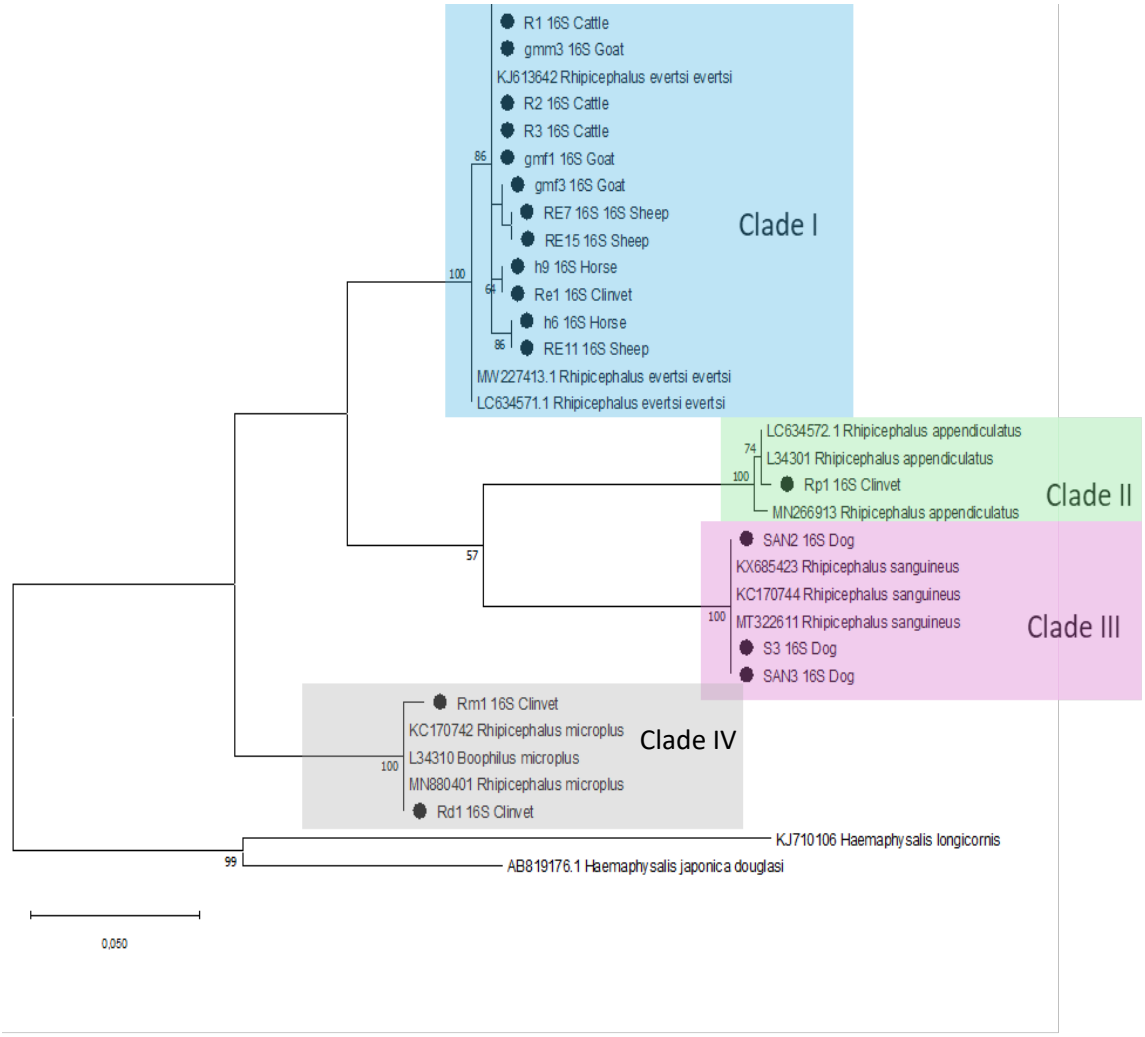


Figure 3.3: Phylogenetic analysis of the tick *16S rRNA* gene using the Maximum Likelihood (ML) method with 1000 bootsrap percentage replicates. Sequences of this study are indicated by a black bullet and contain the sample ID The tree was inferred using the aligned 370 nucleotide data set of the *16S rRNA* gene region. Coloured boxes indicate clades where blue = "*R. e. evertsi*"; green = "*R. appendiculatus*"; purple = "*R. sanguineus*" and grey = "*R. microplus*".

3.3.2.5 Amplification of the tick *ITS2* gene

The amplification of *ITS2* gene region was successful with an expected fragment size between 1000 -1200 bp. The average sequence length used for analysis in the present study was 1200 bp. The BLASTn search was performed where sequences of this study were compared to other sequences on the NCBI database. Similar sequences returned on the NCBI database with the top ranked hits were highly regarded as identification of the tick species. BLASTn search results from *R. e. evertsi* ticks collected from cattle (sample ID: R1, R2 and R3) on this study were similar to *R. e. evertsi* sequences (GenBank accession numbers: KY457503.1 and KY457502.1) (Appendix 8.3) on the NCBI database with 99% query covers & 99.48% matching identity for sample R1; 100% query covers with 99.56% matching identity for sample R2 and 100% query covers with 99.83% matching identity for sample R3. BLASTn search for *R. e. evertsi* collected from sheep (Re7, Re11, and Re15) matched with *R. e. evertsi* sequences (GenBank accession numbers: KY457503.1 and KY457502.1) (Appendix 8.3) on the NCBI database with 99% query covers and 99.82% matching identity for sample Re7, 100% query covers with 99.74% matching identity for sample Re11 whilst sample Re15 has 100% query covers and 99.65% matching identity. For the *R. e. eversti* samples collected from horses (sample ID: h6, h7 and h9), the BLASTn search results showed similarities to *R. e. evertsi* sequences (GenBank accession numbers: KY457503.1 and KY457502.1) on the NCBI database with 100% query covers and matching identity of 99.74% for all three samples. For the *R. e. evertsi* ticks collected from goats (Sample ID: gmf3), BLASTn search results showed similarities to *R. e. evertsi* sequences (Genbank accession numbers: JQ412127.1 and MZ408104.1) (Appendix 8.3) on the NCBI database with 100% query covers for all goat samples and 99.53% matching identity. The BLASTn search results for *R.*

sanguineus collected from dogs (sample ID: S3 and SAN2) showed similarities to *R. sanguineus* sequences (Genbank accession numbers: JQ412127.1 and MZ408104.1) (Appendix 8.3) on NCBI database with 100% query cover for all samples and matching identity range of 99.55 - 99.64%. For clinvet samples, BLASTn search results for *R. e. evertsi* (sample ID: Re1) were identical to *R. e. evertsi* sequences (GenBank accession numbers: KJ613642.1 and KY457538.1) on the NCBI database with 100% query covers and 99.72% matching identity. *Rhipicephalus decolaratus* (sample ID: Rd1) and *R. microplus* (sample ID: Rm1) matched with *R. microplus* sequences (GenBank accession numbers: MG721035.1 and KY457506.1) on the NCBI database with 100% query covers and 99.73% matching identity for both samples. Lastly *R. appendiculatus* (sample ID: Rp1) was identical to *R. appendiculatus* sequences (GenBank accession numbers: KY457500.1 and KX276952.1) on the NCBI database with 100% query covers and 100% matching identity. The amplified DNA from *R. appendiculatus* collected from vegetation (sample ID: RPM3, RPM4 & RPF5) failed to produce sequence results targeting the ITS2 gene region.

3.3.2.6 Phylogenetic analysis of the *ITS-2* gene

Phylogenetic analysis was done using the Maximum likelihood (ML) method based on Tamura 3-parameter model (T92) (Nei and Kumar 2000) with sequences from the *Rhipicephalus* genera. Three tick samples from each host species-horses, sheep, dogs, cattle and goats- were sequenced using the *ITS2* gene as a molecular marker. All three samples collected from horses, sheep, cattle yielded high-quality sequences. Of the three samples collected from dogs, two produced high-quality sequences, while one failed to yield a usable sequence. In the case of goat-derived tick samples, only one generated a high-quality sequence, with the remaining two failing to produce

usable results. Additionally, four samples from laboratory-reared ticks were sequenced, of which three generated high-quality *ITS2* sequences, and one failed to produce a usable sequence. In total Fifteen sequences were obtained and subsequently used for phylogenetic analysis. (Table 3.5). These were combined with 12 sequences from NCBI database to create a homologous dataset of 30 sequences used for phylogenetic analysis. The *Haemaphysalis longicornis* (Genbank Accession number: FN296270.1) (Appendix 8.3) and *H. parva* (Genbank accession number: FN286282.1) were used as outgroups. Additionally, Sequences generated in this study indicated by a black bullet are all submitted to the NCBI database under the accession numbers PQ122538-PQ122552 (Appendix 8.9). The accession numbers for *R. e. evertsi* are PQ122543-PQ122552. For *R. microplus*, the accesion numbers were PQ122539 and PQ122540; For *R. sanguineus*, PQ122538 and PQ122542, and for *R. appendiculatus* PQ122541.

x

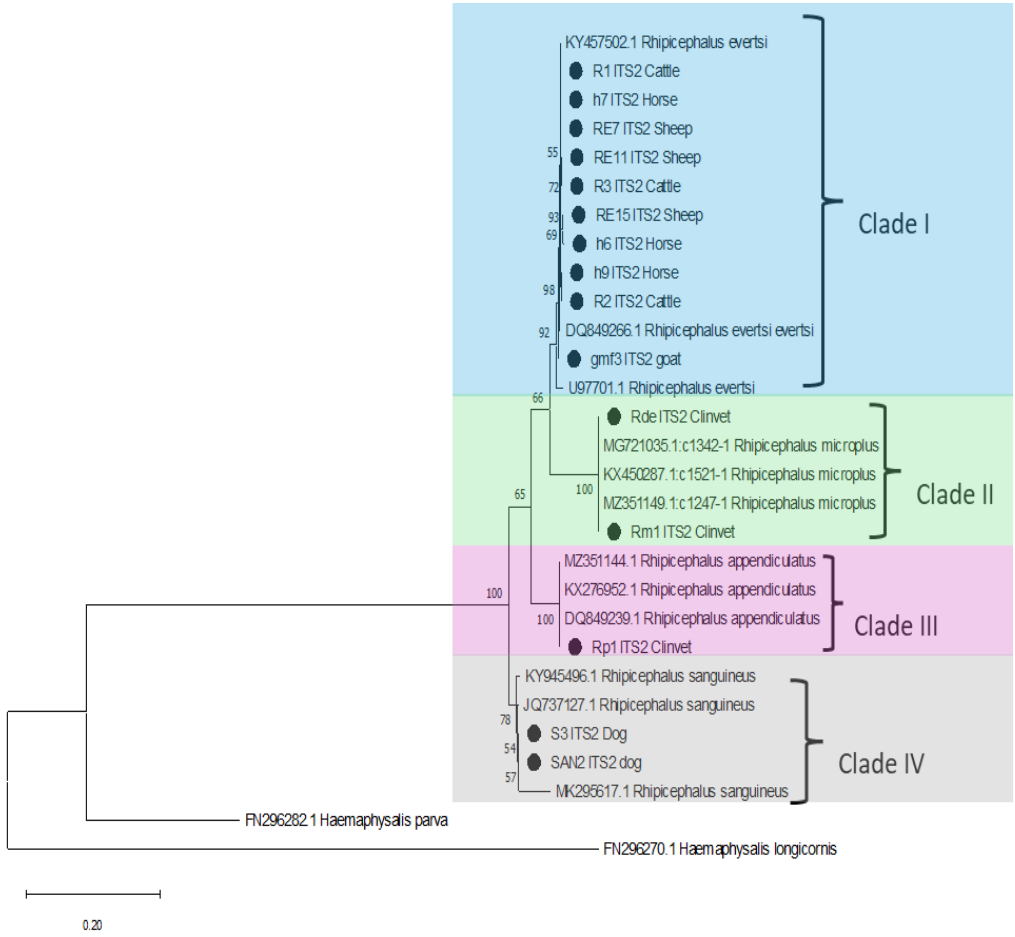


Figure 3.4: Phylogenetic analysis of the *ITS2* gene using the Maximum Likelihood (ML) method with 1000 bootsrap percentage replicates. Sequences of this study are indicated by a black bullet and contain the sample ID The tree was inferred using the aligned 1203 nucleotide data set of the *ITS2* gene region. Coloured boxes indicate clades where blue = "*R. e. evertsi"*; green = "*R. microplus*"; purple = "*R. appendiculatus*" and grey = "*R. sanguineus*".

3.3.2.7 Amplification of the tick*18S rRNA* gene

Amplification of the *18S rRNA* gene region was also successful targeting a product size of 780 bp. The average sequence length used for analysis in the present study was 809bp. BLASTn search was performed where sequences of this study were compared to other sequences on the NCBI database. Similar sequences obtained on the NCBI database with the top ranked hits were regarded as identification of the tick species. BLASTn search results from *R. e. eversti* ticks collected from cattle (sample ID: R1, R2 and R3); sheep (sample ID: Re7, Re11 and Re15); horse (sample ID: h6, h7 and h9); goat (sample ID: gmf1.gmf3 and gmm3); dog (sample ID: S3, SAN2 and SAN3) and Clinvet (sample ID: Re1); *R. decolaratus* (sample ID: Rd1); *R. microplus* (sample ID: Rm1); and *R. appendiculatus* collected from vegetation (sample ID: RPM2) and Clinvet (sample ID: Rp1) in the present study were all similar to *R. zambeziensis* sequences (GenBank accession numbers: KY457509.1 and KY457507.1) (Appendix 8.4) on the NCBI database with query covers ranging from 99%-100% and matching identity ranging from 99%-100%. BLASTn results did show similarities with corresponding species, however the query coverage and matching identity values were low.

3.3.2.8 Phylogenetic analysis of the tick *18S rRNA* gene

Phylogenetic analysis was done using the Maximum likelihood (ML) method based on Kimura 2-parameter and non-uniform evolutionary rates among sites modelled using discrete Gamma distribution (K2 +G) (Nei and Kumar 2000) with sequences from the *Rhipicephalus* genera. Three tick samples from each host species-horses, sheep, dogs, cattle and goats- were sequenced using the *18S rRNA* gene as a molecular marker, all samples produced high-quality sequences. Additionally, four laboratory-

reared tick samples were sequenced, also yielding high-quality*18S rRNA* sequences. In total, nineteen sequences were obtained and used for subsequent phylogenetic analysis (Table 3.5). These were combined with 29 sequences from NCBI database to create a homologous dataset of 48 sequences used for phylogenetic analysis. The *Argas africolumbae* (Genbank accession number: KJ133632.1) (Appendix 8.4) and *A. walkerae clone* (Genbank accession number: KJ133636.1) (Appendix 8.4) was used as an outgroup. Additionally, sequences generated in this study indicated by a black bullet are all submitted to the NCBI database under the accession numbers PQ114275-PQ114292 (Appendix 8.10). The accession numbers for *R. e. evertsi* are PQ187065-187068; PQ187071-PQ187076 and PQ187081-PQ187083. For *R. microplus*, the accesion numbers were PQ187077 and PQ187078; For *R. sanguineus*, PQ187069, PQ187070 and PQ187080; and for *R. appendiculatus* PQ187079.

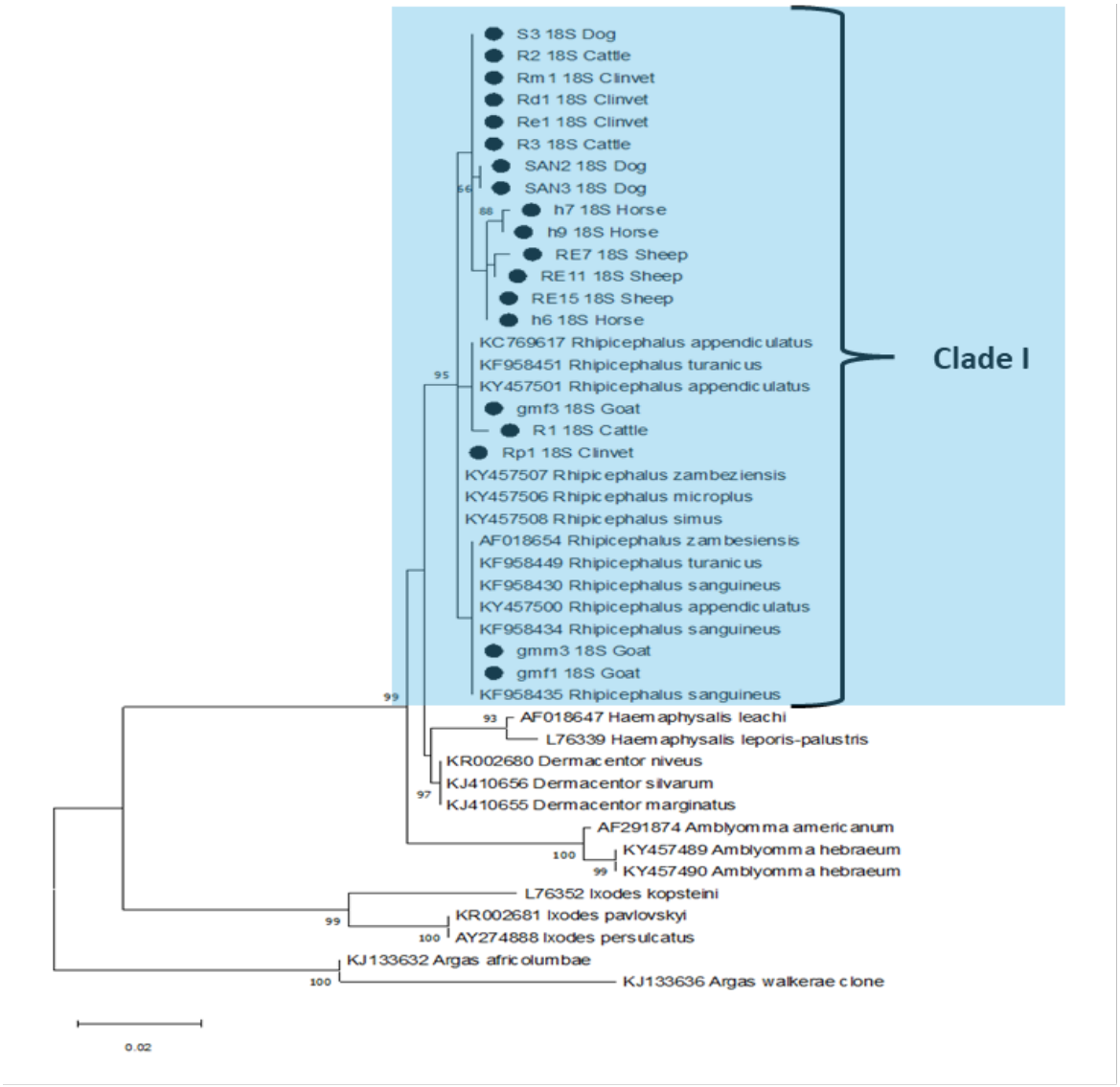


Figure 3.5: Phylogenetic analysis of the tick *18S rRNA* gene using the Maximum Likelihood (ML) method with 1000 bootsrap percentage replicates. Sequences of this study are indicated by a black bullet and contain the sample ID. The tree was inferred using the aligned 809 nucleotide data set of the *18S rRNA* gene region. Coloured box indicates a clade where blue = the genus "*Rhipicephalus".*

Table 3.5: A summary of samples that where successfully sequenced targeting four different gene regions used in this study for tick characterization.

HOST	SAMPLE ID	*CO1*	*16S*	*ITS2*	*18S*
Cattle	R1	+	+	+	+
	R2	+	+	+	+
	R3	+	+	+	+
Sheep	Re7	+	+	+	+
	Re11	+	+	+	+
	Re15	+	+	+	+
Goat	Gmf1	+	+	-	+
	Gmf3	+	+	+	+
	Gmm3	+	+	-	+
Horse	H6	+	+	+	+
	H7	+	+	+	+
	H9	+	+	+	+
Dog	S3	-	+	+	+
	SAN2	+	+	+	+
	SAN3	+	+	-	+
Vegetation	RPM3	-	-	-	-
	RPM4	-	-	-	-
	RPF5	-	-	-	-
Laboratory reared	Rd1	+	+	+	+
	Re1	+	+		+
	Rm1	+	+	+	+
	Rp1	+	+	+	+

Blue (+) = successful sequences white (-) = No successful sequences

3.4 Discussion

Five tick species from the genus *Rhipicephalus* were identified following published taxonomic keys. These include *R. e. evertsi*, *R. sanguineus* & *R. appendiculatus* adult ticks *and R. (B) microplus* & *R. (B) decoloratus* nympths. These findings align very well with a survey conducted in North West Province, South Africa in 2011 were similar ticks from the genus *Rhipicephalus* were identified in livestock (cattle, sheep and goats) (Spickett *et al*., 2011). Furthermore, the same tick species were previously recovered from livestock and vegetation in Gauteng, KwaZulu-Natal, North West and Limpopo (Walker *et al*., 2000; Walker *et al*., 2003; Nyangiwe *et al*., 2013; Mapholi *et al*., 2016; Nyangiwe *et al*., 2017; Horak *et al*., 2018). To cornfirm morphological identification, DNA barcoding has been employed in previous studies as a method to efficiently describe the species (Miller, 2007; Chen *et al*., 2012; Mahlobo-Shwabede *et al*., 2022). The present study used mitochodrial DNA (*CO1* and *16S rRNA*) and nuclear DNA markers (*ITS2* and *18S rRNA*) similar to previous studies (Song *et al*., 2011, Burger *et al*. 2013; Lv *et al*., 2014b; 2014; Burger *et al*., 2014, Binetruy *et al*., 2019) to confirm morphological identification of collected tick species. Most molecular biologists favor the use of *Mitochondrial DNA* (*mtDNA*) in species identification especially for studying evolutionary genetics (Brown, 1985; Xiong and Kocher, 1991) hence mitochondrial markers were used. Reasons for using *mtDNA* as a marker of choice by many molecular biologists could be the markers high mutation rate compared to nuclear DNA, its highly conserved and variable regions, the fact that the inheritance is clonal (marternal) (Galtier *et al*., 2009). The accuracy in delimiting species strongly depends on the type of DNA marker selected and finding the appropriate marker is a challenge which is a subject of ongoing discussions (Rach *et al.,* 2017).

In this study *R. e. evertsi* was the most abundant species collected from the different hosts (Horses, sheep, goats, and cattle). Similarly, *R. e. evertsi* distribution was common throughout the livestock (donkeys, sheep, horses, cattle etc.) collected in different districts of Lesotho (Mahlobo-Shwabede *et al*., 2022). Horak *et al*., (2017) also reported the presence of *R. e. evertsi* in nine provinces surveyed in South Africa. The overall occurrence of *R. e. evertsi* was at 63.15% from all species collected. Tick infestation was more prevalent on horses and vegetation at 27.34%, followed by ticks collected from cattle at 13%, goat 12.11%, sheep 10.42% and the least infestation was observed on dogs at 9.51% prevalence (Table 2.3). The low tick prevalence in dogs is similar to findings made by Mahlobo-Shwabede *et al*., (2022) in Lesotho. In the same study, *R. appendiculatus* was collected only on vegetation, which is also the case in the present study. However, this does not mean that *R. appendiculatus* cannot occur on other hosts in South Africa. Spickett *et al*., (2011) reported the presence of *R. appendiculatus* on cattle, goats and sheep in North West Province of South Africa. Horak *et al*., (2015) even suggested that *R. appendiculatus* is endemic to South Africa. It was also recorded that *R. appendiculatus* prefers woodland and woodland savannah regions with good vegetation (Horak *et al*., 2002). Walker *et al*., (2000) reported the presence of *R. appendiculatus* in KwaZulu-Natal Province similar to the present study. The *R. sanguineus* tick was collected only on dogs. This is not surprising since this tick is known to be host specific (Walker *et al*., 2003; Madder *et al*., 2013). In this study, *R. (B.) decoloratus* and *R. (B.) microplus* were not detected in the different lifestock species, including cattle. This is positive news for the small-scale farmers involved since these two species are known to transmit *Babesia bigemina* a causative agent of African redwater and *B. bovis* the causative agent Asiatic redwater disease respectively (Yawa *et al*., 2019). However, the high presence of *R. e. evertsi* cannot

be ignored since ticks from this species are known to transmit infections like *B. caballi* and *T equi* causing equine piroplasmosis (Montes Cortés *et al*., 2017, Horak *et al.,* 2017); *A. marginale* causing bovine anaplasmosis in cattle (Teshale *et al*., 2016) and toxicosis causing spring lamb paralysis (Mans *et al*., 2004). *Rhipicephalus e. evertsi* adaptation to infest diverse host, increases chances of disease transmission and disease maintainance therefore giving much reason of concern for livestock farmers. The *R. sanguineus* on the other hand is known for transmitting *E. canis* causing canine monocytic ehrlichiosis; *A. platys* causing canine infectious cyclic thrombocytopenia; additionally *Rickettsia* species belonging to the spotted fever group like *R. rickettsia* (the cause of Rocky Mountain spotted fever); *R. conorii* (the cause of Mediterranean spotted fever), *R. parkeri* and *R. massiliae* (Dantas-Torres, 2008; Dantas-Torres *et al*., 2012; Chomel, 2011; Latrofa *et al*., 2014; Galay *et al*., 2018).

The 5' region of *COI* gene is known as the standard universal barcoding marker for all animals (Herbet *et al*., 2003; Xai *et al*., 2012). This gene is continually examined for identification of Ixodida ticks (Lv *et al*., 2014b; Rach *et al*., 2017). The *CO1* primers used in the current study (LCO1490/HCO2198) could not produce quality sequences for some of the *Rhipicephalus* species which were identified morphologically. However, the quality of the sequences produced was very good, straightfoward and there were no gaps within the alignment. This is similar to a previous study where attempts to sequence some ixodid ticks using *CO1* was not successful (Lv *et al*., 2014a). The same was true in a study conducted targeting three regions in mitochondrial genome (*COI*, *COII* and *Cytb*) of Aphids (Chen *et al*., 2012). The reason for this challenge in sequencing could be due to the gene being highly variable. Results of this study suggest that the universal primer pair LCO1490/HCO2198 may not be

efficient in sequencing for some tick species although amplification was successful in all attempts. To resolve this challenge, one can simulteneously use other mitochondrial primer pairs like COI-F/COI-R, Cox1F/Cox1R for more improved results although we cannot confidently say which primerset is the most efficient in recovering *COI* sequences across ixodid tick species (Lv *et al*., 2014a).

The *16S rRNA* mitochondrial gene region is a highly utilized gene region to discriminate species in ticks. The sequencing quality of *16 rRNA* was highest among the four markers in the current study, this is similar to findings from a previous study (Lv *et al*., 2014a). Of all the markers used in the current study, *16S rRNA* and *18S rRNA* had the best sequence recovery rate at 86.36 % compared to *CO1* 81.81%, and *ITS2* 68.18%. DNA marker *18S rRNA* and *16S rRNA* amplified and produced good sequences for all samples collected from different hosts. However, the sequence alignment for *18S rRNA* had more gaps compared to *16S rRNA* which had fewer gaps. Furthermore, the sequence length for *16S rRNA* was shorter and reproducible with just a single sequencing reaction. Short DNA sequences of a standardized gene region have been highly promoted for their fast and reliable identification of specimens of unknown species origin (Rach *et al*., 2017).

There was no clear separation of species using the *18S rRNA* marker from the *Rhipicephalus* genus in the current study. This is in agreement with previous studies suggesting that *18S rRNA* marker is not highly informative in resolving species taxonomy, by not having sufficient phylogenetic information for resolving relationships among Metastriata complex, this marker is mostly significant in resolving relationships

among higher taxa (Lv *et al*., 2014a; Burger *et al*., 2014; Low *et al*., 2015, Mahlobo-Shwabede *et al*., 2022).

In comparison to the other three gene regions, *ITS2* marker produced high-quality sequences for only a few of the 19 samples that were sequenced. The *ITS2* sequence length was longer than the other fragments (*CO1*, *16S rRNA* and *18S rRNA*) exceeding 1000bp, requiring two reactions to sequence completely, this could likely reduce the quality of sequence compared to the other shorter fragments that require a single reaction for sequencing (Lv *et al*., 2014a).

Sequences from this study matched the morphological determinations on the NCBI database and clustered with relevant species on the ML phylogenetic tree. Although, sequence results could not be obtained for all samples. Morphological traits used for identification of *Rhipicephalus* ticks and genetic identification were consistent with each other except for laboratory reared *R. (B) decoloratus* and *R. (B) microplus* nymph of laboratory reared specimens from CLINVET which were morphologicaly described as separate species but molecularly identified as *R. (B) microplus* using NCBI BLASTn search results. Ticks from these two species (previously reported as separate species morphologically) clustered together with reference sequences from NCBI Genbank of *R. (B) microplus* in one monophyletic clade for the above markers (*CO1*, *16S rRNA* and *ITS2* genes). Identification of closely related species in the early stages of life using morphology alone has been reported to be challenging (Olson *et al*., 1991). The morphological identification of the nymphs in the present study is likely to be inconsistent because traits used to separate these two species were equivocal, not all

features have developed in the nymph stages, and it is possible to make mistakes even by seasoned taxonomist.

BLASTn search results showed that *18S rRNA* marker used in the current study is not reliable in the identification of *Rhipicephalu*s ticks to species level. This could be due to the marker being too conserved as suggested in previous studies and recommended as a tool reliable for higher taxonomic classification (Dabson & Baker, 1999; Klompen *et al*., 2007; Mans *et al*., 2011, Lv *et al*., 2014b). Morphologically identified tick samples in this study clustered together in one monophyletic clade (Clade I) with other *Rhipicephalus* ticks from Genbank. This supports the idea that *18S rRNA* is indeed good in identification of tick species to genus level (Mahlobo-Shwabede *et al*., 2022).

CHAPTER 4

Microbial communities harboured by *Rhipicephalus* tick species of domestic animals

4.1 Introduction

Infectious diseases in humans and animals are often associated with arthropod vectors and ticks are the most important blood-sucking vectors second to mosquitoes known to transmit bacteria, protozoa and viral zoonotic pathogens (Berrada and Telford, 2009; Yu *et al*., 2011). Ticks support transmission of pathogens between hosts through their different life cycle. These include mainly the two-host and the three-host life cycle ticks that can also feed on accidental hosts (Carmichael and Fuerst, 2006). Pathogenic transmission in ticks with a one-host life cycle is also not excluded. Every motile life stage of ixodid ticks require a blood meal from a vertebrate host increasing the chances of pathogen transmission (Varela-stokes *et al*., 2017). An individual tick can carry multiple pathogenic agents and symbiotic bacteria (Khoo *et al*., 2016). The commonly known pathogenic agents transmitted by ticks include species from the genera *Coxiella*, *Borrelia*, *Rickettsia*, *Francisella*, *Ehrlichia*, *Anaplasma* (de la Fuente *et al*., 2008; Dantas-torres *et al*., 2012; Brites-Neto *et al*., 2015; Greay *et al*., 2018).

Knowledge of bacterial communities in ticks beyond pathogenic species is limited although the association between non-pathogenic bacteria and ticks was documented at the beginning of the 20th century (Cowdry, 1925; Andreotti *et al*., 2011). A few studies documented the ability of ticks in harbouring bacterial endosymbionts which can have commensal, mutualistic or parasitic relationships with their tick host (Burgdorfer *et al*., 1973; Scoles, 2004, Clay *et al*., 2008; Vilcins *et al*., 2009). The most common endosymbiotic bacteria known to be habored by hard ticks include species

of *Coxiella*-, *Francisella*-, *Rickettsia- Arsenophonus*-like symbionts, *Wolbachia* and *Candidatus Midichloria mitochondrii* (Ahantarig *et al*., 2013). Tick pathogens and the non-pathogenic bacterial genera coexist within a tick and some studies suggest that bacterial symbionts influence survival rate, reproduction, immunity, and nutritional adaptation of ticks (Bonnet *et al*., 2017; Bonnet and Pollet, 2021; Narisimhan *et al*., 2021; Wu-Chuang *et al*., 2021). In short, the tick bacterial composition is influenced by a variety of components including tick species, host blood-meal, host species, tick immunity, seasons, tick developmental stages, and physiological stress by environmental traits (Wu-Chuang *et al*., 2021; García-De la Peña *et al*., 2024, Sambodo *et al*., 2024). An organisms's microbiome is typically composed of many microbial species (Andreotti, 2011; Kim *et al*., 2015) and one needs to employ good molecular techniques to identify as many microbial species as possible.

Different molecular methods such as conventional PCR and qPCR has given us opportunities to characterize several microbial pathogens, however these traditional molecular diagnostic methods allow the detection of only one or a few targets at a time in one sample. Whilst with multiplex PCR (mPCR) can evaluate many molecular targets at once, in one reaction from one sample (Han, 2012). The possibility of identification of multiple species at the same time from a single procedure offers a great advantage. This was achieved by the development of next-generation sequencing technologies that enables genes and genomes of single cells and communities of microorganisms to be explored in a massively parallel, rapid and cost –effective manner (Greay *et al*., 2018; Couper *et al*., 2019; Papa *et al*., 2020). Today huge diversity of bacterial species are discovered using techniques such as *16S rRNA* profiling where no bacterial culturing is required (Chandra *et al*., 2021). This study sought to characterize the bacterial communities associated with *Rhipicephalus* ticks

(*R. sanguineus, R. appendiculatus, R. e. evertsi, R. (B) decoloratus,* and *R. (B) microplus*) through *16S rRNA* V3-V4 hypervariable region amplicon sequencing using the Illumina next-generation sequencing platform. Additionally, the study assessed the impact of seasonal variation on the composition of these bacterial communities.

4.2 Materials and methods

4.2.1 Tick samples and identification

Tick samples used in this study were sampled from domestic animals including cattle, sheep, goats, horses, dogs as well as from vegetation in four different provinces of South Africa. Figure 3.1, Chapter 3, section 3.2.2 contains detailed information.

Morphological and genetical identification of ticks used in this study is described in detail in in Materials and methods of Chapter 3 section 3.2.2 and 3.2.3. All information pertaining to the collected ticks (sex, life stage, host) was noted for future use. All female ticks that laid eggs were stored at -4°C freezer until DNA extraction was performed. Biological samples (Adult ticks, nympth, eggs laid) were all stored following correct storage procedures (Chapter 3 section 3.2.3.).

4.2.2 DNA extraction

To get rid of external environmental contaminants, ticks were washed once with 70% ethanol and three times with distilled water (Carpi *et al*., 2011) prior to DNA extractions. DNA from collected ticks was extracted following Qiagen Dneasy Blood and Tissue kit (Qiagen Hilden, Germany) extraction protocol and the Chelex method (Chapter 2, section 2.2.4). Eggs were weighed using AS 220.R2 PLUS Analytical balance-Radway (Lasec (pty) Ltd), put inside 1.5 tubes and labelled before processing. Eggs in each

tube were dissected using a fine forceps to break the tissues. The forceps were washed in ethanol and distilled water after every use to avoid contamination. DNA was then extracted from eggs following Qiagen Dneasy Blood and Tissue kit (Qiagen Hilden, Germany) extraction protocol detailed in chapter 2. The extracted DNA was measured using Qubit ® 3.0 Fluorometer (Life Technologies, California, USA) then stored at -4°C until use.

4.2.3 The 16S library preparation workflow

4.2.3.1 Initial amplicon PCR

The library preparation was performed according to the illumina preparation guide where hypervariable regions V3 and V4 of the *16S rRNA* gene were amplified using universal bacteria primers 341F forward (5' TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG 3') and 805R reverse primer (5' GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C 3') (Klindworth *et al*., 2013). Each gene-specific primer (Forward and Reverse) had overhang adapters attached. Namely, Forward overhang: TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG and Reverse overhang: GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G, respectively (Illumina Inc., United States). Library preparation steps were performed as described previously (Mashiane *et al*., 2017; Van Wyk *et al*., 2017; Ezekiel *et al*., 2019). Each PCR reaction contained 12.5 µl of 2X KAPA Hifi HotStart ReadyMix [composed of KAPA HiFi HotStart DNA Polymerase (1 U/µL), Fidelity Buffer (5X), GC Buffer (5X), high-quality dNTPs (10 mM each) and $MgCl_2$ (25 mM)] (KAPA Biosystems, Massachusetts, USA), 5 µl of 1 µM Amplicon PCR Forward primer, 5 µl of 1 µM amplicon PCR Reverse primer and 2.5 µl of microbial DNA. The total reaction volume was 25 µl in 96- well 0.2 ml PCR plates

(Illumina, 2013). The PCR reaction was amplified according to the following conditions: initial denaturing at 95°C for 3 minutes and subjected to 25 cycles of denaturing at 95°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 30 seconds with the final extension at 72°C for 5 minutes and final holding temperature at 4°C ∞. To visualize the size of the PCR product 1 µl of the PCR product was viewed using Bioanalyzer DNA 1000 chip (Agilent Technologies, Waldbronn, Germany).

4.2.3.2 Initial PCR clean-up

PCR amplicons were purified using Agent Court AMPure XP beads (Beckman Coulter, Brea, CA, United States). The AMPure XP beads were equilibrated to room temperature prior to use. The amplicon PCR plate was centrifuged at 3 500 rpm at 20°C for a minute to collect condensation and to carefully remove seal. The AMPure XP beads was then vortexed for 30 seconds to ensure that the beads are evenly dispersed (Illumina, 2013). Then 20 µl of AMPure XP beads was added to each PCR amplicon in the PCR plate. The mixture was mixed by pipetting the entire volume up and down 10 times. The samples were incubated at room temperature for 5 minutes without shaking to allow the DNA to bind to the beads. Then the plate was placed on a magnetic stand for 2 minutes until the supernatant has cleared. The multichannel pipette was used to remove and discard the supernatant. With the amplicon PCR plate on the magnetic stand, the beads were washed twice with freshly prepared 80% ethanol. A 200 µl of freshly prepared 80% ethanol was added to each sample well, then the plate was incubated for 30 seconds on the magnetic stand and the supernatant discarded. Excess ethanol was removed. The beads still in the amplicon PCR plate was allowed to air dry at room temperature for 10 minutes, thereafter, the amplicon PCR plate was removed from the magnetic stand. The DNA was eluted in

52.5 µl of 10 mM Tris-Cl buffer (pH 8.5). This was mixed gently by pipetting the mix up and down 10 times to make sure that the beads are fully resuspended. Tips were changed after each column. Thereafter, the amplicon PCR plate was incubated at room temperature for 2 minutes. The plate was then placed on a magnetic stand for 2 minutes until the supernatant has cleared. Then 50 µl of the supernatant from the Amplicon PCR plate was transferred into a new 96-well PCR plate changing tips between samples to avoid cross contamination.

4.2.3.3 Index PCR

From the 50 µl of purified PCR amplicon, a multichannel pipette was used to transfer 5 µl of the PCR amplicon into a new 96- well plate. The remaining 45 µl of purified PCR amplicons was stored for future use. The index primers 1 and 2 were arranged in the following order: Index 2 primer tubes with white caps and a clear solution were vertically aligned with rows A through H whereas, the Index 1 primer tubes with orange caps and yellow solution were horizontally aligned with columns 1 through 12 in the TruSeq Index Plate Fixture (Illumina, 2013). Subsequently, the 96-well PCR plate with the 5 µl of resuspended PCR amplicons was placed in the TruSeq Index Plate Fixture. A PCR reaction mixture with the final volume of 50 µl was prepared. The reaction contained 5 µl of Nextera XT Index Primer 1 (N7xx), 5 µl Nextera XT Index Primer 2 (S5xx), 25 µl of 2X KAPA HiFi HotStart Ready Mix [composed of KAPA HiFi HotStart DNA Polymerase (1U/µL), Fidelity Buffer (5X), GC Buffer (5X), high-quality dNTPs (10 mM each) and MgCl2 (25 mM)] (KAPA Biosystems, Massachusetts, USA),10 µl of PCR grade water and 5 µl of DNA. The contents were mixed thoroughly by pipetting up and down 10 times and thereafter the plate was covered with a Micro- seal ‘A’. The plate was then centrifuged at (1,000 x g) 3 500 rpm for 1 minute at 20°C. The PCR

conditions used were as follows; Initial denaturing at 95°C for 3 minutes subjected to 8 cycles of denaturing at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds followed by final extension at 72°C for 5 minutes and a final holding temperature at 4°C-∞.

4.2.3.4 PCR clean-up 2

The Index PCR plate was centrifuged at 2 500 rpm for 1 minute at a temperature of 20°C to collect condensation. As done before in the first clean-up, the AMPure XP beads were vortexed for 30 seconds to ensure that the beads are evenly dispersed (Illunima, 2013). Then 56 µl of AMPure XP beads were added to each well of the Index PCR plate. The mixture in the 96-well PCR plate was mixed thoroughly by gently pipetting the mix up and down 10 times. The Index PCR plate was incubated at room temperature for 5 minutes with no shaking to allow the DNA to bind to the beads. The Index PCR plate was then placed on a magnetic stand for 2 minutes until the supernatant was clear. A multichannel pipette was then used to remove and discard the supernatant while the Index PCR plate remained on the magnetic stand. With the amplicon PCR plate on the magnetic stand, the beads were washed twice with freshly prepared 80% ethanol. A 200 µl of freshly prepared 80% ethanol was added to each sample well, then the plate was incubated for 30 seconds on the magnetic stand and the supernatant discarded. Excess ethanol was removed. The beads still in the amplicon PCR plate was allowed to air dry at room temperature for 10 minutes, thereafter, the amplicon PCR plate was removed from the magnetic stand. The DNA was eluted in 27.5 µl of 10 mM Tris-CL buffer (pH 8.5). This was mixed gently by pipetting the mix up and down 10 times. Until the beads were fully resuspended, changing tips after each column. Thereafter, the amplicon PCR plate was incubated

at room temperature for 2 minutes then the plate was placed on a magnetic stand 2 minutes until the supernatant has cleared. Then 25 µl of the supernatant from the Index PCR plate was transferred into a new 96-well PCR plate changing tips between samples to avoid cross contamination.

4.2.3.5 Library quantification, normalization and pooling

DNA concentration was calculated and converted to nano molar (nM) based on the size of DNA amplicons as determined by Agilent Technologies 2100 Bioanalyzer trace (Agilent Technologies, Waldbronn, Germany):

$$\frac{\text{Concentration in ng/ml}}{660 \text{ g/mol} \times \text{average library size}} \times 10^6 = \text{Concentration in nM}$$

The determined concentrations were diluted with 10 Mm Tris pH 8.5 to reach a concentration of 4 nM for the library preparation (Illumina, 2013). From each library, 5 µl of the diluted DNA from each library was mixed with aliquots for pooling libraries with unique indices.

4.2.3.6 Library denaturation and MiSeq sample loading

A heat block suitable to contain 1.7 ml microcentrifuge tubes was set to 96°C. The MiSeq v3 reagent cartridge (Illumina, USA) was removed from -15°C to -25°C storage and thawed at room temperature. Then ice water bath containing 3 parts of ice and 1 part of water was prepared in an ice bucket (Illumina, 2013). Then 5 µl of freshly diluted 0.2 N NaOH was added to 5 µl of 4 nM pooled library in a microcentrifuge tube. The sample solution was mixed briefly by vortex and centrifuged at (280 x *g*) 2

500 rpm at 20°C for a minute. The solution was then incubated at room temperature for 5 minutes to allow all DNA to denature into single strands. Finally, 990 µl of pre-chilled HT1 was added into 10 µl of the denatured DNA and the samples were placed on ice until final dilution stage.

4.2.3.7 Denaturation and dilution of PhiX control

To dilute the PhiX library to 4 nM, 2 µl of 10 nM PhiX library was mixed with 3 µl of 10 mM Tris pH 8.5. Thereafter, 5 µl of 4 nM PhiX library and 5 µl of 0.2 N NaOH was mixed in a microcentrifuge tube. To mix the 2 nM PhiX library solution, the mixture was briefly vortexed and incubated for 5 minutes at room temperature to denature the PhiX library into single strands. To prepare a 20 pM PhiX library, 10 µl of denatured PhiX library was added into 990 µl of pre-chilled HT1. The solution was mixed by inverting several times and then pulse centrifuged the DNA solution. The denatured and diluted PhiX was placed on ice.

4.2.3.8 Combined amplicon library and PhiX control

To combine the two libraries, 30 µl of the denatured and diluted PhiX control and 570 µl of denatured and diluted amplicon library was added in a microcentrifuge tube. The combined solution was then placed on ice (Illumina, 2013) until one is ready to heat denature the mixture immediately before loading it into the MiSeq reagent catridge. Using a heat block the combined library and PhiX control was incubated at 96°C for 2 minutes, then inverted twice and placed in the ice water bath for 5 minutes. Then the samples were loaded into MiSeq flow cell (Illumina, USA) and analysed using the MiSeq Reported Software (MRS) using the metagenomics workflow option from the MiSeq system.

4.2.4 Sequencing data analysis

All metabarcoding sequences used in this study were recovered from the MiSeq system in FASTQ format. For analysis of data, Open source bioinformatics pipeline Quantitative insights into Microbial Ecology (QIIME) (Caporaso *et al.,* 2010, Nguyen *et al.,* 2016) was used for metagenomic analysis.The QIIME2 analysis was performed using the following strategy: construct an Amplicon Sequence Variant (ASV) table with paired-end sequencing reads, and then, taxonomic information was assigned using SILVA database, following the QIIME2 pipeline (version 2020.2) The data processing and taxonomic assignment based on the QIIME2 pipeline were performed as follows (1) DADA2 was used for joining paired-end reads, filtering, and denoising; (2) assigning taxonomic information to each ASV using a naive Bayes classifier in the QIIME2 classifier with the *16S rRNA* V3-V4 gene regions from the SILVA database to determine the identity and bacterial composition.

4.2.5 Core microbiome determination

The core microbiome was identified to determine bacteria taxa consistently present across the tick samples, independent of host or season. Analysis was performed using MicrbiomeAnalyst (Dhariwal *et al*., 2017) based on the prevalence threshold set at 20%, the detection threshold (relative abundance) set at 0.01% and a blanket (global) analysis was applied to identify core taxa across all samples, irrespective of categorical grouping (e.g., host or season).

4.2.6 Alpha diverisity analysis

Statistical comparison of alpha diversity values were conducted to determine whether microbial richness differed significantly between seasons of tick collection and between host species from which ticks were collected. The Analysis odf Variance (ANOVA) statistical test was performed for group comparisons (seasonal and host comparison).

4.2.7 Beta diversity analysis

Beta diversity metrics were computed to assess the dissimilarity of microbial communities among tick samples collected in different seasons and from different hosts. The Bray-Curtis dissimilarity index was used to calculate pairwise distances between samples based on ASV abundance profiles. These distance matrices were then subjected to Analysis of similarities (ANOSIM) to statistically test differences in microbial community composition between seasons and between host species. A PERMDISP (Permutational Analysis of Multivariate Dispersions) was also conducted using the Bray-Curtis distance matrix to investigate microbial dispersion between ticks sampled in different seasons and ticks collected from different hosts. To further validate the influence of seasonality and host species on microbial community structure, Permutational Multivariate Analysis of Variance (PERMANOVA) was performed. In short, a single Bray-Curtis distance matrix was used for all tests. PCoA was run once, and then sample labels were changed to highlight either season or host. The tests (ANOSIM, PERMANOVA, PERMDISP) were applied to that same distance matrix, so the results relate to the same ordination space

4.2.8 Sequencing depth assessment and rarefaction analysis

To evaluate evaluate whether sequencing depth was sufficient to capture microbial diversity across all samples, rarefaction curves were generated using QIIME2. The curves plotted the number of observed ASVs against sequencing depth for each sample. Good's coverage estimate were used to further assess sequencing completeness.

4.2.9 Data normalization and differential abundance testing

Processed ASV tables were imported into MicrobiomeAnalalyst (Dhariwal *et al*., 2017) for statistical analysis. Prior to differential abundance testing, data were normalized using total sum scaling (TSS) to correct for sequencing depth variability. Differential abundance analysis was performed to identify bacterial genera that varied significantly between samples based on season of tick collection and Host species from which ticks were collected. The Kruskal-Wallis non-parametric test was used for multiple group comparisons. For each genus, a false discovery ratev (FDR) correction was applied to adjust for multiple testing (Benjamini-Hochberg procedure), ensuring statistical robustness.

4.3 Results

Metabarcoding was carried out on 221 tick DNA samples collected from cattle, sheep, goat horse, dog, environment and laboratory reared ticks from Clinvet. Only 105 Fastq sequences passed the quality check to generate amplicon sequence variants (ASVs.) The summary table showing the final number of samples used for Metabarcoding analysis is shown below (Table 3.2). The OUT/ASV number were 689, while the OTUs/ASV with ≥ 2 counts was 371. The total read counts were 1590249 and the average counts per sample were 15290. The maximum counts per samples were 92844 while the minimum counts per sample was 3. All sequences generated in this study were uploaded on the Sequence Read Archive (SIR), as submission number SUB14667322, NCBI database. The submission processing is in progress.

Table 4.1: Samples used for final metabarcoding analysis

Host	Sequenced samples	Samples used for final analysis
Cattle	42	10 (4 eggs & 6 adult)
Sheep	29	13 (1 egg & 12 adult)
Goat	27	20 (adult)
Horse	50	49 (adult)
Dog	49	7 (3 eggs & 4 adult)
Environment	20	4 (adult)
Clinvet	4	2 (adult)
TOTAL	**221**	**105**

4.3.1 Overall bacteria detected from ticks collected from different hosts in four provinces of South Africa

There were a total of 27 bacterium phyla recovered from the overall tick samples in the present study include; Proteobacteria, Actinobacteria, Firmicutes, Epsilonbacteraeota, Bacteroidetes, Planctomycetes, Verrucomicrobia, Synergistetes, Chloroflexi, Patescibacteria, Chlamydiae, Spironchaeota, Fusobacteria, Latescibacteria, Tenericutes, Armatimonadetes, Acidobacteria, Deinococcus-Thermus, Dependentiae, Elusimicrobia, Fibrobacteres, Gemmatimonadetes (1.3%), Kiritimatiellaeota, Nitrospirae, FBP, BRC1 and Cyanobacteria. A total of 65 classes; Acidobacteriia, Blastocatellia (Subgroup 4), Holophagae, Subgroup 5, Subgroup 6, Acidimicrobiia, Actinobacteria, Coriobacteriia, MB-A2-108, Nitriliruptoria, Rubrobacteria, Thermoleophilia, Fimbriimonadia, uncultured bacterium, Bacteroidia, Ignavibacteria, Rhodothermia, Chlamydiae, AD3, Anaerolineae, Chloroflexia, Dehalococcoidia, Gitt-GS-136, JG30-KF-CM66,KD4-96, Ktedonobacterria, TK10, Melainabacteria, Oxyphotobacteria, Sericytochromatia, Deinococci,Babeliae, Elusimicrobia, Camphylobacteria, Metagenome, Uncultured actinobacterium, Uncultured bacterium, uncultured organism, Uncultured soil bacterium, Fibrobacteria, Bacilli, Clostridia, Erysipelotrichia, Negativicutes, Fusobacteriia, Gemmatimonadetes, Longimicrobia, S0134 terrestrial group, Kiritimatiellae, Nitrospira, Gracilibacteria, Microgenomatia, Parcubacteria, Saccgarimonadia, W56 (Dojkabacteria), Phycisphaerae, Planctomycetacia, Alphaproteobacteria, Deltaproteobacteria, Gammaproteobacteria, Leptospirae, Spirochaetia, Synergistia, Mollicutes and Verrucomicrobiae.

The overall bacteria Order's recovered were 145 and they include; Acidobacteriales, Solibacterales, Subgroup 2, Blastocatellales, DS100, Pyrinomonadales, Subgroup 7, Acidobacteria bacterium WX27, Unknown Order, Uncultured Acidobacteriales bacterium, Uncultured microorganism, Wastewater metagenome, Microtrichales, Actinomycetales, Bifidobacteriales, Corynebacteriales, Frankiales, Kineosporiales, Micrococcales, Micromonosporales, Propionibacteriales, Pseudonocardiales, Streptomycetales, Streptosporangiales, Coriobacteriales, Uncultured Actinomycetales bacterium, Nitriliruptorales, Rubrobacterales, Gaiellales, Solirubrobacterales, Fimbriimonadales, Uncultured bacterium, Bacteroidales, Chitinophagales, Flavobacteriales, SM1A07, Sphingobacteriales, Ignavibacteriales, OPB56, Rhodothermales, Chlamydiales, Uncultured clostridium sp., Anaerolineales, Ardenticatenales, Caldilineales, SBR1031, SJA-15, Chloroflexales, Kallotenuales, Thermomicrobiales, S085, Uncultured caldilinea sp., C0119, Ktedonobacterales, Gastranaerophilales, Obscuribacterales, Nostocales, Oxyphotobacteria incertae sedis, Phormidesmiales, Uncultured Soil bacterium, Deinococcales, Babeliales, Lineage IV, Camphylobacterales, Metagenome, Uncultured Actinobacterium, Fibrobacterales, Bacillales, Lactobacillales, Clostridiales, M55-D21, Erysipelotrichales, Selenomonadales, Fusobacteriales, Gemmatimonadales, Longimicrobiales, WCHB1-41, Nitrospirales, Absconditabacteriales (SR1), JG1 0000069-P22, Candidatus pacebacteria, Candidatus woesebacteria, Candidatus nomurabacteria, GWA2-38-13b, Uncultured parcubacteria group bacterium, Saccharimonadales, phycisphaerales, Tepidisphaerales, Gemmatates, Isosphaerales, Pirellulales, Planctomycetates, Acetobacterales, Azospirillales, Caulobacterales, Elsterales, Holosporales, Micavibrionales, Paracaedibacterales,Puniceispirillales, Reyranallales, Rhizobiales, Rhodobacterales,

Rhodospirillales, Rhodovibrionales, Rickettsiales, Sphingomonadales, Tistrellales, Bdellovibrionales, Bradymonadales, Desulfobacterales, Desulfovibrionales, Myxococcales, Oligoflexales, SAR324 clade (marine group B), Syntrophobacterales, AEROMONADALES, Alterromonadales, Betaproteobacteriales, Cardiobacterials, Cellvibrionales, Coxiellales, Diplorickettsiales, EPR3968-08a-Bc78, Enterobacteriales, Francisellales, Gammaproteobacteria incertae sedis, Legionellales, Oceanospirillales, Plasteurellales, Pseudomonadales, R7C24, Salinisphaerales, Steroidobacterales, Xanthomonadales, Leptospirales, Spirochaetales, Synergistales, Mycoplasmatales, Chthoniobacterales, Opitutales and lastly Verrucomicrobiales. The overall bacterium class recovered were 64, order 145, family 234 and genera 580. Further discussion of recoverd bacterium family and genera below (Table 3.3).

Table 4.2: A summary of the most dominant bacterial phylum, family and genus for ticks collected from the various host's species used in the present study

HOST	TICK SPECIES	PHYLUM	FAMILY	GENUS
Cattle	*R. e. evertsi*	Proteobacteria 90%	Coxiellaceae 55%	*Coxiella* 55%
Sheep	*R. e. evertsi*	Proteobacteria 54%	Coxiellaceae 19%	*Coxiella* 19%, V3 19%
Goat	*R. e. evertsi*	Proteobacteria 88%	Moraxellaceae 30%	*Acinetobacter* 30%
Horse	*R. e. evertsi*	Firmicutes 48%	Staphylococcaceae 45%	*Staphylococcus* 44%
Dog	*R. sanguineus*	Proteobacteria 100%	Coxiellaceae 71%	*Coxiella* 71%
Environment	*R. appendiculatus*	Proteobacteria 88%	Pseudomadaceae 50%	*Pseudomonas* 50%
Clinvet	*R. e. evertsi* *R. appendiculatus*	Proteobacteria 75%	Enterobacteriaceae 50%	*V3 75%

*V3 = unnamed bacteria

4.3.2 Bacteria detected from *Rhipicephalus* tick species collected from cattle

All ticks collected from cattle were morphologically identified as *R. e. evertsi*. The identification was further confirmed molecularly using a subset of the morphologically identified ticks targeting the mitochondrial and nuclear gene regions. The most abundant phylum of bacteria found from these ticks was Proteobacteria at 90% and all the other phyla added up to 10% (Figure 4.1**A**). A total of 5 bacterial families were obtained. The most abundant bacteria family was Coxiellaceae (55.0%) followed by V14[unnamed bacteria] (20.0%); the bacteria family Pseudomonadaceae and Staphylococcaceae were all ranging the same at 10% each; whilst Fracisellaceae was at 5% (Figure 4.1**B**). A total of 5 bacterial genera were obtained. The dominant bacterial genera included *Coxiella* at 55.0% followed by *V3*[unnamed genus] at 20.0%, *Pseudomonas* and *Staphylococcus* at 10.0% each; *Francisella* had an abundance of 5.0% (Figure 4.1**C**).

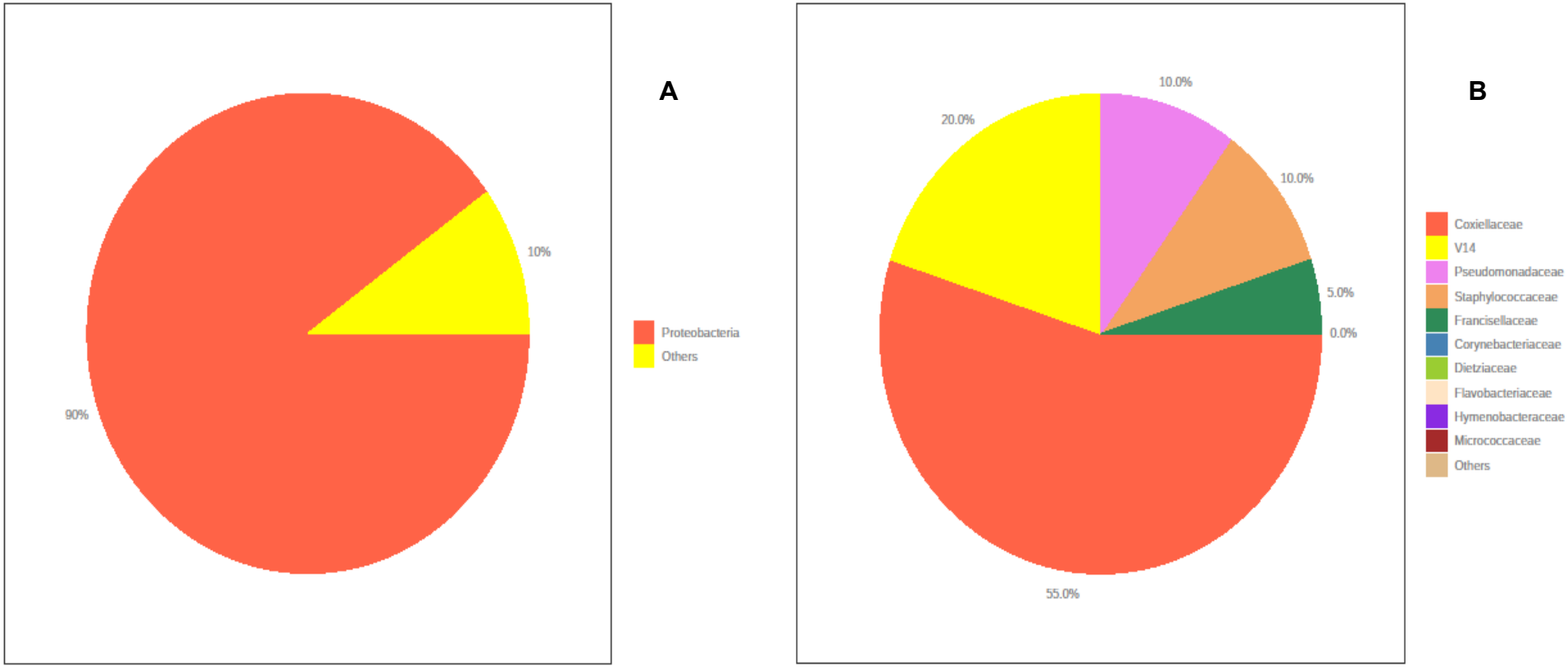


Figure 4.1: *Cont.*

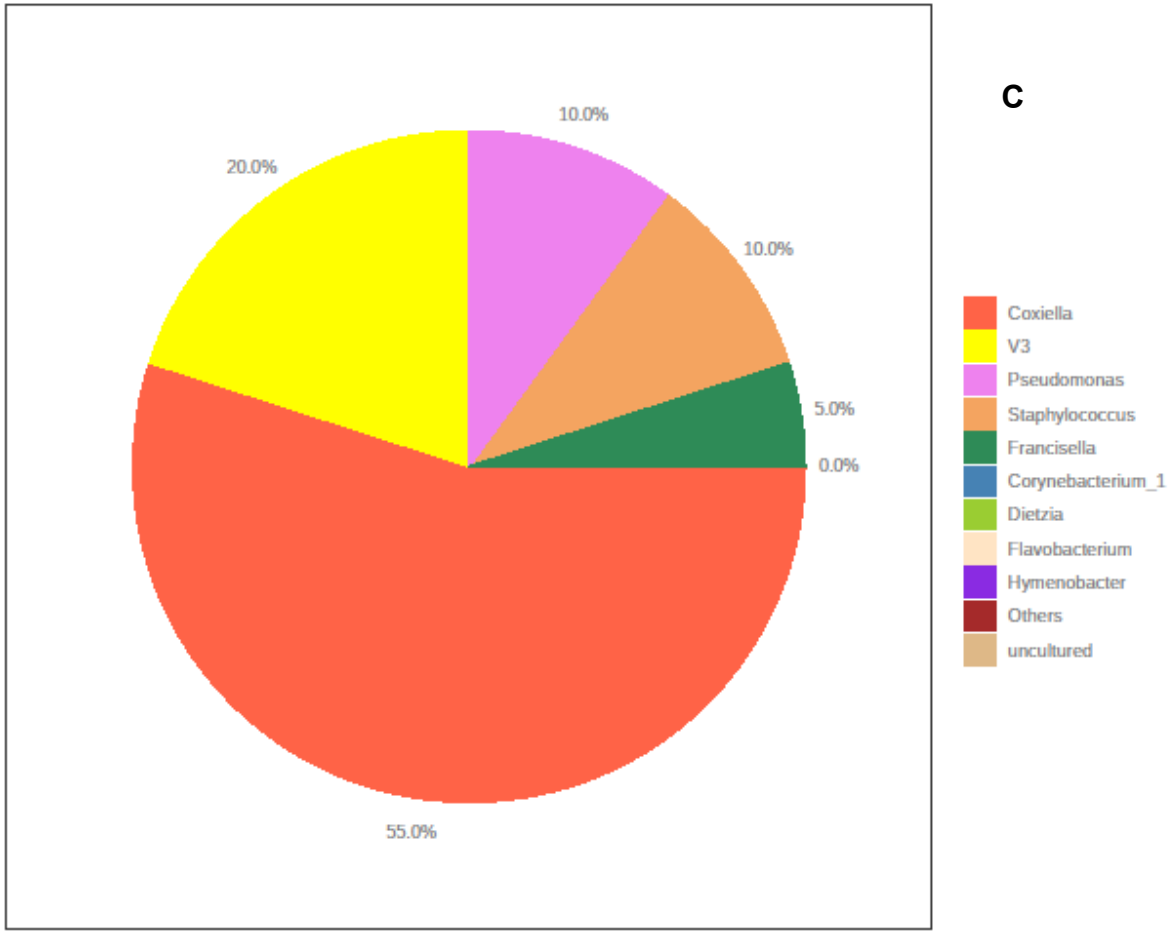


Figure 4.1: Bacterial phyla (**A**), family (**B**), and genera (**C**) detected from *R. e. evertsi* ticks collected from cattle.

4.3.3 Bacteria detected from *Rhipicephalus* ticks collected from goats

All ticks collected from goats were identified morphologically as *R. e. evertsi*. A subset of these ticks were further confirmed genetically by amplified mitochondrial and nuclear gene regions. A total of 3 bacterial phyla were obtained from *R. e. evertsi* ticks collected from goats. The most dominant bacterial phyla included Proteobacteria with 88.0% followed by Firmicutes (7%) and Bacteroidetes (5%) (Figure 4.2**A**). Abundant bacterial families detected from ticks collected from goats in the present study were 10. These included Moraxellaceae (30%), Pseudomonadaceae (20%), Enterobacteriaceae (15%), V14 [unnamed bacteria] (12%), Coxiellaceae (7%), Staphylococcaceae (5%), Aerococcaceae (3%), Caulobacteraceae (3%), Flavobacteriaceae (3%) and lastly Sphingobacteriaceae (3%) (Figure 4.2**B**). A total of 11 bacterial genera were obtained from *R. e. evertsi* ticks collected from goats. The most abundant bacterial genera included *Acinetobacter* (30%), *Pseudomonas* (20%), V3 [unnamed genus] (15%), *Coxiella* and *Escherichia-Shigella* at 7% each. All the the other unnamed bacterial genera and *Proteus* had similar abundance at 5%; the rest of the remaining bacterial genera had an abundance of 3% each including *Aerococcus*, *Flavobacterium*, *Sphingobacterrium* and *Staphylococcus* (Figure 4.2**C**).

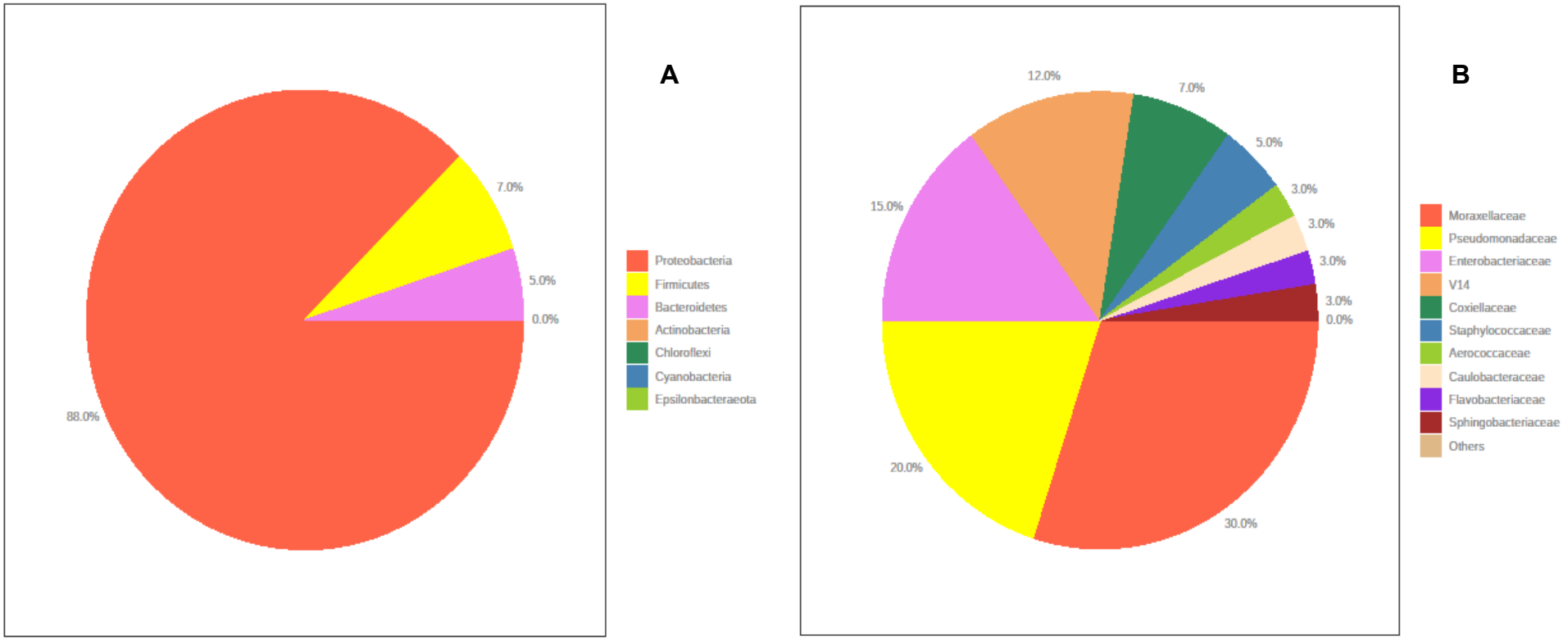


Figure 4.2: *Cont.*

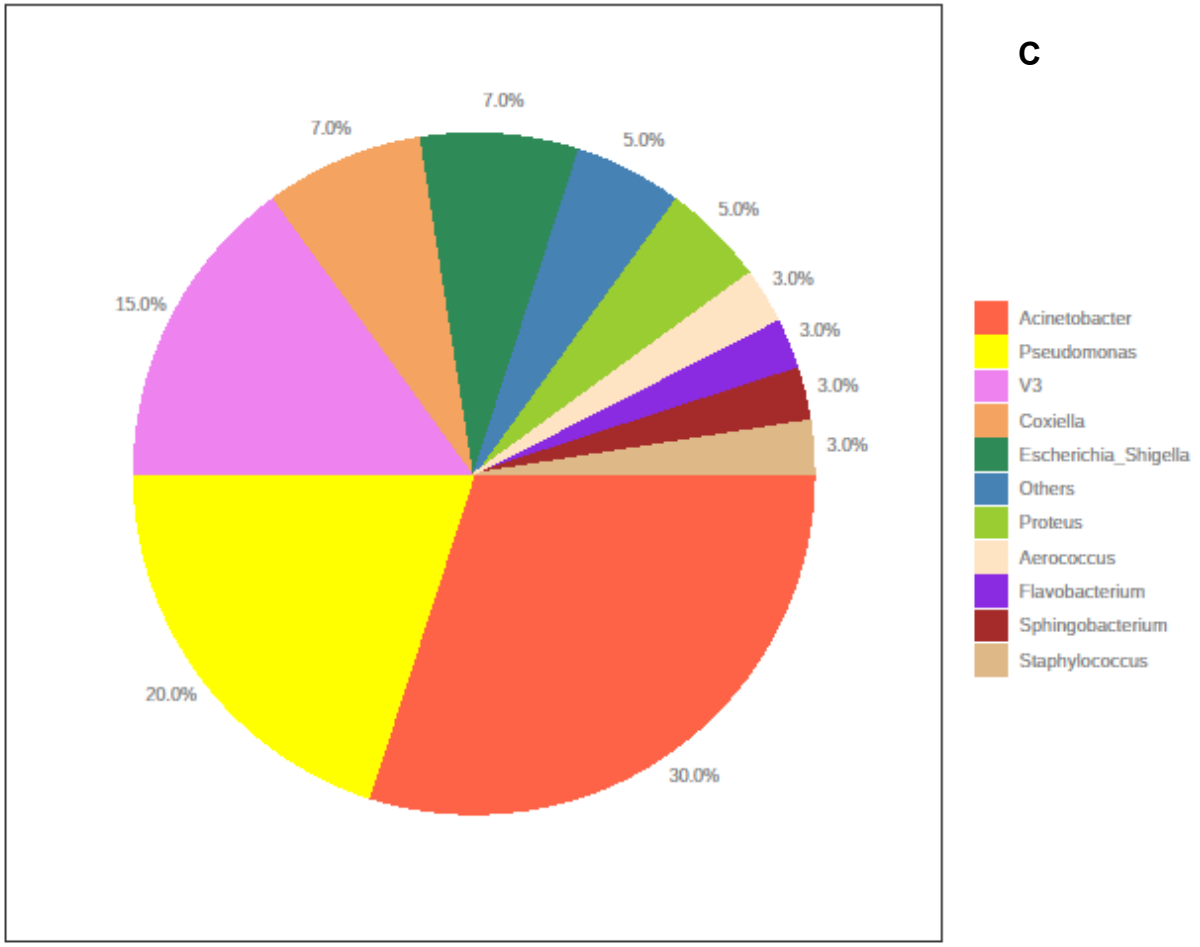


Figure 4.2: Bacterial phyla (**A**), family (**B**), genera (**C**) detected from *R. e. evertsi* ticks collected from goats.

4.3.4 Bacteria detected from *Rhipicephalu*s ticks collected from sheep

All ticks collected from sheep were identified morphologically as *R. e. evertsi*. A subset of these ticks were further confirmed genetically by amplified mitochondrial and nuclear gene regions. A total of 3 phyla were obtain from *R. e. evertsi ticks* collected from sheep. The most dominant bacterial phyla detected included Proteobacteria at 54.0% followed by Firmicutes at 42.0% and Epsilonbacteraeota at 4.0% (Figure 4.3**A**). Abundant bacterial families detected in ticks collected from sheep included the family Coxiellaceae at 19.0% followed by Anaplasmataceae, Bacillaceae, Planococcaceae and Staphylococcaceae at 12.0% each; additionally, other bacterial families detected were Pseudomonadaceae, V14[unnamed bacteria] and all other unnamed bacteria families added up to 8.0%; followed by Camphylobacteraceae, Clostridiaceae_1 and Peptostreptococcaceae at 4.0% each (Figure 4.3**B**). A total of 11 bacteria genera were obtained from *R. e. evertsi* ticks collected from sheep. The most abundant genera included *Coxiella* and V3[unnamed genus] at 19.0% each, all other unnamed bacteria genera detected added up to 15.0%; whilst *Ehrlichia*, *Lysinibacillus* and *Pseudomonas's* relative abundance was at 8.0% each. These was followed by *Camphylobacter*, *Clostridium_Sensu_stricto_13*, *Oceanobacillus* and *Salinicoccus* at 4.0% each (Figure 4.3**C**).

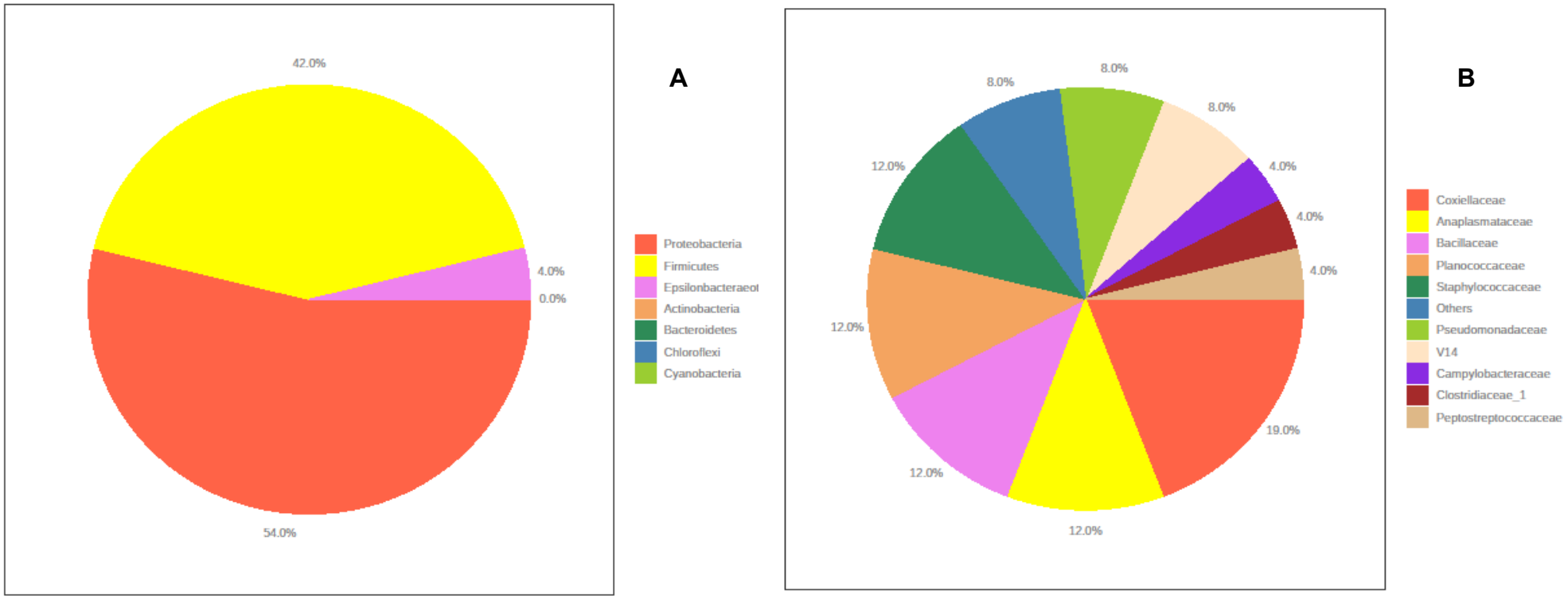


Figure 4.3: *Cont.*

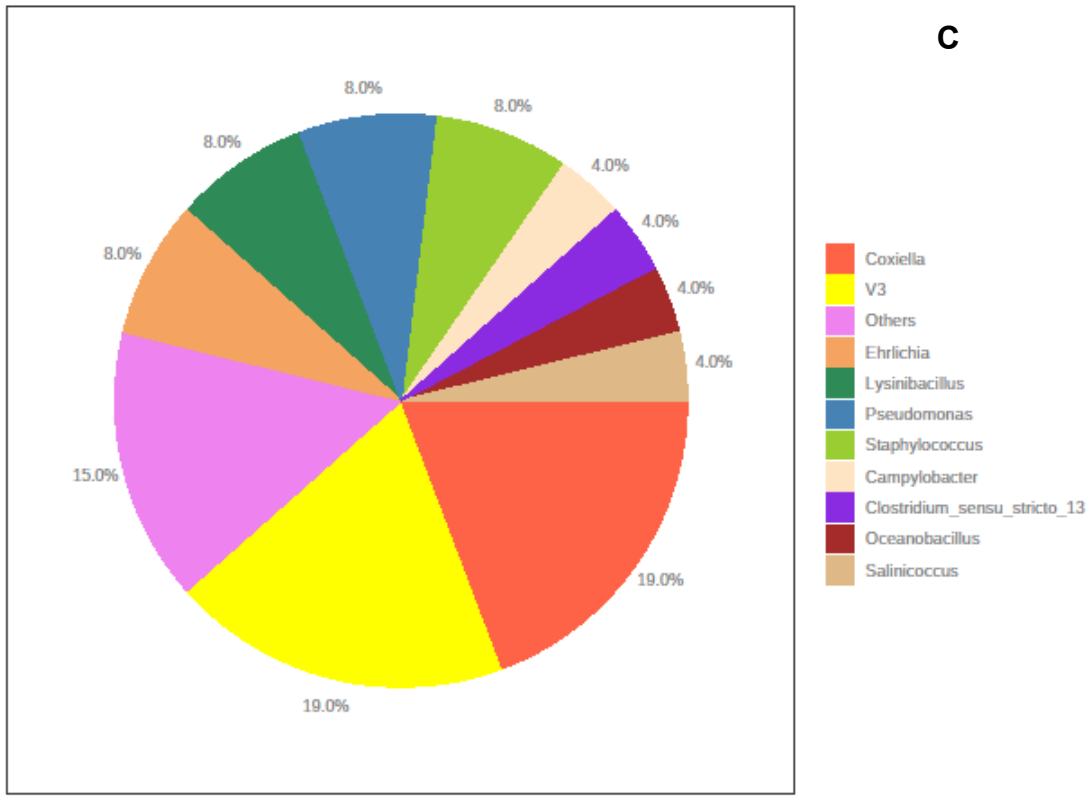


Figure 4.3: Bacterial phyla (**A**), family (**B**) and genera (**C**) detected from *R. e. evertsi* ticks collected from sheep.

4.3.5 Bacteria detected from *Rhipicephalus* ticks collected from horses

All ticks collected from horses were identified morphologically as *R. e. evertsi*. A subset of these ticks were further confirmed genetically by amplified mitochondrial and nuclear gene regions. A total of 5 bacterial phyla were obtained from *R. e. evertsi* tick species from horses. The most abundant bacterial phyla detected were Firmicutes at 48.0%, followed by Proteobacteria (38.0%), Actinobacteria (11.0%), Bacteroidetes (2.0%) and Cyanobacteria (1.0%) (Figure 4.4**A**). Abundant bacterial families obtained were 11. This included Staphylococcaceae at the highest abundance of 45.0%, followed by Coxiellaceae (13.0%), Corynebacteriaceae (10.0%), Moraxellaceae (7.0%), Enterobacteriaceae (6.0%) and V14 [unnamed bacteria family] (5.0%); the family Burkholderiaceae, Caulobacteraceae, and Xanthomonadaceae had an abundance of 2.0% each; the family Dietziaceae had the abundance of 1.0%; all the other unnamed bacteria families detected added up to 6.0% (Figure 4.4**B**). A total of 11 bacterial genera were obtained from *R. e. evertsi* ticks collected from horses. The most abundant bacteria genera detected included *Staphylococcus* (44%), *Coxiella* (13.0%), *Corynebacterium_1* (10.0%), *Acinetobacter* (7.0%), *V3* [unnamed genus] (6.0%), *Serratia* (5.0%) and uncultured bacteria at 3.0%; the genus *Burkholderia_Caballeronia_Paraburkholderia* and *Stenotrophomonas* showed an abundance of 2.0%, whislt *Dietzia's* abundance was at 1.0%. All the other unnamed bacteria genera detected added up to 6.0% (Figure 4.4**C**).

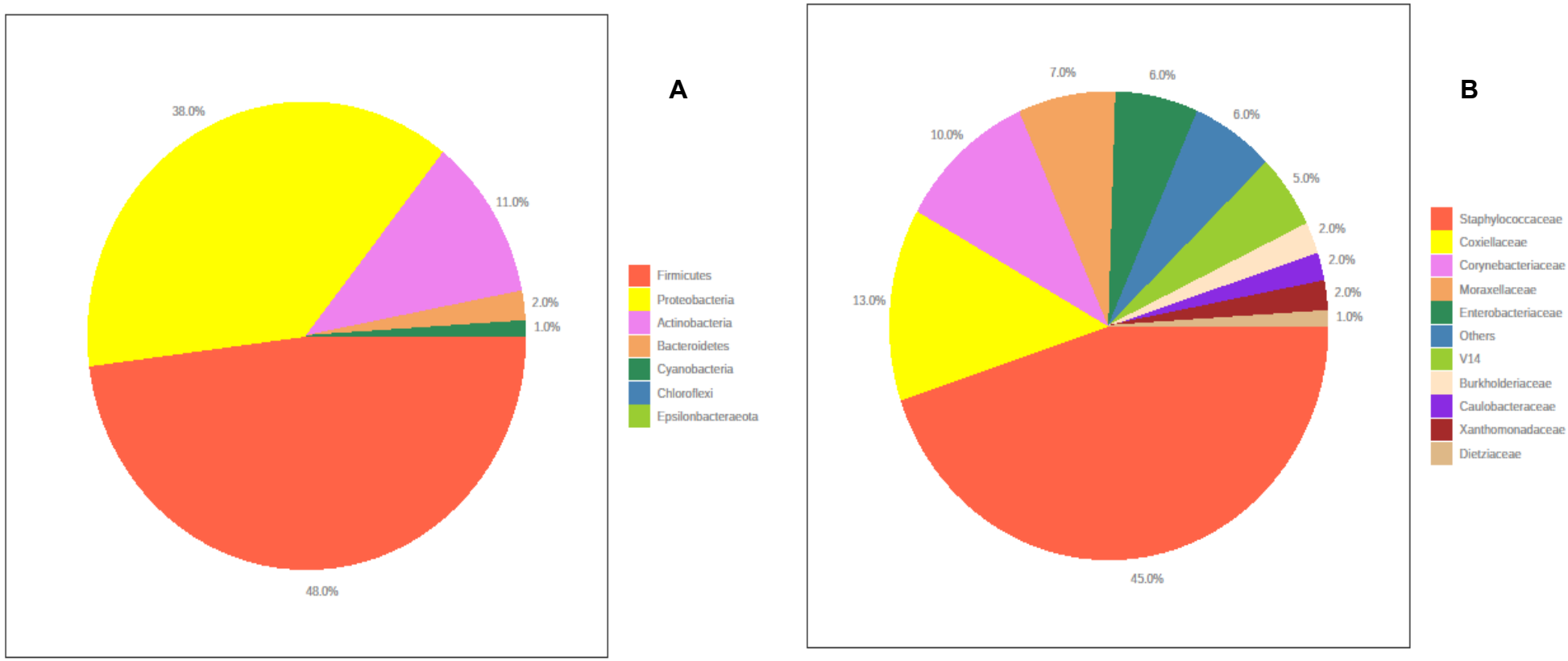


Figure 4.4: *Cont.*

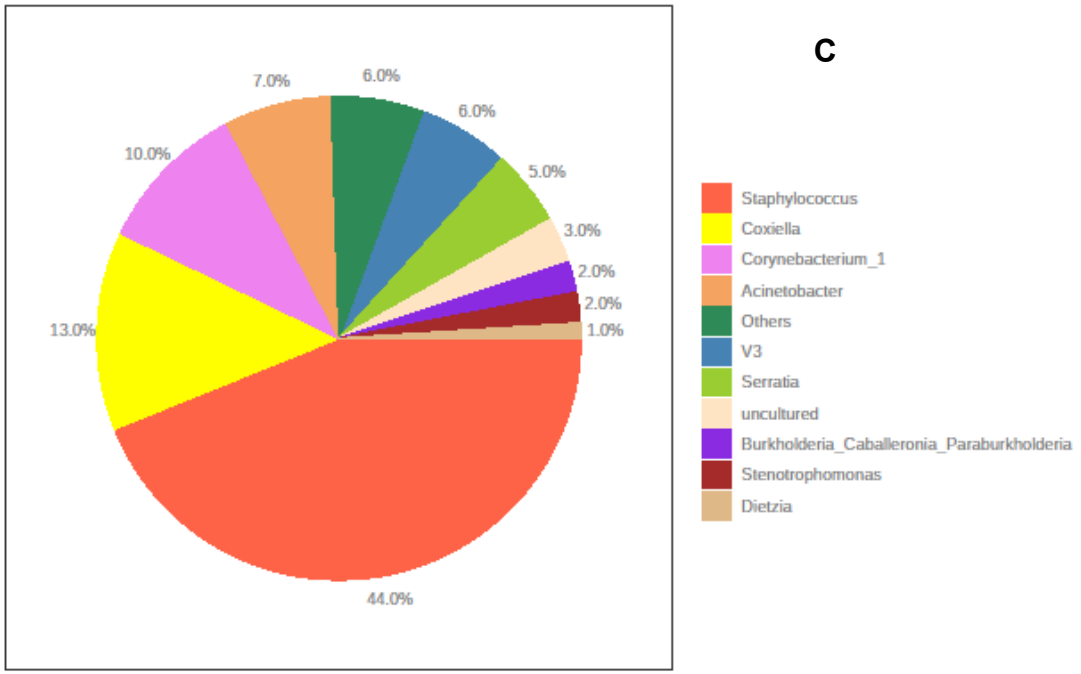


Figure 4.4: Bacterial phyla (**A**), family (**B**), genera (**C**) detected from *R. e. evertsi* ticks collected from horses.

4.3.6 Bacteria detected from *Rhipicephalus* ticks collected from dogs

All ticks collected from dogs were identified morphologically as *R. sanguineus.* A subset of these tick species were further confirmed genetically by amplified mitochondrial and nuclear gene regions. The bacteria phyla obtained were Proteobacteria at an abundance of 100% (Figure 4.5**A**). A total of 4 bacteria families were obtained from *R. sanguineus* ticks collected from dog. The most abundant bacteria family included Coxiellaceae (71.0%), V14 [unnamed bacteria] (14.0%), Moraxellaceae (7%) and an unknown_family of bacteria at 7.0% (Figure 4.5**B**). Abundant bacteria genera obtained from *R. sanguineus* were *Coxiella* (71.0%), V3 [unnamed genus] (14.0%), *Acidibacter* (7.0%) and *Acinetobacter* (7.0%) (Figure 4.5**C**).

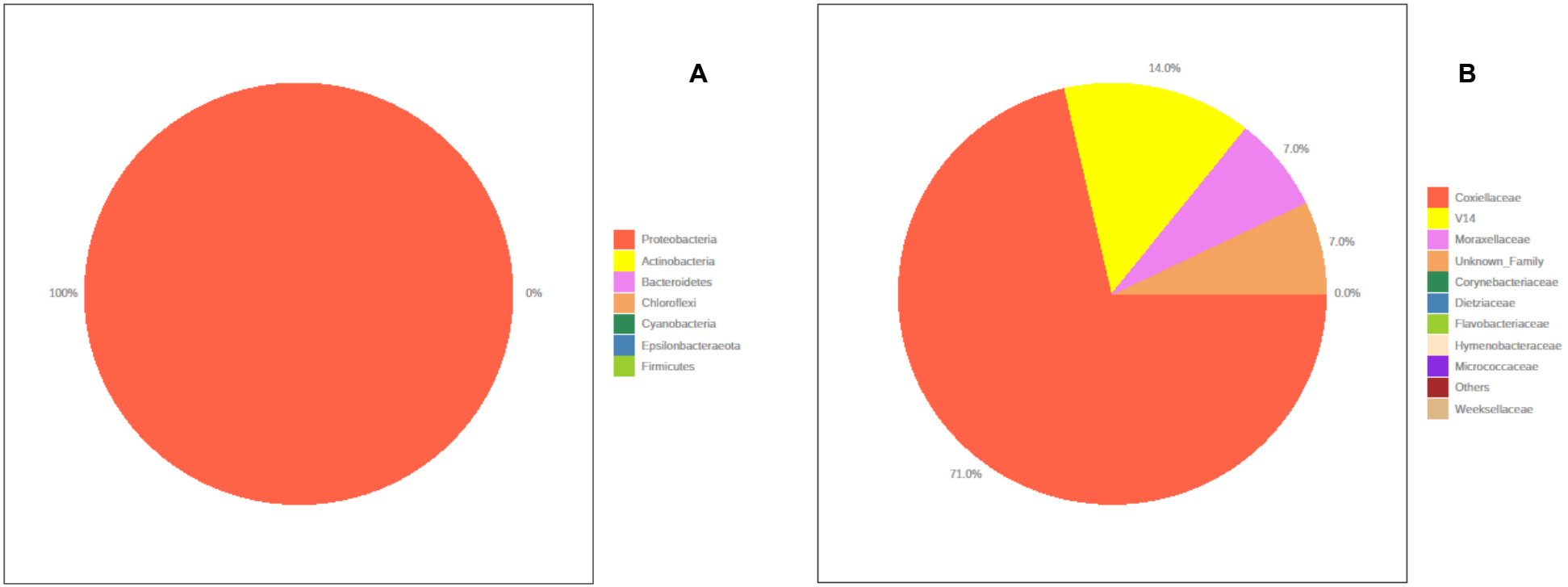


Figure 4.5: *Cont.*

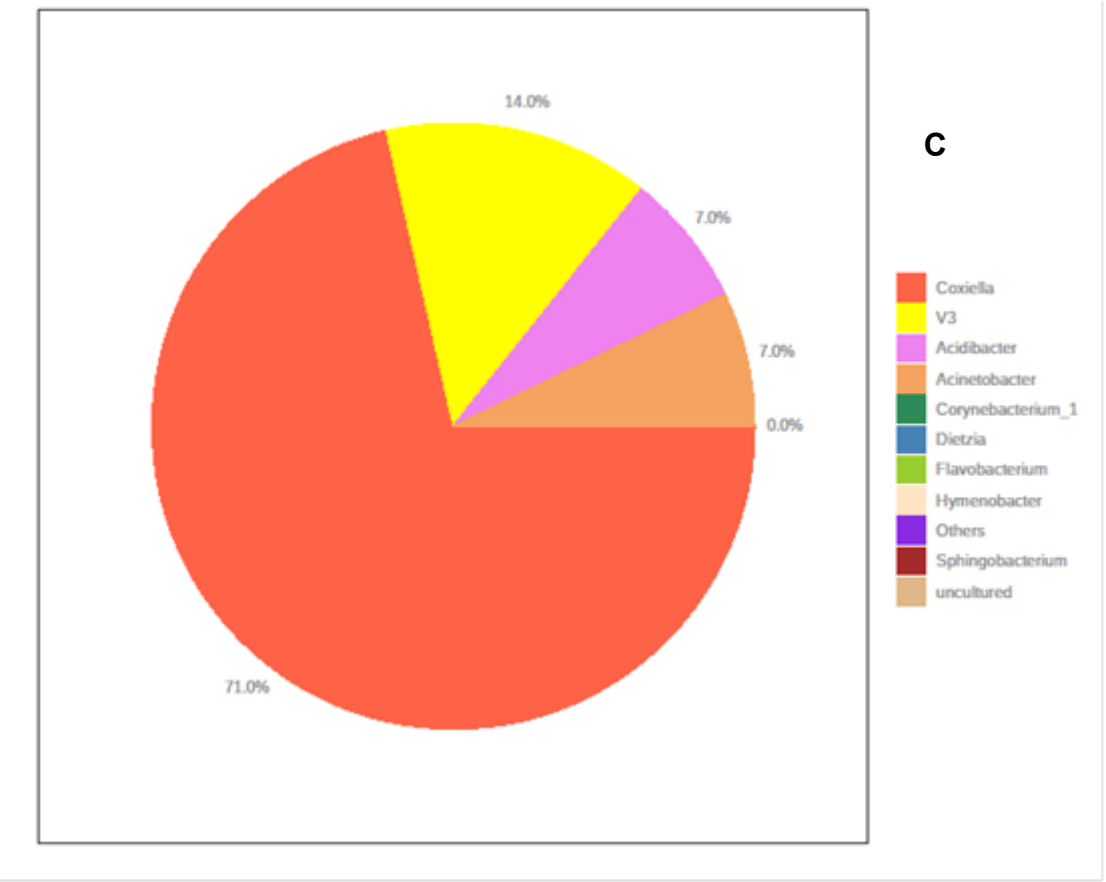


Figure 4.5: Bacterial phyla (**A**), family (**B**), genus (**C**) detected from *R. sanguineus* ticks collected from dogs.

4.3.7 Bacteria detected from *Rhipicephalus* ticks collected from vegetation

All ticks collected from vegetation was identified morphologically as *R. appendiculatus.* A subset of these tick species was further confirmed genetically by amplified mitochondrial and nuclear gene regions. Two bacterial phyla were obtained from *R. appendiculatus* ticks collected from vegetation. The most abundant bacterial phylum was Proteobacteria at 88.0% followed by Actinobacteria at 12.0% (Figure 4.6**A**). The abundant bacterial families detected were Pseudomonadaceae (50.0%), V14 [unnamed bacteria] (25%), Micrococcaceae (12.0%) and Rhodobacteraceae (12.0%) (Figure 4.6**B**). A total of three bacterial genera were detected these included *Pseudomonas* (50.0%), followed by V3 [unnamed genus] (38.0%) and *Paracoccus* at 12.0% (Figure 4.6**C**).

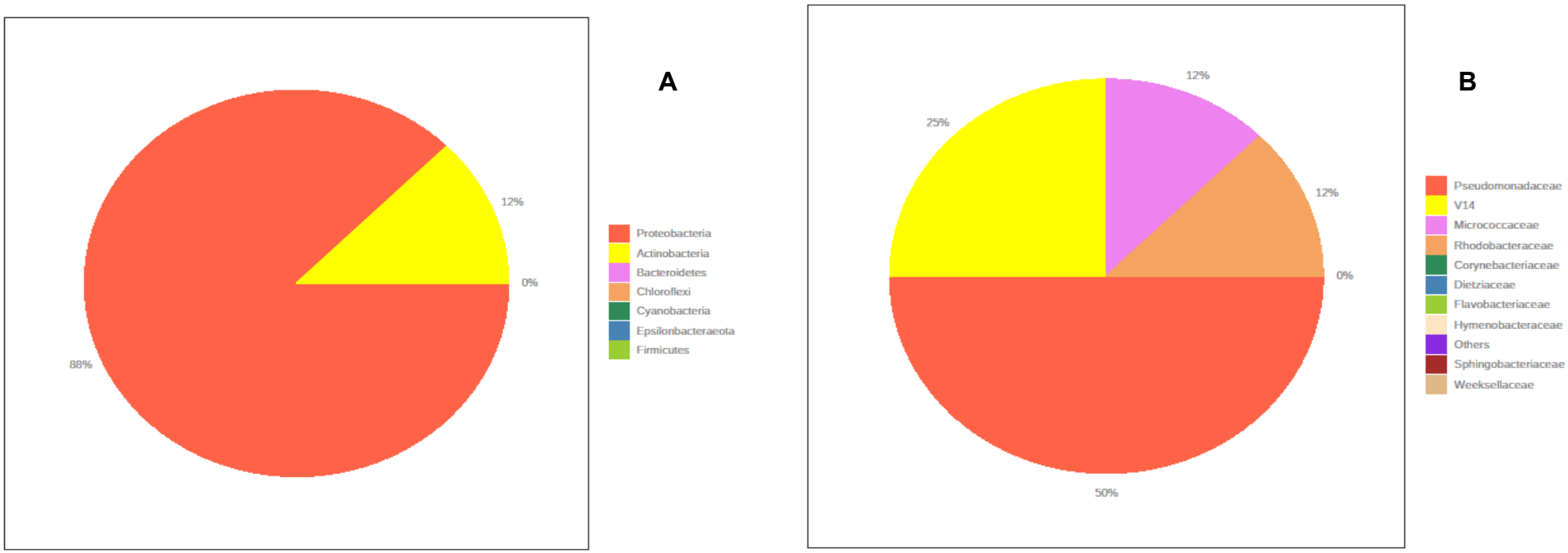


Figure 4.6: *Cont.*

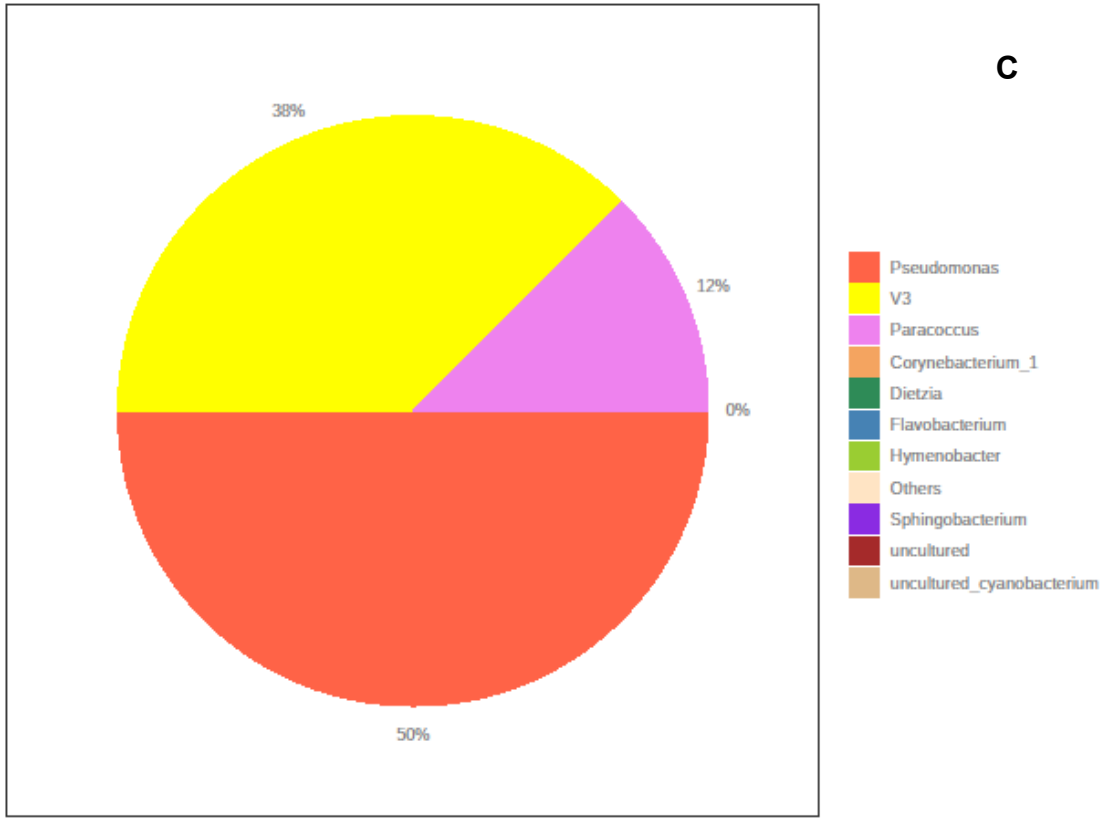


Figure 4.6: Bacterial phyla (**A**), family (**B**) and genera (**C**) detected from *R. appendiculatus* ticks collected from vegetation.

4.3.8 Bacteria detected from colony *Rhipicephalus* ticks

Laboratory reared colony ticks from Clinvet that passed the filtering to good quality ASV files used in final metabarcoding analysis were ticks identified morphologically as *R. e. evertsi* and *R. appendiculatus.* These tick species identifications were confirmed genetically by amplified mitochondrial and nuclear gene regions. A total of 2 bacterial Phyla were detected from *Rhipicephalus* spp. That were laboratory reared at Clinvet laboratories. These included the bacterial phylum Proteobacteria (75.0%) and Chloroflexi (25.0%) (Figure 4.7**A**). Three bacterial families were obtained. The most abundant bacterial families detected included Enterobacteriaceae at 50.0%, followed by Ktedonobacteraceae at 25.0% and Xanthomonadaceae also at 25.0%. (Figure 4.7*B*). The most abundant bacteria genera detected in laboratory reared ticks were bacteria from the *V3* [unnamed genus] at 75.0% followed by bacteria from the *Stenotrophomonas* at 25.0% (Figure 4.7**C**).

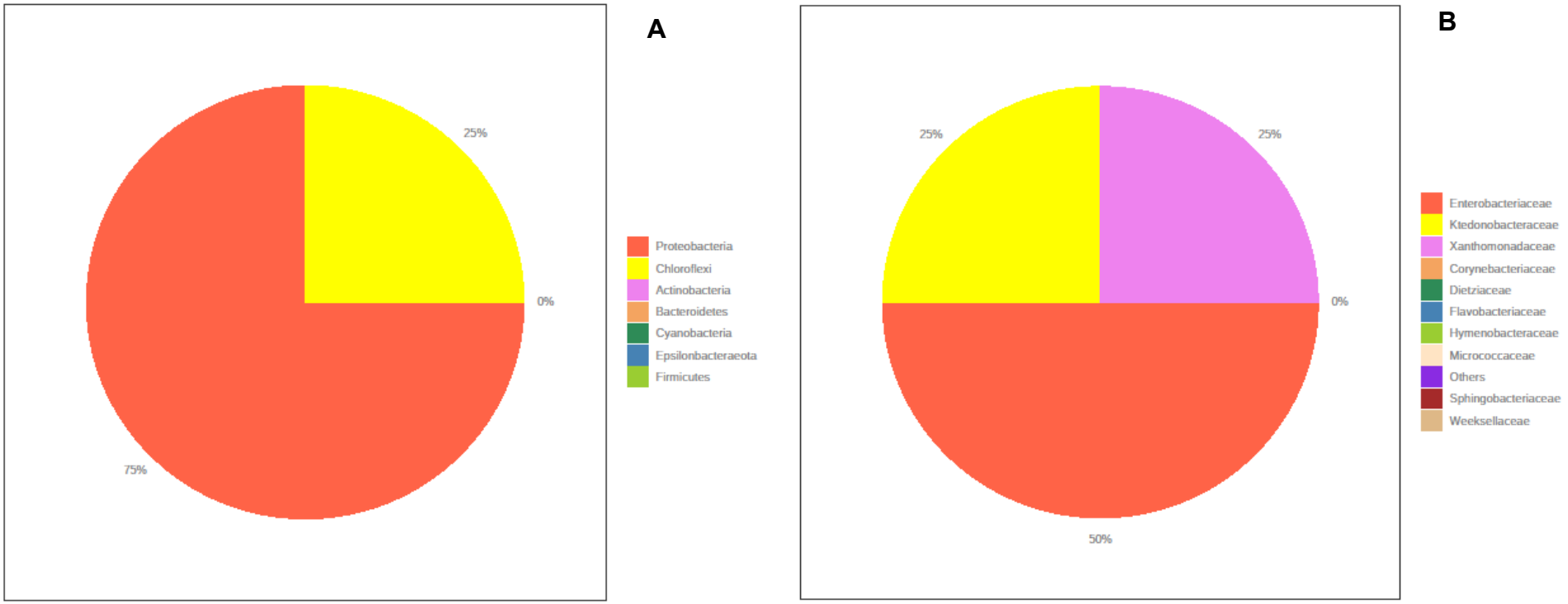


Figure 4.7: *Cont.*

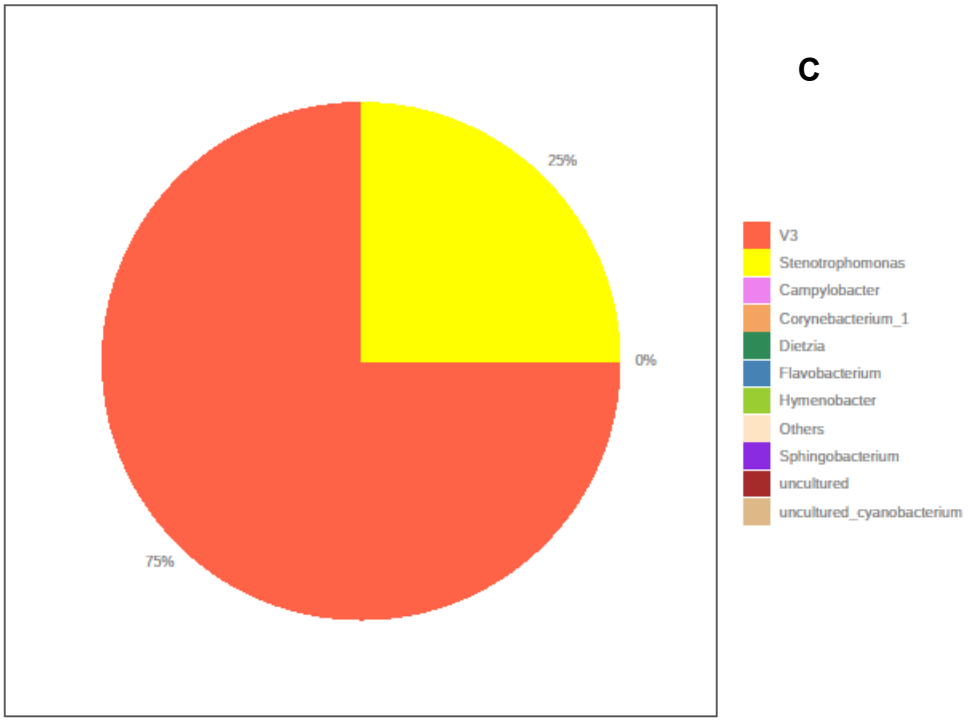


Figure 4.7: Bacterial Phyla (**A**), Family (**B**) and genera (**C**) detected *R. e; evertsi* and *R. appendiculatus* laboratory reared ticks

4.3.9 Seasonal classification of bacteria detected from *Rhipicephalus* ticks collected

The bacterial community composition in *Rhipicephalus* spp. ticks collected in this study varied across seasons. During autumn, the most abundant bacteria phylum was Proteobacteria (76) %, followed by Firmicutes (12%), Epsilonbacteraeota (6%), Actinobacteria (4%) and Bacteroidetes (2%) (Figure 4.8**A**). In summer, Firmicutes were predominant (50%), with Proteobactera (33%), Actinobacteria (13%), Bacteriodetes (3%) and Cyanobacteria (1%) also detected (Figure 4.8**B**). In spring, Proteobacteria were the most abundant (70%), followed by Firmicutes (21%), Bacteroidetes (5%) and Actinobacteria (4%) (Figure 4.8**C**). Lastly, in winter, Proteobacteria dominated at 86%, with Firmicutes at 10%, and both Actinobacteria and Bacteroidetes present at 2% each (Figure 4.8**D**). All bacterial phyla with relative abundance below 1% were excluded from the figures across all seasons to prevent prevent visual overcrowding and enhance data clarity.

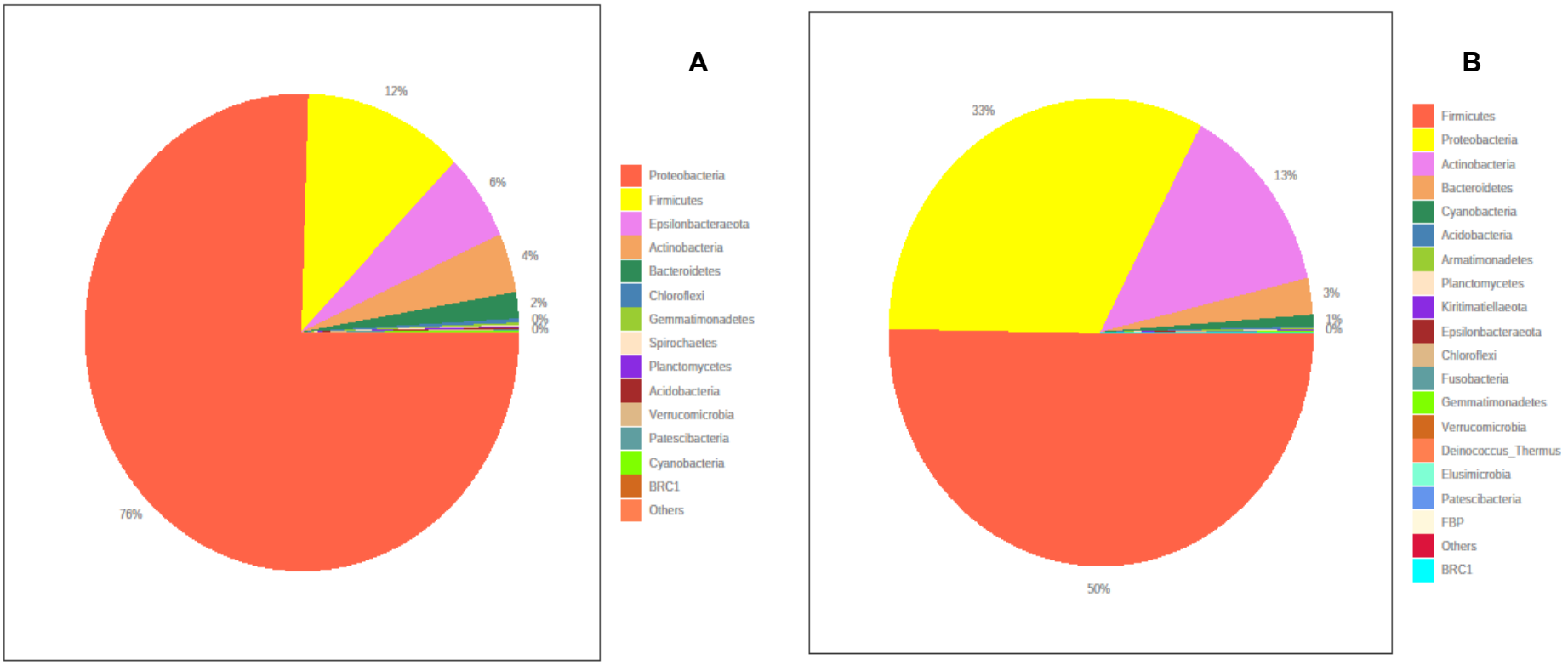


Figure 4.8: *Cont.*

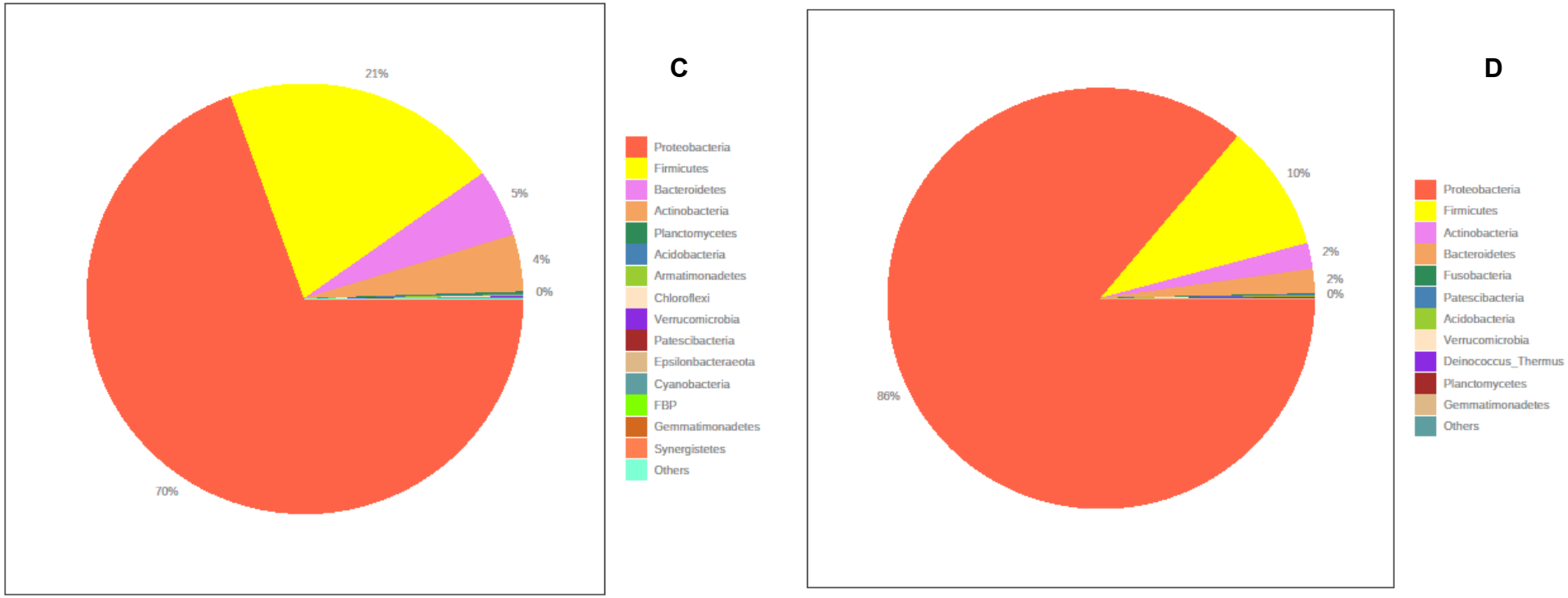


Figure 4.8: Bacterial phyla detected in *Rhipicephalus* species collected in autumn (**A**), summer (**B**), spring (**C**) and winter (**D**) seasons.

4.3.10 Core microbiota

The determination of the core microbiota was carried out across relative abundance/prevalence thresholds with the blanket analysis based on various signal and prevalence. For the core microbiome analysis, the prevalence threshold was set to 20% and detection threshold of relative abundance was 0.01% (Figure 4.9). The core microbiome of *Rhipicephalus* spp. collected in autumn comprised one genus with a high prevalence of 83.33% (*Coxiella*), followed by a 50.00% prevalence (*Staphylococcus*), 44.44% prevalence (*Corynebacterium*), 27.78% (*Erhlichia*) and three genera (*Acinetobacter*, *Camphylobacter* and *Aerococcu*s) with prevalences of 22.22% (Figure 4.9**A**). The core microbiome of *Rhipicephalus* spp. ticks collected in summer comprised of three genera with high prevalences, *Staphylococcus* (95.92%), *Corynebacterium* (89.79%)*, Coxiella* (75.51%), followed by *Acinetobacter* with a prevalence of 46.94%, and then two genera *(Serratia* and *Pseudomonas)* with the lowest prevalences of 22.44% (Figure 4.9**B**). The core microbiome of *Rhipicephalus* spp. collected in spring comprised two genera with high prevalences of 66.67% *Pseudomonas* and 63.33% *Acinetobacter*; these were followed by two genera (*Brevundimonas* and S*taphylococcus*) with prevalences of 46.67% each, then *Corynebacterium* at 40% and lastly *Escherichia-Shigella* 36.67% (Figure 4.9**C**). The core microbiome of *Rhipicephalus* ticks collected in winter comprised of one genus *Coxiella* with a highest core microbiome prevalences of 100%, followed by S*taphylococcus* (42.86%) and the rest of the genera (*Acinetobacter, Sphingobium, Corynebacterium* and the unknown bacteria from the phylum Proteobacteria) at a prevalence of 28.57% each (Figure 4.9**D**).

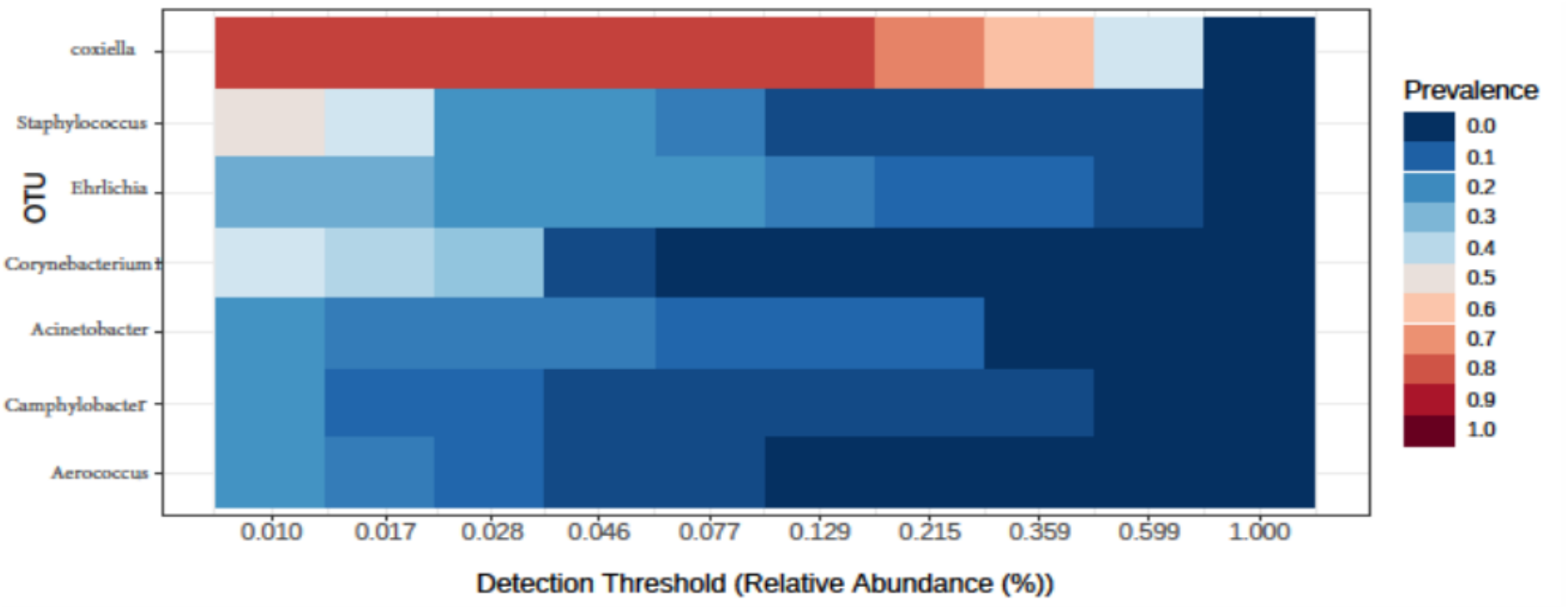


Figure 4.9: *Cont.*

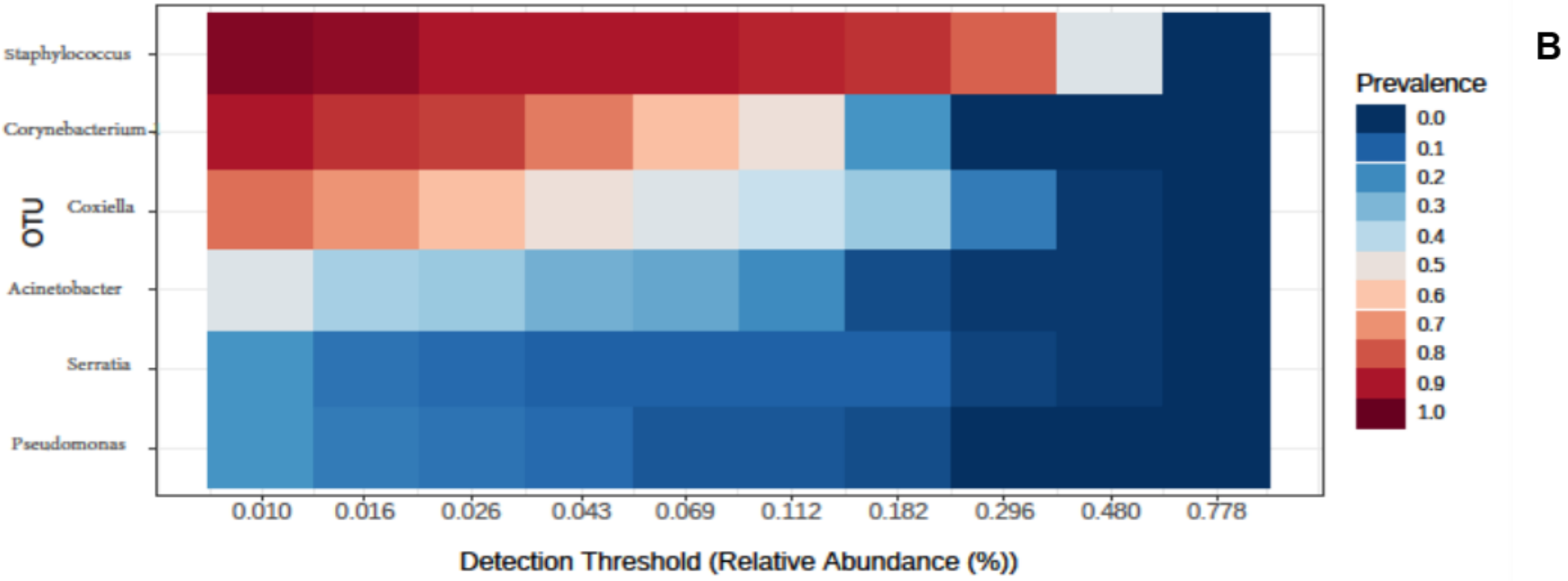


Figure 4.9: *Cont.*

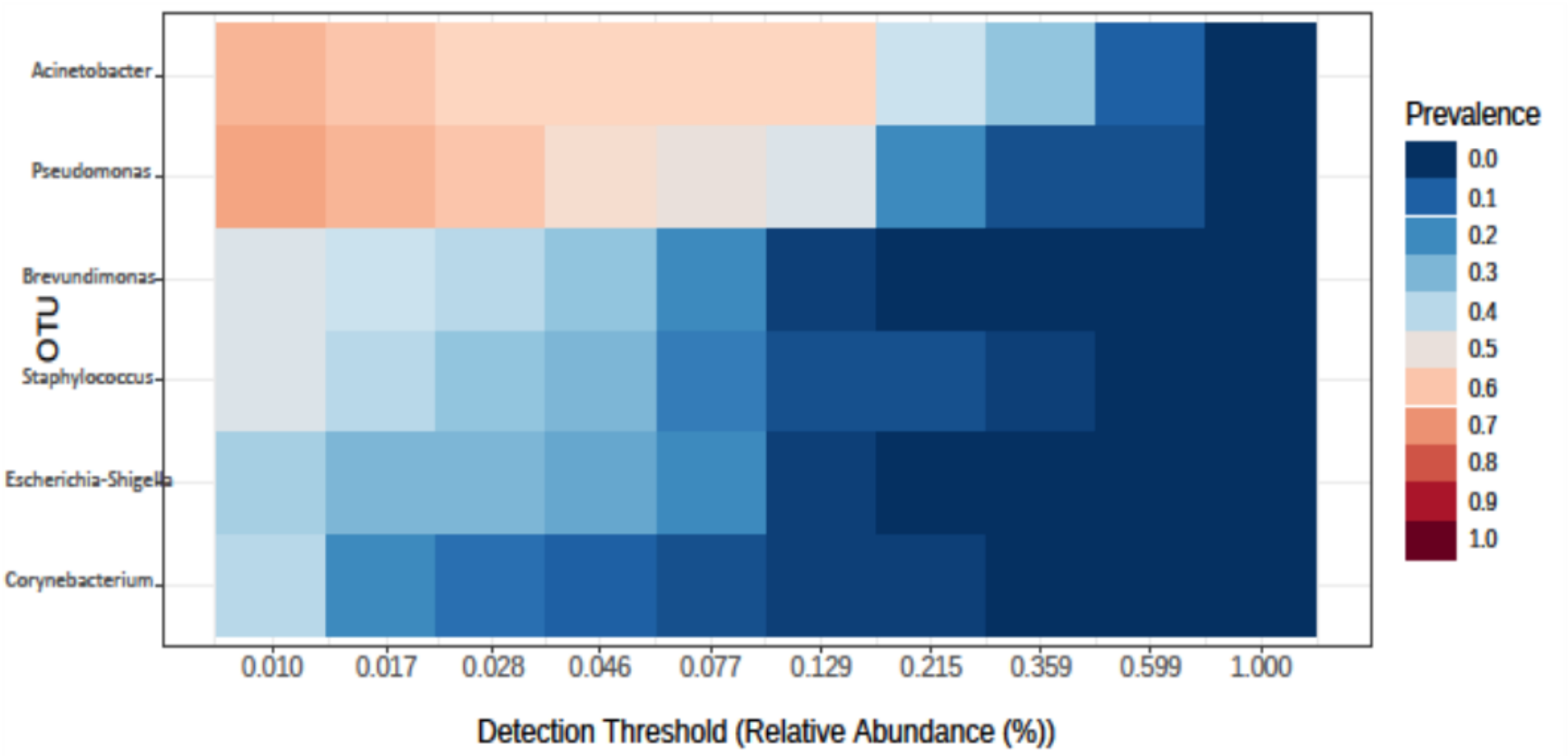


Figure 4.9: *Cont.*

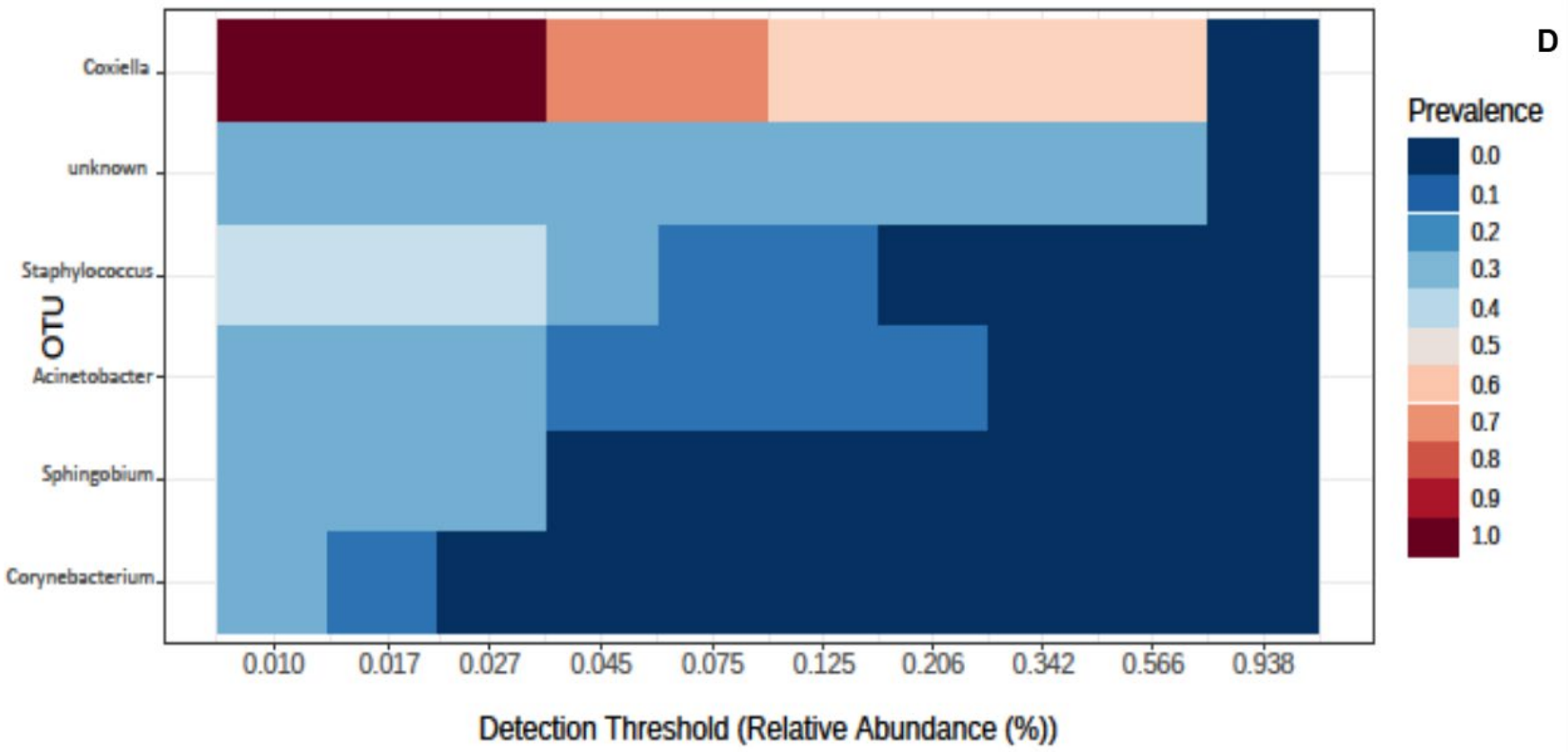


Figure 4.9: Core-microbiome of bacteria genera from *Rhipicephalus* tick species collected in autumn (**A**), summer (**B**), spring (**C**) and winter (**D**) seasons. Red tonality shows the prevalence from 50% to 100% and blue tonality from 0% to 40%.

4.3.11 Diversity measures

The results of alpha-diversity of *Rhipicephalus* ticks collected in different seasons and from different hosts showed no statistically significant difference in the microbiota using Chao1 index. Results from the Chao1 index are shown below for seasons (*p-value* = 0.20328; ANOVA F-value: 1.563) (Figure 4.10**A**) and between hosts (*p-value* = 0.3802; ANOVA F-value: 1.0794) (Figure 4.10**B**).

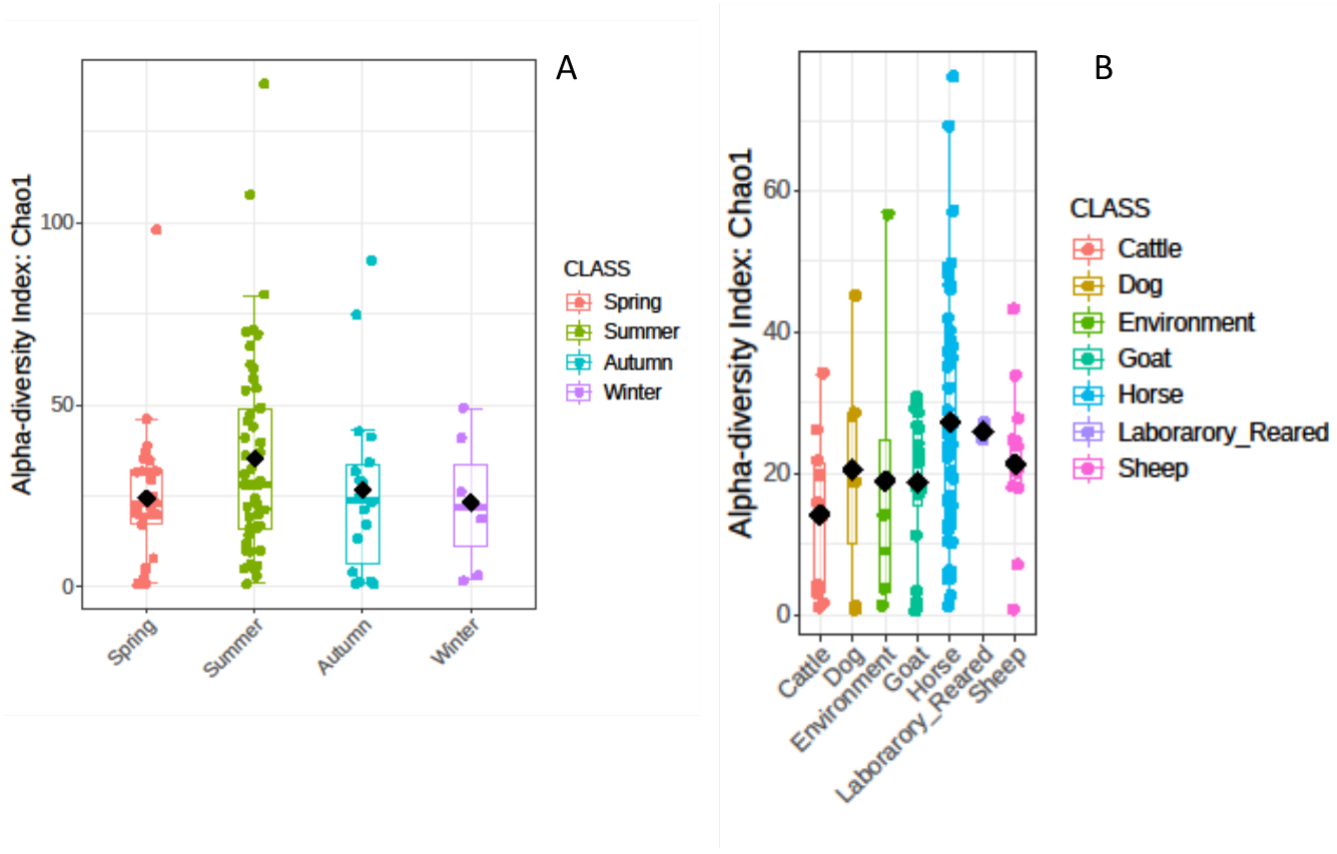


Figure 4.10: Alpha-diversity of *Rhipicephalus* ticks collected in different seasons (**A**) and from different hosts (**B**) using the Chao1 index.

Beta diversity analysis revealed a clear separation of samples according to both the seasons of collection and the host species from which the ticks were collected (Figure 4.11). Statistical analysis of beta diversity further confirmed significant divergence in microbial communities among *Rhipicephalus* ticks sampled in different seasons, as shown by the Bray-Curtis IndexANOSIM test (*R*: 0.49957; *p*-value < 0.001) and among ticks collected from different hosts (*R:* 0.56778*; p-value* < 0.001). These results suggest that the observed differences in microbial beta diversity are unlikely to have occurred by chance. PERMDISP analysis also revealed significant difference in the dispersion of microbial communities across ticks sampled in different seasons (*F*-value 4.5593*; p*-value < 0.0048963), but not across ticks from different hosts (*F-value* 1.6694*; p-value* < 0.13667). This p-value exceeds the 0.05 threshold, indicating that any observed differences in microbial communities between host species are likely due to random variation rather than a true effect. Additionally, PERMANOVA results showed statistically significant variation in microbal communities by season (*F*-value 12.532*, R-squared: 0.27323; p-value:* 0.001) and and by host species (*F-value* 7.2093*, R-squared:* 0.30841*; p-value:* 0.001). This means that, although the composition of microbial communities may differ by host (as indicated by the ANOSIM and PERMANOVA results), the variability within groups is similar across hosts.

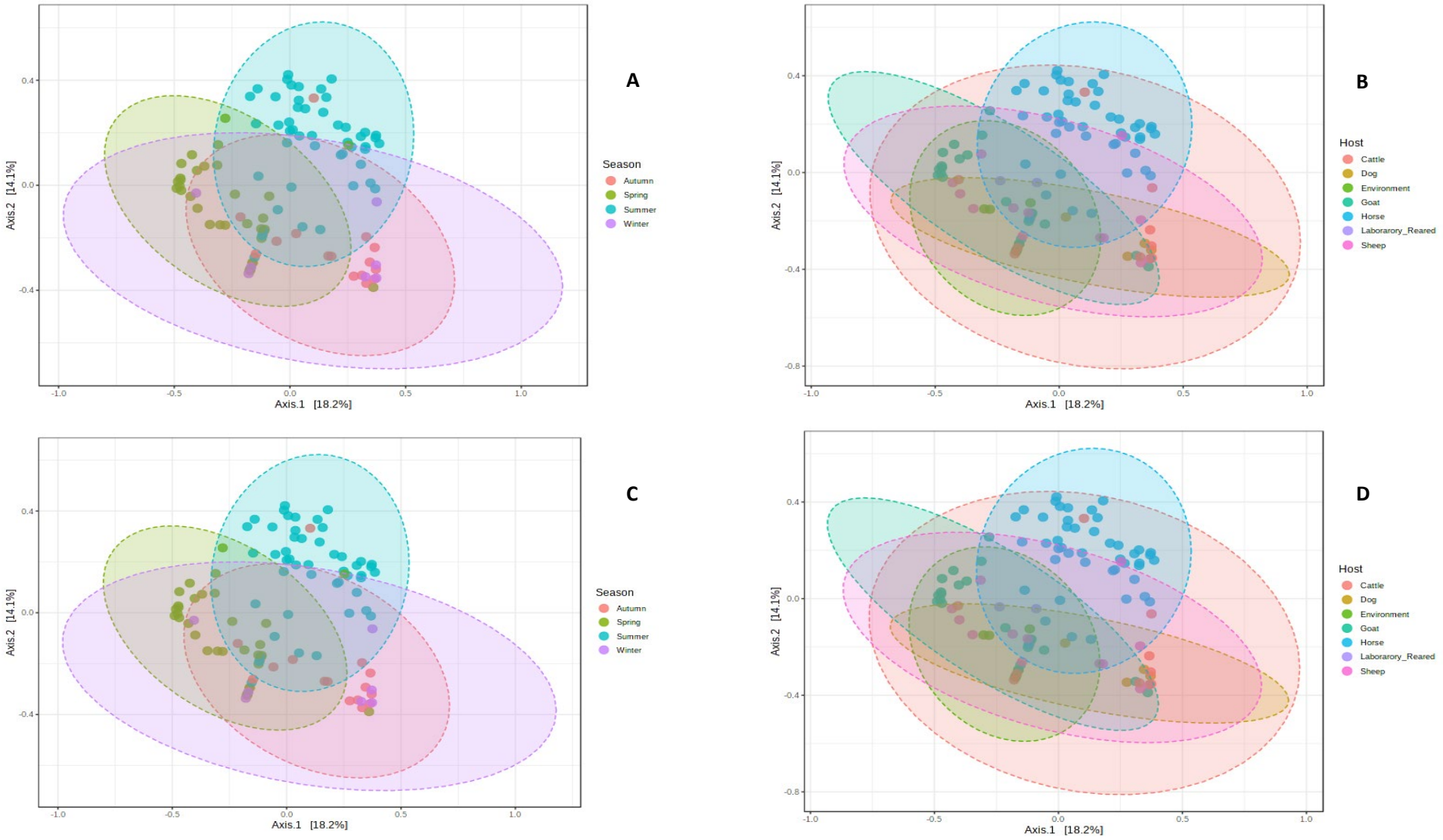


Figure 4.11: *Cont.*

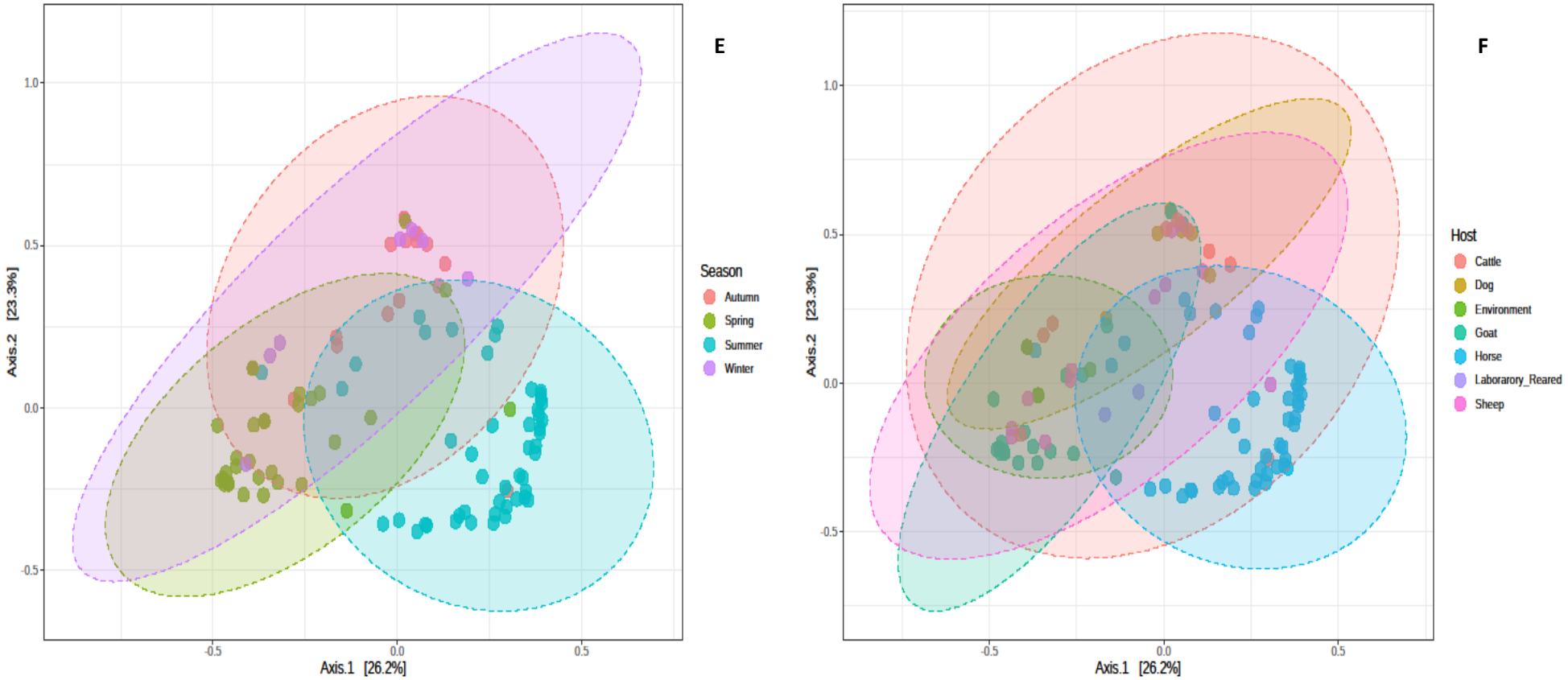


Figure 4.11: Principal coordinate analysis (PCoA) plots based on the Bray-Curtis index distance method; i) using ANOSIM test on the ticks collected in different seasons (**A**) and tick samples collected from different hosts (**B**); ii) using PERMDISP test for for ticks collected in different seasons (**C**) and tick samples collected different hosts (**D**); lastly iii) using Permanaova test for ticks collected in different seasons (E) and samples collected from different hosts (**F**).

Rarefaction curves showed that plateau level was reached in all sampling seasons. Sequences generated in spring, autumn and winter were not more than 30 000 whilst sequences generated in summer were more than 30 000 showing a Good's coverage (plateauing indicates enough sampling depth, which probably is a true representative for each season) (Figure 4.12).

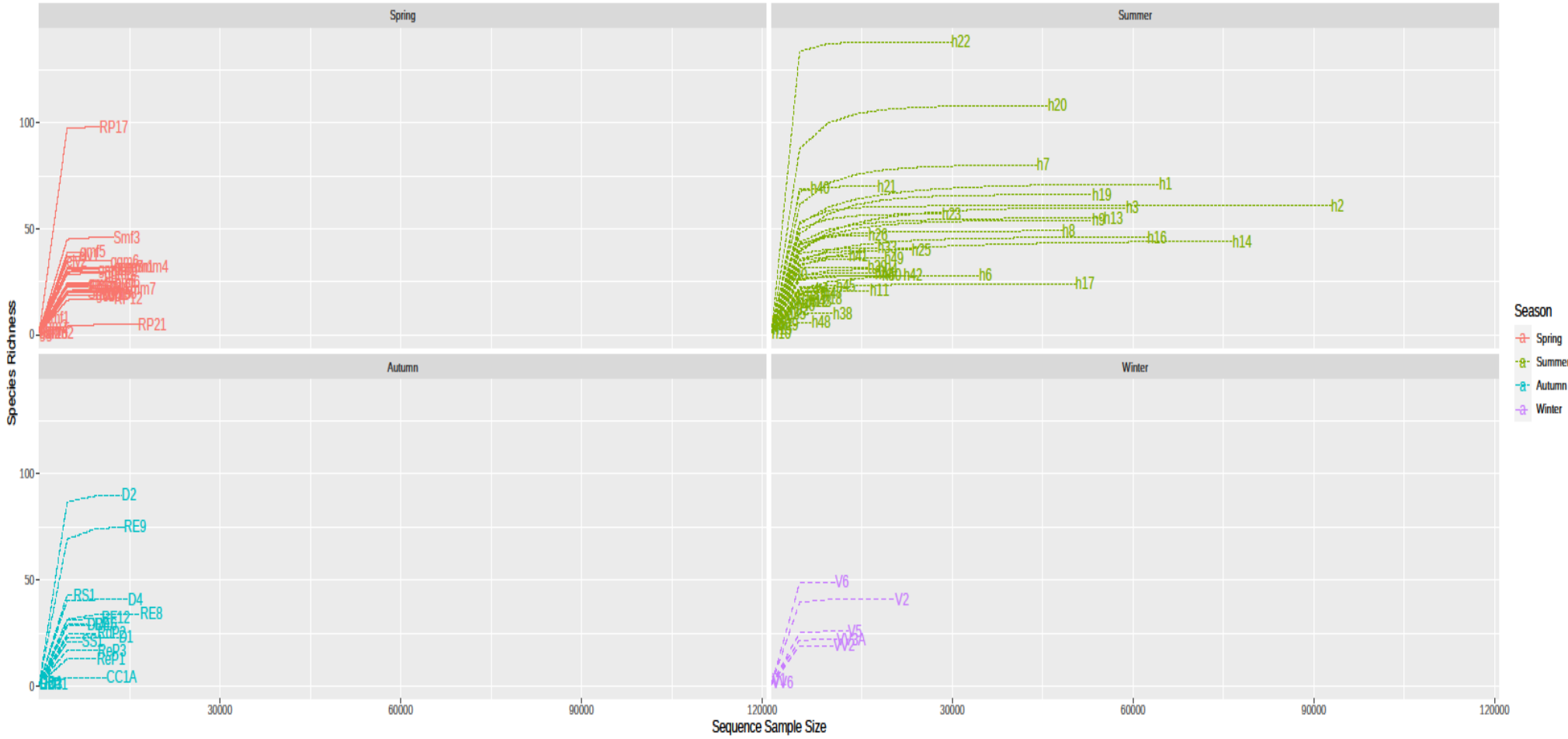


Figure 4.12: Rarefaction curves showing level plateau in all sampling seasons (winter, summer, spring and autumn).

4.3.12 Univariate statistical comparisons

Differential abundance testing between all samples for the different seasons were done by a nonparametric test (Kruskal-Wallis) as it is used in MicrobiomeAnalyst (Dhariwal *et al*., 2017). The result of this analysis clearly indicated significant difference for 7 genera, genus *Family XIII AD3011 group* (*p*-value = 7.183 E-7*; FDR* 2.6649E-6), *Sphingobium* (*p*-value = 4.7198E-6*; FDR* 4.8458E-4), *Staphylococcus* (*p*-value = 4.4676E-6*; FDR* 4.8458E-4), *Brevundimonas* (*p-value* = 5.2246 E-6; *FDR* 4.8458E-4) *Corynebacterium* (*p*-value = 6.4529E-5*, FDR* 0.0047881), *Ehrlichia* (*p*-value = 9.0108E-4*; FDR* 0.0417871) and unnamed genera from the phylum Fusobacteria (*p*-value =1.9423E-4*; FDR* 0.011506) (Figure 3.13). Although the unnamed genus from the phylum *Fusobacteria* lacks formal nomenclature, they were consistently classified at the genus rank by the SILVA database and thus included in the genus-level differential abundance analysis. On the other hand, six genera [*Hafnia-Obesumbacterium* (*p*-value = 2.658E-15; FDR 1.1544E-9), *Globicatella* (*p*-value = 3.2538E-5*;* FDR 4.2221E-2), *Staphylococcus* (*p*-value = 3.263E-4, FDR 1.2106E-24), *Corynebacterium* 1 (*p*-value = 9.155E-17*; FDR* 1.6983E-14), *Tissierella* (*p*-value =*.* 1.2061E-4 *FDR* 0.0014238 and *Escherichia-Shigella* (*p-value* = 1.3109E-4 *FDR* 0.0014737)] showed significant difference when differential abundance test was conducted on bacterium genera obtained from tick samples taken from the different hosts. (Fig 3.14).

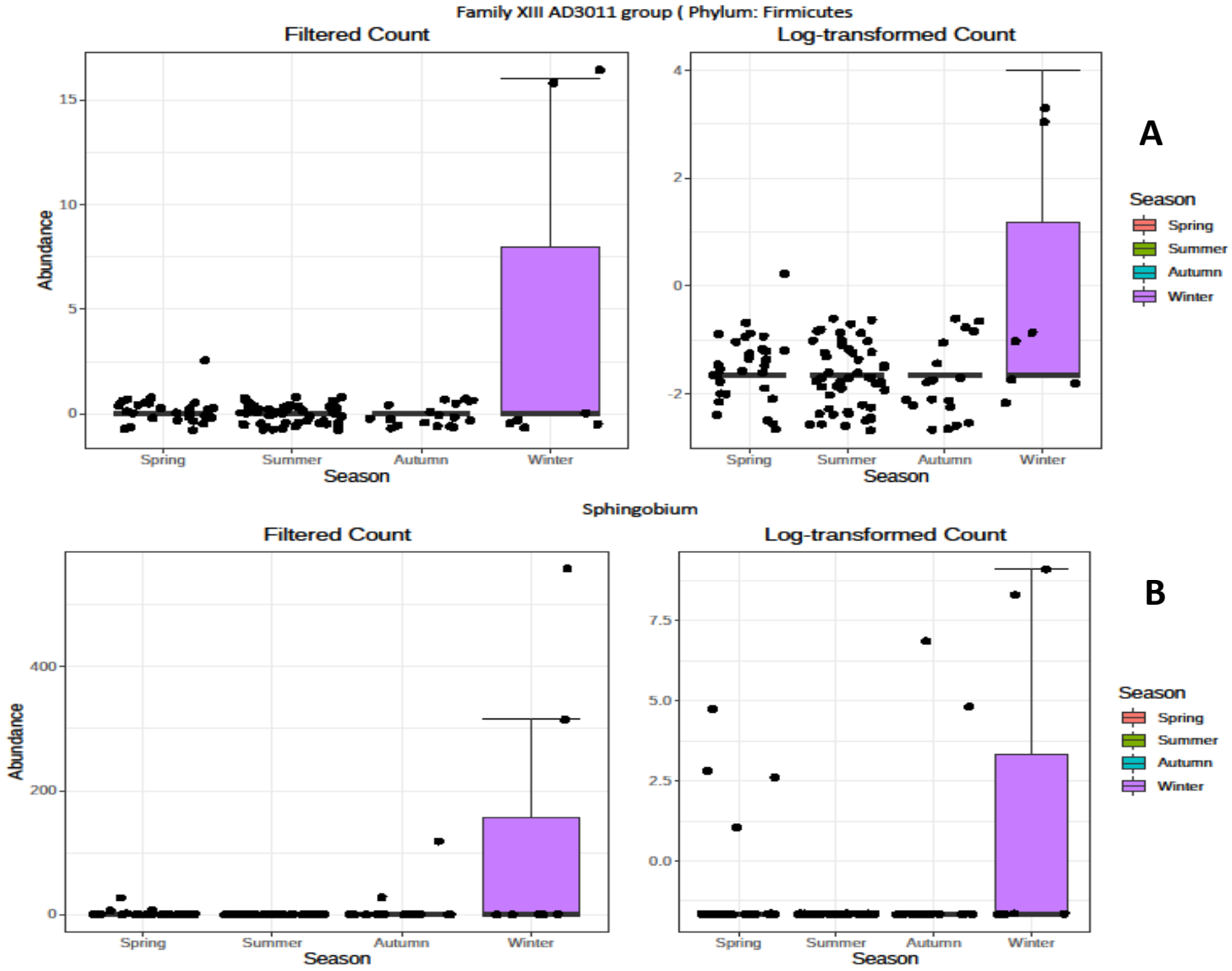


Figure 4.13: *Cont.*

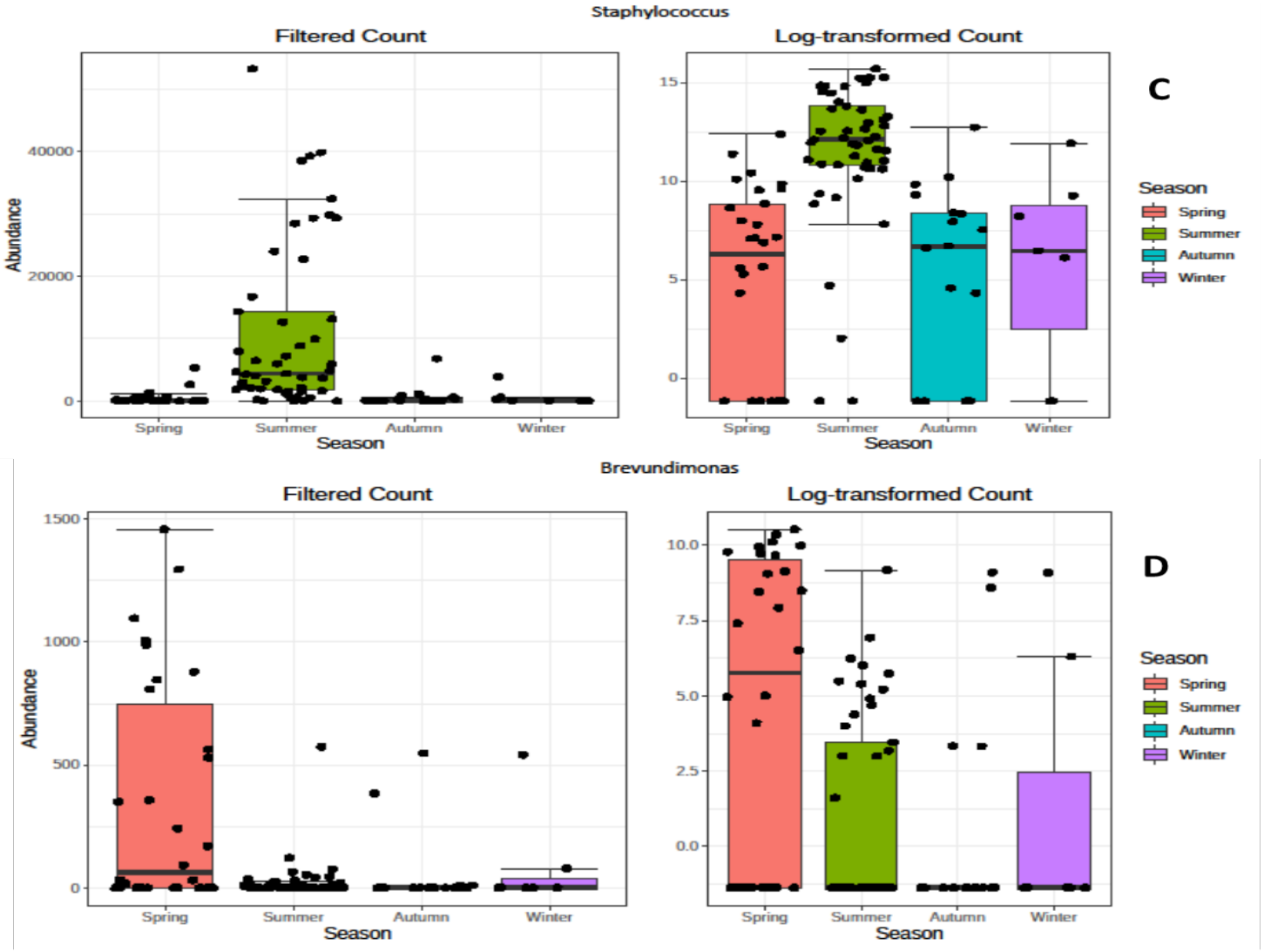


Figure 4.13: *Cont.*

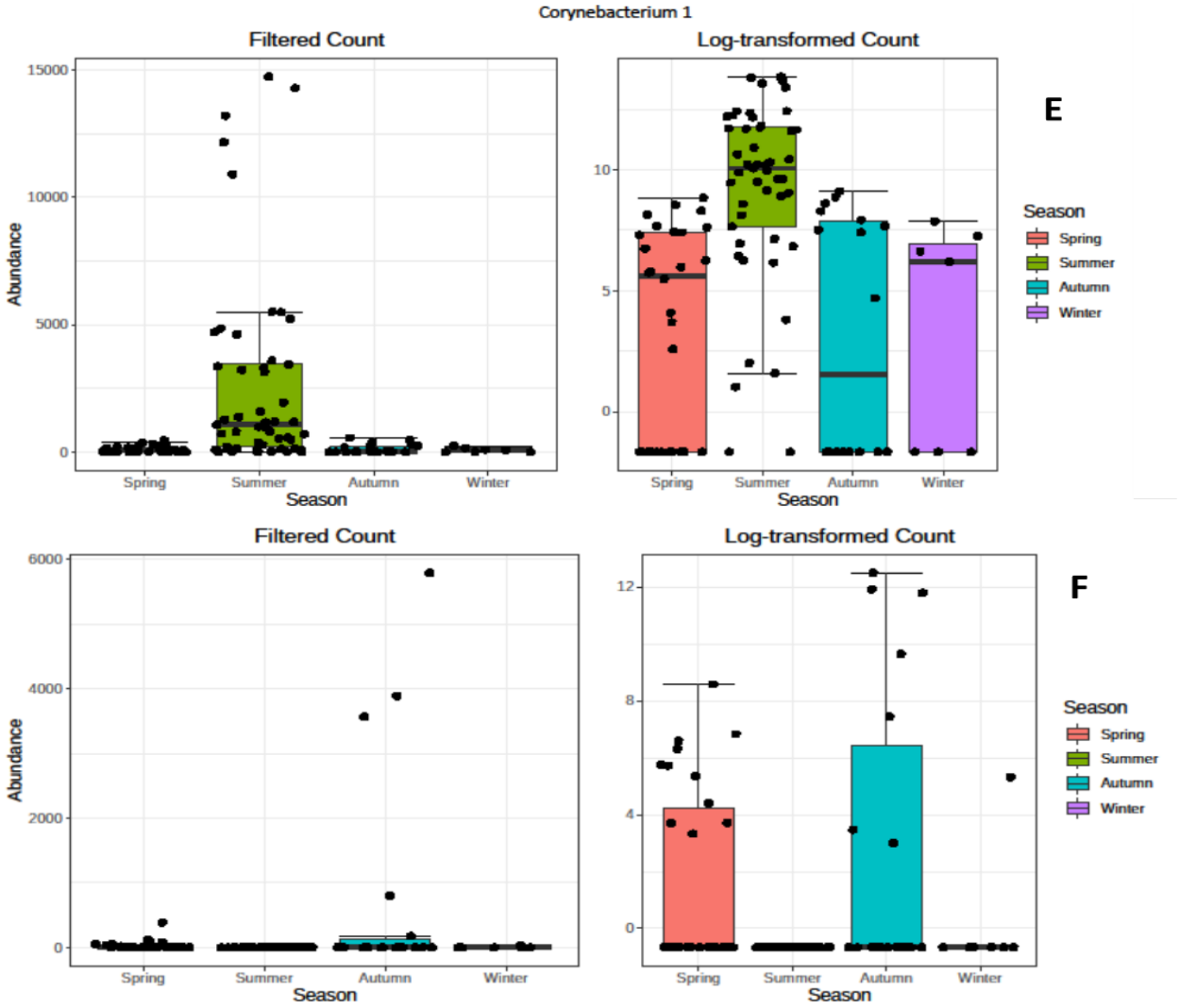


Figure 4.13: *Cont.*

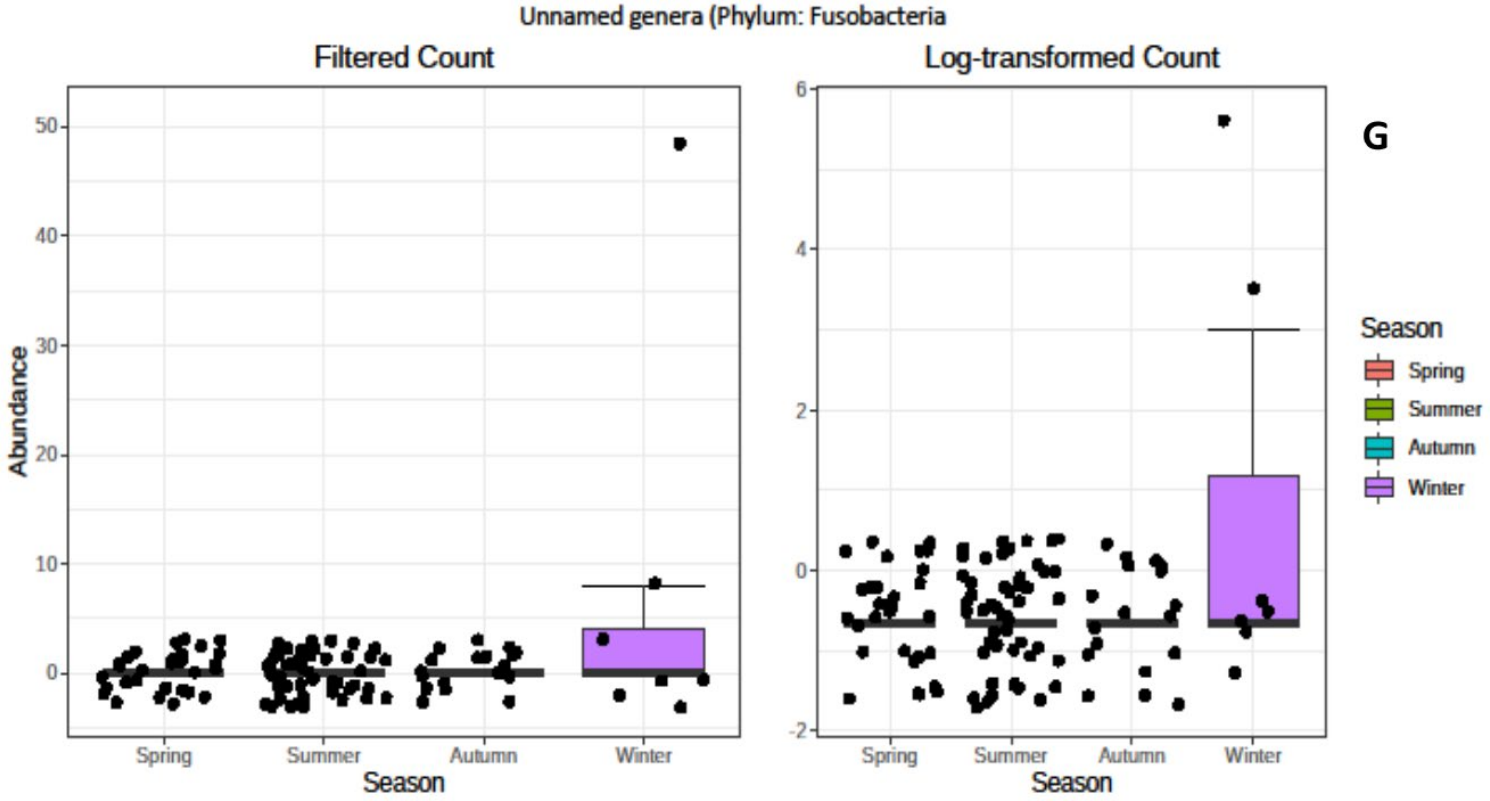


Figure 4.13: Seasonal differential abundance analysis done by a nonparametric test (Kruskal–Wallis test) as it is implemented in Microbiome Analyst (Dhariwal *et al.,* 2017). Differential abundance analysis of the genus (**A**) genus Family *XIII AD3011 group*, (**B**) *Sphingobium*, (**C**) *Staphylococcus*, (**D**) *Brevundimonas*, (**E**) *Corynebacterium*, (**F**) *Ehrlichia* and (**G**) unnamed genera from the phylum Fusobacteria.

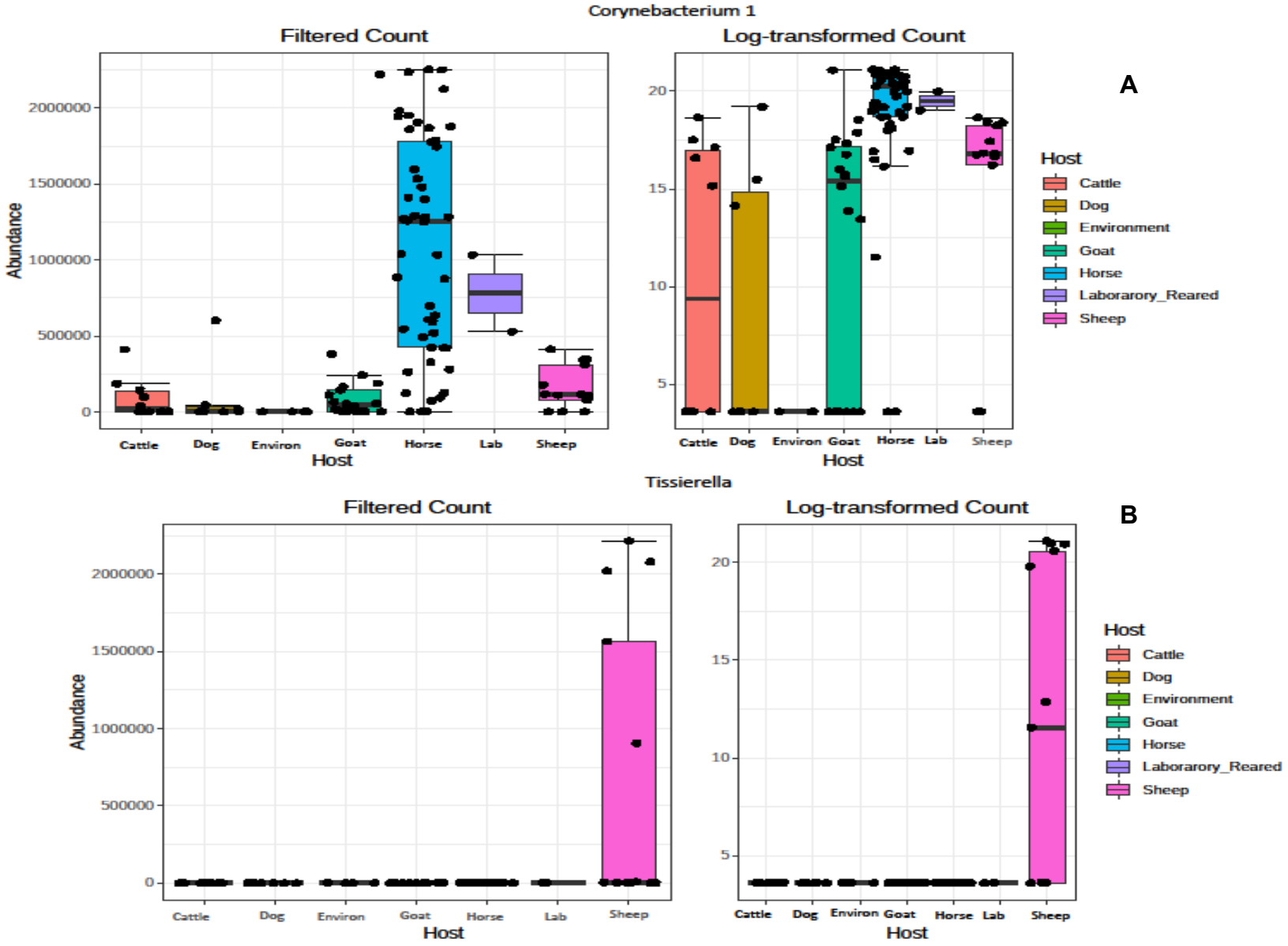


Figure 4.14: *Cont.*

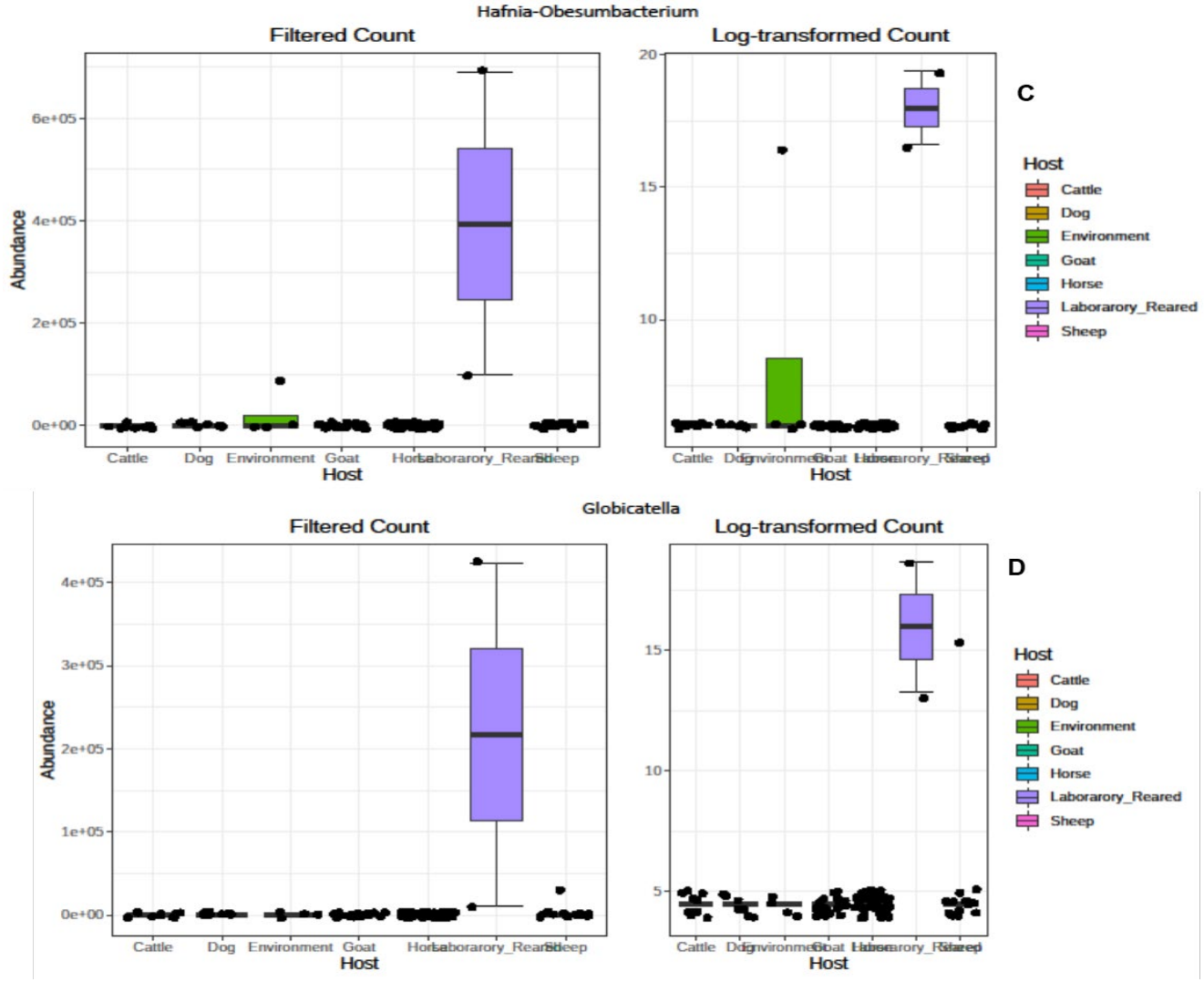


Figure 4.14: *Cont.*

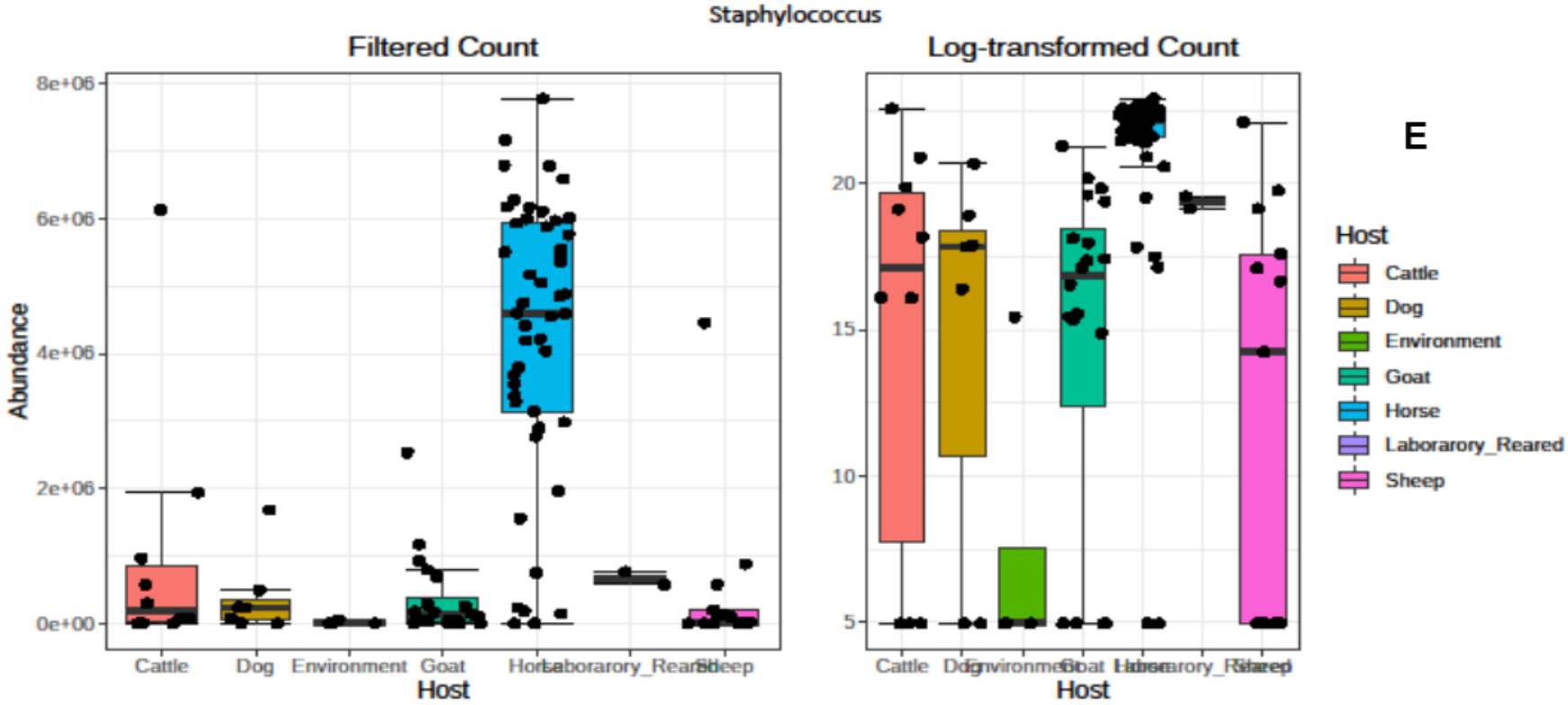


Figure 4.14: Differential abundance analysis done by a nonparametric test (Kruskal-Walli's test) as it is implemented in Microbiome Analyst (Dhariwal *et al*., 2017). Differential abundance analysis of the genus (**A**) *Corynebacterium*, (**B**) *Tissierella*, (**C**) *Hafnia-Obesumbacterium*, (**D**) *Globicatella*, (**E**) *Staphylococcus*.

Table 4.3: Potential pathogenic genera found in the microbiota of *Rhipicephalus* ticks collected from 5 different hosts, laboratory reared and vegetation

GENUS	SIGNIFICANCE	REFERENCE
Coxiella (n=77)	Species from *Coxiella* can cause Query fever (Q-fever) in pets, livestock, and humans	Cooper *et al*., 2011; Mtshali *et al*., 2017,
Anaplasma (n=5)	*Anaplasma* species infect many wild, domestic and humans causing diseases like human granulocytic anaplasmosis, tick-borne fever, canine cyclic thrombocytopenia etc.	Han *et al*., 2019, Yan *et al*., 2022.
Ehrlichia (n=19*)*	*Ehrlichia canis* parasitizes circulating monocytes causing canine monocytic (monocytotropic) ehrlichiosis in dogs. Emerging infections in humans and infection in canines are also caused by *E. chaffeensis* and *E. ewingii.*	Kamesh *et al*., 2005; Peleg *et al*., 2010
Rickettsia (n=8)	Species of *Rickettsia* are known to cause spotted fever, typhus in livestock and human infections characterised by fever, myalgia, rash, lymphadenopathy, and severe systematic manifestations potentially resulting in death.	Parola *et al*., 2013; Colombo *et al*., 2020
Escherichia shigella (n=30)	*Shigella* spp. can cause gastroenteritis in humans, while other species may lead to sepsis and various soft tissue diseases. Symptoms of Shigellosis include diarrhea and/or dysentery with frequent mucoid bloody stools, abdominal cramps, and tenesmus.	Niyogi 2005; Kirecci *et al*., 2015
Staphylococcus (n=82)	Staphylococcal infections may present as folliculitis or as impetigo (udder dermatitis) in cattle, chorioptic mange or contagious pustular dermatitis in goat and Ovine staphylococcal dermatitis in sheep. *Staphylococcus. aureus* causes superficial skin lensions (boils, styes), osteomyelitis, endocarditis, furunculosis. nosocomial, food poisoning, toxic shock syndrome. S, saprophyticus causes urinary tract infections in humans. *Staphylococcus lugdunensis, S haemolyticus, S warneri, S schleiferi* and *S intermedius* are infrequent pathogens.	Foster 1996; Foster 2012; Ghalehnoo 2018
Francisella (n=9)	Species of *Francisella* can cause zoonotic disease tularemia, *F. tularensis* sub sp. *tularensis* (type A) and *holarctica* (type B) can sometimes be mortal in humans.	Petersen *et al*., 2009; Duzlu *et al*., 2016
Pseudomonas (n=65*)*	*Pseudomonas* sp. is one of the pathogens involved in Bovine mastitis in the livestock sector affecting the dairy industry	Thompson and Dahl 2012; Aguayo *et al*., 2020
Streptococcus (n=30)	*Streptococcus* spp cause mastitis in dairy cows while y *Streptococcus equi* causes strangles in horses (a highly pathogenic and contagious infection).	Swerczek 2019; Bochniarz *et al*., 2021
Campylobacter (n=9)	The bacterium causes enzootic infectious infertility in cattle owing to *Campylobacter fetus* subsp. *venerealis* and abortions in sheep, goats, and cattle. *Campylobacter fetus* subsp. *Fetus* and *C. jejuni* Septic abortion, usually epizootic in sheep. Human campylobacteriosis is caused principally by *C. jejuni* and *C. coli*.	Stanley and Jones 2003; Sheppard *et al*., 2009; Sahin *et al*., 2017
Clostridium-sensu_Stricto_1 (n=20)	*Clostridium (C.) botulinum* causes a syndrome of ‘‘chronic’’ or ‘‘visceral’’ botulism involving unspecific clinical symptoms, reduced performance of dairy cows and massive animal losses in the affected herd. *Clostridium perfringens* type E is considered a rare toxinotype and an infrequent cause of enterotoxemia of lambs, calves, and rabbits	Redondo *et al*., 2013; Seyboldt *et al*., 2015;

Table 4.4: Co-infections with various pathogens found in *Rhipicephalus* ticks taken from 5 different hosts, laboratory reared and vegetation

HOST (n=105)	***Coxiella***	***Anaplasma***	***Ehrlichia***	***Rickettsia***	***Escherichia_Shigella***	***Staphylococcus***	***Streptococcus***	***Francisella***	***Pseudomonas***	***Campylobacter***	***Clostridium_sensu_stricto_1***
Cattle	8.6%		1.9%			6.7%	3.8%	1.0%	2.9%	1.0%	1.0%
Goat	8.6%		9.5%		14.3%	14.3%	1.9%		14.3%	1.9%	1.0%
Sheep	6.7%	3.8%	6.7%		4.8%	6.7%	1.0%		6.7%	2.9%	3.8%
Horse	42.9%			7.6%	6.7%	43.8%	19.0%	7.6%	32.4%	2.9%	10.5%
Dog	4.8%					3.8%	1.9%		1.9%		1.0%
Laboratory reared	1.9%				1.9%		1.9%		1.9%		1.0%
Vegetation	1.0%				1.0%	1.0%	1.0%		2.9%		1.0%
TOTAL	**74.5**%	**3.8**%	**18.1**%	**7.6%**	**28.7**%	**76.3**%	**30.5**%	**8.6**%	**63%**	**5.9**%	**19.3**%

Key: n= number of samples

4.4 Discussion

Ticks are vectors for a variety of microorganisms, many of which infest animals and humans. There are seven major genera of ticks in southern Africa. These include ticks from the genera *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *ixodes* and *Rhipicephalus* (Jongejan and Uilenberg 2004); with members of the genus *Rhipicephalus* being the most predominant (Tawana *et al.,* 2022; Makwarela *et al.,* 2023, Lilak *et al*., 2024). Despite the threats they pose, a comprehensive report on microbial communities associated with *Rhipicephalus* ticks infested in livestock and vegetation has not been documented in South Africa. Ticks from this genus feed on a variety of hosts (Mutai *et al*., 2022) and transmit multiple zoonotic pathogens (Kimemia *et al*., 2024), making them a crucial element of One Health.

In the present study, *R. e. evertsi* (67.4%) was the most abundant tick species collected across the four provinces (Limpopo, Gauteng, North West and KwaZulu-Natal) from domestic livestock. *Rhipicephalus e. evertsi* is a two-host tick known to transmit *B. caballi* and *T. equi* causing equine piroplasmosis in horses (Onyiche *et al*., 2019). It also transmits *A. marginale*, the pathogen behind bovine anaplasmosis in cattle (Teshale *et al*., 2016) and members of *Rickettsia* spp. that cause African tick bite fever or spotted fever (Kimemia *et al*., 2024). Ticks from this species are also known to transmit *C. burnetti, a pathogen* that causes Q-fever in livestock (Guo *et al*.,2019; Iweriebor *et al*., 2020; Kimemia *et al*., 2024).

The second most abundant tick species collected in the present study was *R. sanguineus* (22.2%) known to transmit *Rickettsia* sp., *E. canis* and *Coxiella* spp. (Van Wyk *et al.,* 2022), followed by *R. appendiculatus* (9.0%) known to transmit *Theileria parva*, an apicomplexan protozoan parasite that causes the most economically

important and lethal disease of cattle (Obara *et al*., 2020). The number of ticks sampled in the present study were uneven for the different hosts in the different provinces (Table 4.1). This was due to the lack of willingness from the small-scale farmers in allowing tick sampling from their livestock.

This study sought to detect bacterial communities and zoonotic pathogens haboured by *Rhipicephalus* tick species infesting domestic animals (cattle, dogs, sheep, goats, and horses) and ticks questing on vegetation in four provinces (Limpopo, Gauteng, North-West and Kwazulu Natal) of South Africa. High-throughput detection and sequencing technologies are recently highlighted as one of the best methods of pathogen detection, these method complements traditional and molecular methods by improving scalability while maintaining sensitivity (Cabezas Cruz *et al.,* 2018; Greay *et al.,* 2018; Bonnet and Pollet 2021), every technique has its limitation. High-throughput methods face issues like database gaps and species identification constraints (Cabezas Cruz *et al.,* 2018; Hestetun *et al*., 2020). Non-molecular methods remain essential for culturing and identifying unknown bacteria, which can then be sequenced conventionally to improve references (Houpikian and Raoult 2002). The *16S rRNA* Metabarcoding was applied in the current study, and a variety of potential pathogenic and non-patogenic bacteria were identified. Since the *16S rRNA* V3-V4 approach can't distinguish species within genera, to be cautious the genera known to consist pathogenic species were labelled as potentially pathogenic. From the sequences retrieved in this study, there was a total of 689 ASV/OTU's documented, these composed of 27 bacterial phyla; 64 classes; 145 bacterial order, 234 bacterial family and 580 bacterial genera. Similarly, a study conducted in ticks collected from domestic livestock in Kenya identified 27 bacterial phyla (Mutai *et al*., 2022). The dominat phyla detected from ticks collected from the different livestock and vegetation

in the present study included Proteobacteria (30.8%) Firmicutes (18.3%), Actinobacteria (14.9%) and Bacteroidetes (13.8%). In alignment with the findings of the current study, Egan *et al*., (2021) reported Proteobacteria as the most prevalent phylum at 37.3%, followed by Firmicutes at 27.8%, Actinobacteria at 14.7%, and Bacteroidetes at 10.7%. The same order (61.2%; 15.9%; 15.6% and 11.6% respectively) of phyla prevalence was reported by Mutai *et al*., (2022) in ticks collected from domestic livestock in Kenya. The remaining 23 phyla in this study contributed to 22.2% of the overall ASV sequences and each were in low abundance. Differential abundance testing across seasonal groups revealed significance variation in seven bacterial genera: f*amily XIII AD3011 group Sphingobium*, *Staphylococcus*, *Brevundimonas*, *Corynebacterium*, *Ehrlichia* and Unnamed genus from the phylum *Fusobacteria.* Similarly, when comparing bacterial genera across different host species, six genera were found to be differentially abundant, these included *Hafnia-Obesumbacterium, Globicatella, Staphylococcus, Corynebacterium 1, Tissierella and Escherichia-Shigella.*

The alpha diversity showed that there was no statistically significant difference in the variability of microbiota for the different tick species of the same genus sampled from different hosts and in different seasons. A study done in Kenya, found no statistical difference in tick microbiota at different taxonomic levels for the different tick species, suggesting that there is similar distribution of bacterial communities among the different tick genera (Kimemia *et al*., 2024). The same authours suggest that the similarity in the overall bacteriome could be due to the abundance of commensal and endosymbitic bacteria. Previous research suggests that the environment, host type and trans-ovarian factors influences tick microbiome (Pollet *et al.,* 2020). The Beta

diversity test indicated a clear separation of microbiota in samples according to seasons of collection and the different hosts where the ticks were collected (Figure 4.11) contradicting findings by Kimemia *et al.,* (2024). Results from the present study showed significant separation of the tick microbial communities by season and host species using ANOSIM and PERMANOVA statistical tests. Additionally, Significant dispersion of the microbial communities was detected using the PERMDISP test only by season and not by host. These results suggest that seasonal factors (e.g. temperature, humidity, tick life cycle stage, environmental exposure) and Host-related factors (e.g., host immunity, skin microbiota, blood composition etc) may influence microbial community structure in *Rhipicephalus* ticks. However, seasonality introduces greater variability in microbial communities, whereas host-associated differences are more stable and consistent. These findings align with a study on *Ixodes ricinus* ticks collected from vegetation, where seasonal changes were shown to influence microbiome composition (Aivelo *et al*., 2022). Among abiotic factors, temperature has been the most studied environmental variable affecting tick microbiota composition (Thapa *et al*., 2019; Wu-Chuang *et al*., 2021). Experimental studies on *Ixodes scapularis* ticks have demonstrated that temperature can alter the tick bacterial microbiome under controlled laboratory conditions (Thapa *et al*., 2019). Seasonal variations in tick activity (phenology) have been reported to influence pathogen transmission dynamics (Sambodo *et al*., 2024), further emphasizing the need for research on seasonal effects on tick microbiota. Additionally, environmental factors such as vegetation/natural environment and soil have been identified as contributors to tick microbiota composition (Zolnik *et al*., 2016; Pollet *et al*., 2020; Ogden *et al*., 2021; Sambodo *et al*., 2024). Research on the seasonal effects on tick microbiota remains limited and requires further investigation.

Similar to the present study, microbial richness was found to differ significantly among tick species (*Amblyomma auricularium, A. dissimile, A. geayi, A. longirostre, A. mixtum, A. naponense, A. oblongoguttatum, A. ovale, A. pacae, A. sabanerae, A. tapirellum, A. varium, H. juxtakochi, I. affinis,* and *O. puertoricensis*) based on PERMANOVA analysis (Chicana *et al*., 2019; Kueneman *et al*., 2021). This aligns with previous research reporting significant differences in microbial diversity and composition across tick species, including *Ixodes pacificus, Ixodes angustus, Dermacentor variabilis, Dermacentor occidentalis, Dermacentor albipictus,* and *Haemaphysalis leporispalustris* (Chicana *et al*., 2019). Previous studies indicate that microbial diversity in ticks is more pronounced when comparing different developmental stages (Zolnik *et al*., 2018; Chicana *et al*., 2019; Kueneman *et al*., 2021). Future research on the genus *Rhipicephalus* should consider comparisons across different tick life stages, collection sites, or between male and female ticks factors to provide a more comprehensive understanding of bacterial diversity, which could inform the development of effective tick control strategies. The lack of significancant differences in alpha diversity alongside beta diversity may suggest that *Rhipicephalus* ticks harbor a similar number of microbial taxa, regardless of host or season but the composition and relative abundance of those microbes differs significantly across seasons and hosts.

4.4.1 Phylum Proteobacteria

Proteobacteria are the largest phylum within the bacteria domain divided into 6 classes: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria, and Zetaproteobacteria (*Rizzatti et al.,* 2017). Narasimhan and Fikrig (2015) reports the phylum Proteobacteria as the predominant phylum of bacteria across all genospecies of hard ticks. Which was also the case in the present study (Table 4.2) across the three different *Rhipiciphelus* tick species identified except in *R. e. evertsi* ticks collected from horses, were phylum Firmicutes was the most dominant. Three of the above classes were identified in the current studies. These include Alphabateria at 44.3%; Deltaproteobacteria at 11.8% and finaly Gammaproteobacteria at 43.9%.

Amongst the 153 bacteria genera identified under the phylum Proteobacteria, some genera are known to contain potential pathogenic species. These are *Sphingobium*, *Achromobacter*, *Ralstonia*, *Neisseria*, *Proteus*, *Legionella*, *Haemophilus*, *Paracoccus*, *Acinetobacter*, *Moraxella*, *Pseudomonas* and *Escherichia-Shigella*. Several reports indicate the presence of these genera in ticks (Trout Fryxell and DeBruyn 2016; Van Wyk 2019; Rojas-Jaimes *et al.,* 2021; Ergunay *et al*., 2022; Mutai *et al*., 2022; Kimemia *et al*., 2024). Previous research has reported the genera *Acinetobacter*, *Sphingobium* and *Pseudomonas* as soil and environmental microorganisms (Hernández-Jarguín *et al.,* 2018). *Sphingobium paucimobilis* and *S. wittichi* are known to cause infections in immunocompromised patients (Martínez *et al.,* 2011; Moreno-Forero and Van-Der-Meer 2015). In this study the genera *pseudomonas* (50%) and *Acinetobacter* (30%) were most abundant in ticks collected from goats (*R. e. evertsi*) and the environment (*R. appendiculatus*) respectively. Species from these genera includes potentially

pathogenic bacteria that have been reported to show high rates of *antibiotic resistance* (Santajit and Indrawattana 2016). Rojas-Jaimes *et al.,* (2021) reported *pseudomonas* as bacteria involved in infections of soft tissues, these include tissues of the respiratory system. Furthermore, *Paracoccus* are coccobacillary bacterium found in a wide range of ecosystems and these were previously reported as human pathoges (Rojas-Jaimes *et al.,* 2021). Members of the genus Neisseria (*N. meningitidis* and *N. gonorrhoeae*) are agents of bacterial meningitis and gonorrhea (Virji, 2009). Whist species from the genus Proteus (*P. mirabilis*) are reported as causative agents of cystitis and pyelonepthritis in patients with abnormalities of the urinary tract (Rocha *et al*., 2007).

Achromobacter spp. can cause a broad range of infections in hosts with underlying conditions, however, members of this genus are primarily isolated from respiratory people with cystic fibrosis (Isler *et al.,* 2020). These species are naturally resistant to several antibiotics. Members of the genus *Haemophylius* are a common cause of bacterial meningitis, pneumonia, epiglottis, and septic arthritis in humans (Wen *et al*., 2020). Additionally, species from this genus may also act as etiological agents of Chancroid infection and endocarditis (Emmerson *et al.,*1981; Daly, 1985). *Legionella pneumophila* causes human lung infections resulting in severe pneumonia (Sharaby *et al*., 2019). Whist members of *Moraxella* species are known to cause rare cases of infective endocarditis (Loannou *et al., 2022*).

Members of the *Ralstonia* genus (*R. pickettii*; *R. insidiosa* and *R. mannitolilytica)* cause infections such as osteomyelitis and meningitis (Ryan and Adley 2014). Species from the genus *Escherichia,* may contain different strains of *E. coli* that can provide health benefits (*E. coli* strain Nissle 1917- Probiotic) or produce deadly diseases (*E. coli* strain O157:H7 -deadly foodborne pathogen) (Cabezas-Cruz *et al.,* 2018).

The genera *Coxiella*, *Rickettsia*, *Francisella* are known to consist of tick endosymbionts providing nutrients to ticks missing in bloodmeals and are believed to be essential in the survival of ticks (Narasimhan and Fikrig 2015; Smith *et al.,* 2015; Gerhart *et al.,* 2016, Chicana *et al*., 2019, Hoffmann *et al.,* 2021). Furthermore, members of the genera *Rickettsiae*, *Coxiella, Francisella, Ehrlichia* and *Anaplasma* detected in the present study are known to contain potential tick-borne zoonotic pathogens. *Coxiella* was the most abundant genera in *R. e. evertsi* ticks collected from cattle (55%), Sheep (19%) and *R. sanguineus* ticks collected from dogs (71%) (Table 4.2), similar to previous studies (Trout Fryxell and DeBruyn 2016, Khoo *et al*., 2016; Zhang *et al.,* 2019; *Mutai et al.,* 2022). *Coxiella* species, particularly *C. burnetti* is known as an agent of Q-fever in livestock (Kimemia *et al.,* 2024). In the present study, *Rickettsia* was identified in *R. e. evertsi* ticks collected from horses in low abundance at <1%. (Appendix 8.6) unlike in a previous study where *Rickettsia* was reported in all sampled ticks (*Amblyomma gemma*, *A. hebraeume*, *A. variegatum*, *Amblyomma* spp., *Hyalomma truncatum*, *Hyalomma* spp., *R. annulatus*, *R. appendiculatus* and *R. pulchellus*) even though the abundance was low (Mutai *et al.,* 2022). Spotted fever group of *Rickettsia* spp have been detected in horses from Central Italy (Ebani 2019). *Francisella* was present in *R. e. evertsi* ticks collected from cattle (5%) and horses at <1%. Members from this genus are Intracellular bacterial pathogens causing tularemia (Hernández-Jarguín *et al.,* 2018). Similarly, members of the genera *Anaplasma* and *Ehrlichia* were reported in relatively low abundance at <1% just like in previous studies (Trout Fryxell and DeBruyn 2016; Mutai *et al.,* 2022). *Ehrlichia* consist of *E. ruminantium* causing heartwater diseases in cattle (Guo *et al*., 2019) or *E. canis*, causing ehrlichiosis in dogs (van Wyk *et al*., 2022). The genus *Anaplasma* may contain tick born pathogens like *A. phagocytophilum*, associated with human and canine

granulocytic anaplasmosis (Mtshali *et al.,* 2017). We cannot say with certainty that the tick-borne pathogen mentioned above contained pathogenic species because of the limitation of the *16S rRNA* (targeting V3-V4 gene region) in resolving taxonomy to species level using Next-generation sequencing (Cabezas-Cruz *et al*., 2018) hence the use of the term “potential” pathogen. More commensal bacteria genera in this study worth noting are *Enhydrobacter*, *Aquabacterium*, *Acidovorax* and *Bradyrhizobium.*

4.4.2 Phylum Firmicutes

The second most abundant phylum in the current study were Firmicutes at 18.3% with 102 bacterial genera. This phylum consisting of Gram- positive bacteria (Mutai *et al.,* 2022) that includes important pathogens that are found in different environments. Pathogenic members of the phylum Firmicutes detected includes *Enterococcus*; *Lactobacillus*; *Streptococcus*; *Aerococcus*; *Clostridium sensu stricto* 1,13,18, and 8; *Staphylococcus*. *Clostridium* is a broad genus of anaerobic, spore-forming, rod-shaped, gram-positive bacteria found in different environments worldwide (Cook *et al*., 2001). Diseases caused by pathogenic members of this genus include botulism, tetanus, enterotoxemia, gas gangrene, necrotic enteritis, pseudomembranous colitis blackleg and black disease (Zaragoza *et al.,* 2019). The genera *Enterococcus* and *Staphylococcus* includes potentially pathogenic bacteria that have been reported to show high rates of antibiotic resistance (Santajit and Indrawattana 2016). Members of the genus *Staphylococcus* and *Enterococcus* are oppotunistic commensal pathogens of skin and mucosal surfaces causing septicemia, food poisoning and meningitis, pneumonia respectively (Hernández-Jarguín *et al.,* 2018). Furthermore, *Staphylococcus* infects soft tissues in the gut of *R. microplus* ticks (Andreotti *et al.,*

2011). *Streptococcus* spp. and *Staphylococcus* spp. are pathogens that cause mastitis in ewe milk reducing production (Knuth *et al*., 2022). These genera are major pathogens of mastitis in livestock and may survive in the environment where livestock is kept.

4.4.3 Phylum Actinobacteria

In this study, Actinobacteria was the third most abundant phylum and contained 14.9% of the bacterial community with 80 bacterial genera detected. This phylum consists of Gram-positive bacteria with high G+C DNA content distributed in both aquatic and terrestrial ecosystems (Barka *et al*., 2016). According to Shivlata and Satyanarayana (2015), microbes in the phylum Actinobacteria are prolific producers of antibiotics, bioactive compounds with clinical applications, and enzymes important for industrial processes. Veterinary, environmental and medically important bacterial genera from the phylum Actinobacteria detected in the current studies include *Streptomyces*, *Micrococcus*, *Rhodococcus*, *Mycobacterium* and *Corynebacterium*. Members of Corynebacteria (*C. diphtheria*) are known to cause human infections and are distributed in nature as part of the animal and human microbiota (Barka *et al*., 2016). *Corynebacterium* species can cause osteomyelitis or produce the diptheria toxin (Wong *et al*., 2010). Members of the genus *Streptomyces* are a prominent source of secondary metabolites, known to produce more than 50% of total Known microbial antibiotics (Shivlata and Satyanarayana 2015), they are obligate chemoautotrophs. Williams *et al.,* (2007) and Zhang *et al.,* (2010) documented the species from the genus *Microbacterium* (*M. maritypicum* and *M. radiodurans* GIMN 1.002T) as alkalitolerant and radiotolerant actinobacteria. *Micrococcus* and *Streptomyces species play a role* in the composting process, a a self-heating, aerobic biodegradation that

supplies humus and nutrients to soil (Rawat and Johri 2013). Members of the genus *Rhodococcus* form part of the indigenous microbial population of Icelandic rocks (Cockell *et al.,* 2013). Their presence in Icelandic rocks suggests that *Rhodococcus* species are environmentally resilient and capable of colonizing diverse habitats. The detection of these bacteria in both the Icelandic environment and South Africa (in the present study), despite differing environmental conditions, further supports their adaptability and broad ecological distribution. One species from this genus has been reported to cause pheumonia in animals (Hernández-Jarguín *et al.,* 2018). Members of the genera *Micrococcus* and *Mycobacterium* may cause human infections, the later causes diseases like leprosy and tuberculosis (Van Wyk 2019).

4.4.4 Phylum Bacteroidetes

Bacteroidetes was the fourth dominant phylum constituting 13.8% of the ASV'S or microbiome. In this phylum, 61 bacterial genera were detected. This phylum consists of Gram-negative bacteria that are found in diverse habitats known to play a role in the degradation of organic matter (Mutai *et al.,* 2022). Porphyromonas genus identified in the present study consist of potentially pathogenic species. *Porphyromonas gingivalis* is a Gram-negative anaerobic microorganism present in the oral cavity, contributing to the pathogenesis of periodontitis and residing among a diverse community of more than 500 bacterial species in the mouth (Mysak *et al*., 2014). Bay *et al*., (2018) Reported the presence of *Porphyromonas* spp. in cattles interdigital hyperplasia with signs of interdigital dermatitis, interdigital phlegmon, complicated sole ulcers, complicated toe ulcers lesions, and complicated white line lesions. Another genus detected worth noting is *Cloacibacterium*, members of this genus are known to be potential human pathogens (Rojas-Jaimes, *et al*., 2021). Bacteria from this genus

proliferate in aquous environments with high content of organid matter (Gay *et al*., 2016).

4.4.5 Other detected significant phyla

Of the 23 remaining phyla, these include the phyla Epsilonbacteraeota, Fusobacteria and Chloroflexi. Members of the Epsilonbacteraeota phylum were reported and in low abundance. The bacteria genus of pathogenic potential detected in this phylum is *Camphylobacter*. According to Costa and Iraola (2019), *Campylobacter* ranks among the top four causes of gastroineritis worldwide and has increased in both developed and developing countries with many reported infections attributed to *Campylobacter jejuni* and, to a lesser extent, *C. coli.* The *Campylobacter* genera are haboured in gastrointestinal tract of domestic animals (cattle, sheep, pigs and paultry), and human infections can be induced by consumption of meat, milk, vegetables, and water (Shange *et al.,* 2019). The presence of *Campylobacter* in *Rhipicephalus* ticks has not been explored.

The phylum Fusobacteria was detected in *Rhipicphalus* tick samples from the current study. Among the recovered genera, *Fusobacterium* was identified as a member with pathogenic potential. According to Brook (2015), Fusobacteria are strict anaerobic, gram-negative, thin, long, filamentous, nonmotile, and nonsporulating bacteria, with clinically significant species including *Fusobacterium necrophorum*, *F. nucleatum*, *F. gonidiaformans*, *F. naviforme*, *F. mortiferum*, and *F. varium*. The *F. nucleatum* is linked to various human dysbiotic diseases and cancers, this species is further subdivided into four subspecies: *F. nucleatum* subspecies *nucleatum* (*Fn. nucleatum*), *animalis* (*Fn. animalis*), *polymorphum* (*Fn. polymorphum*), and *vincentii/fusiforme* (*Fn.*

vincentii) (Krieger *et al*., 2023). Fusobacteria has been identified in stray dogs infested with ticks in Northern Mexico (García *et al*., 2024).

Chloroflexi, once known as 'green non-sulfur bacteria', is a diverse group found in various aquatic and terrestrial environments, including filamentous photosynthetic bacteria (*Chloroflexales*), oxygen-using bacteria that feed on organic matter (*Herpetosiphonales*), and heat-loving bacteria that consume organic compounds (Thermomicrobia) (Campbell *et al*., 2014). This phylum is known to contain several uncultured bacteria (Yokota, 2012) and in the current study uncultured *clostridium* sp. was detected as a potential pathogenic bacterium genus. These findings need further investigation, as bacteria from the genus *Clostridium* were detected again in the phylum Firmicutes (this study). The presence of uncultured *clostridium* sp. in the phylum Chloroflexi does not align with previous research (Yakota, 2012, Campbell *et al*., 2014 and Speirs *et al*., 2019) the reason for this could be database error.

The current study used whole ticks to investigate the overall tick microbiota of ticks from the *Rhipicephalus* genus haboured by domestic animals, similar to previous studies (*Andreotti et al*. 2011; Lalzar *et al*., 2012; Nakau *et al.,*2013; Vayssier-Taussat *et al*., 2013; Narasimhan *et al*., 2014; Ponnusamy *et al*., 2014;). Lastly, ticks from the current study, collected from various hosts, exhibited evidence of zoonotic pathogen co-infections (Table 4.4). This finding is consistent with a study on Lone Star ticks, where the ticks were found to be infected with two or more pathogens (Trout Fryxell and DeBruyn 2016).

CHAPTER 5

Molecular detection of zoonotic pathogens from *Rhipicephalus* ticks collected from domestic animals

5.1 Introduction

South African livestock economy like any other agricultural sector in the tropics and suptropics is affected greatly by ticks and tick-borne diseases (Boucher *et al*., 2020; Githaka *et al*., 2022). Heavy infestations of ticks on livestock and transmission of tick-borne deases is a serious concern (Spickett *et al*., 2011). Tick bites lead to blood loss and damage to skin and teats resulting in development of wounds which predisposes animals to secondary bacterial infection and development of disease (Moyo and Masika, 2009). Ticks affect dairy and beef cattle, as well as small ruminats (Boucher *et al*., 2020). Ticks are not just a threat to livestock and wild animals, 558 ixodid ticks feeding on 194 humans were reported in a study conducted in South Africa (Horak *et al*., 2002). Crimean-Congo haemorrhagic fever virus was reported in South African ticks, cattle and humans (Msimang *et al*., 2021). Human cases of tick bites and pathogen transmission are reported annually in South Africa, with a 25% case fatality rate since the first case report in 1981 (Msimang *et al*., 2021).

Van den Heever *et al*., (2022) reported a total economic impact of ZAR 1 266 million losses caused by heartwater disease on South African livestock industry per annum. Heartwarter is an acute, often fatal, non-contagious tickborne disease of domestic ruminants transmitted by *Amblyomma* ticks (Dinkisa, 2018). Cowdriosis or heartwater disease is known as the most important tick-borne disease in South Africa (Spickett *et al*., 2011) contrary to a report by de Waal, (2000) that suggested anaplasmosis as the

most widely spread disease in South Africa with more than 99% of the total cattle being at risk. Ticks were reported to cost the South African agriculture approximately ZAR 500 million per annum in direct and indirect losses in 1994. (Randela, 2007). InTanzania, a total annual economic loss caused by tick-borne diseases in cattle was estimated to be 364 million US dollars (Kivaria, 2006).

Acaricide resistance has been reported in several *Riphicephalus* tick species in Africa including *R. decoloratus* (Mekonnen *et al*., 2002; Vudriko *et al*., 2016), *R. microplus* (Robbertse *et al*., 2016), *R. geigyi* (Adehan *et al*., 2016) *R. appendiculatus* (Vudriko *et al*., 2016), *R. e. evertsi* and *R. microplus* (Lynen *et al*., 2008, Silatsa *et al*., 2019) and *R. annulatus* (El Ashram *et al*., 2019; Arafa *et al*., 2021b). This is a challenge because some tick species (*R. e. evertsi, R. appendiculatus, R. decoloratus)* listed above are generalists (Horak *et al*., 2018) capable of host switching in their nympthal and adult stage, encouraging disease transmission.

Ticks from the *Rhipicephalus* genus have been listed as part of the most dominant ticks requiring control in African farms in the SADC region (Tawana *et al*., 2022). These include *R. (B.) decoloratus*, *R. (B.) microplus*, known to transmit *Babesia bigemina* and *B. bovis* causing babesiosis and *Anaplasma marginale* causing anaplasmosis; *R. appendiculatus* which transmit *Theileria parva*, the cause of east coast fever (Githaka *et al*., 2022). Similarly, five tick species from the genus *Rhipicephalus; R. (B). decoloratus*, *R. e. evertsi*, *R. follis, R. getrudae* and *R. warburtoni*) were reported on cattle, goats and sheep form the eastern Free state (Mbati *et al*., 2002).

Tick species from the genus *Rhipicephalus* seem to appear in almost every study in africa investigating tick-borne diseases in livestock (Wesonga *et al*., 2010; Lutomiah *et al*., 2014; Ombondi *et al*., 2017; Boucher *et al*., 2020, Tawana *et al*., 2022). The distribution, host preference and role in diseases transmission of these ticks in livestock is quite intriguing and inspired the current study. The objective of this study was to identify zoonotic pathogens detected in *Rhipicephalus* ticks to species level from metabarcoding data generated in Chapter 4.

5.2 Materials and methods

5.2.1 Sampling of *Rhipicephalus* tick species

The samples utilized in Chapter 4 were also employed in the present chapter.

5.2.2 DNA extraction of *Rhipicephalus* tick species

DNA was extracted using the Qiagen DNeasy Blood and Tissue kit following the manufacturer's protocol (Qiagen Hilden, Germany). A detailed protocol for the extraction of DNA is similar to that describes in Chapter 3, section 3.2.4. The extracted DNA was then stored in a -20 freezer until used.

5.2.3 Detection of zoonotic pathogens

5.2.3.1 Detection of *Coxiella* species

A nested PCR assay was conducted targeting the *16S rRNA* and *23S rRNA* gene regions in order to confirm the species of *Coxiella* harboured by *Rhipicephalus* ticks collected in this study. The primers used for amplification of the *16S RNA* gene were

Cox-16SF1/ Cox-16SR2 for the first PCR and Cox-16SF1/ Cox-16SR2 for the second PCR (Table 5.1). For the *23S rRNA* gene, the primer set Cox-23SF1/ Cox-23SR2 was used in the first PCR, while the second PCR was performed using Cox-23SF2/ Cox-23SR1 (Table 5.1). The total volume of 25 µl of the PCR assay was used for the first reaction. This contained 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water and 2 µl of template DNA. The second PCR contained 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water and 2 µl of the 1st PCR product. The PCR conditions for both reactions were that of the mastermix with initial denaturation step of 95°C for 10 minutes; followed by 35 cycles denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds and extension at 72°C for 60 seconds, and the final extension at 72°C for 7 minutes with a final hold at 4°C - ∞. The positive products of nested PCR were purified and sequenced in both direction by Inqaba Biotechnical Industries (Pty) Ltd in Pretoria, South Africa.

Additionally, A PCR assay was conducted targeting the *IS1111 transposon* gene region using primer set *trans-1/trans-2*. A total volume of 25 µl of PCR assay contained 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water and 2 µl of template DNA. The PCR conditions used were that of the mastermix with initial denaturation step of 95°C for 10 minutes; followed by 35 cycles denaturation at 95°C for 30 seconds, annealing at 61°C for 30 seconds and extension at 72°C for 60 seconds, and the final extension at 72°C for 7 minutes with a final hold at 4°C - ∞.

5.2.3.2 Detection of *Rickettsia* species

To confirm the species of *Rickettsia* detected from *Rhipicephalus* ticks collected in this study, A PCR assay was performed to amplify the outer membrane protein A (*OmpA*) gene using primer set Rr190.70p/Rr190.701n and the citrate synthase gene (*gltA*) using the primer set Rp877p/Rp128n. A total volume of 25 µl of PCR assay contained 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water and 2 µl of template DNA. The PCR conditions used were that of the mastermix with initial denaturation step of 95°C for 10 minutes; followed by 35 cycles denaturation at 95°C for 30 seconds, annealing at 46°C for 30 seconds (*OmpA* gene) and extension at 72°C for 60 seconds, and the final extension at 72°C for 7 minutes with a final hold at 4°C - ∞. The annealing temperature for the *gltA* gene was 48°C for 30 seconds. The positive products of PCR were purified and sequenced in both direction by Inqaba Biotechnical Industries (Pty) Ltd in Pretoria, South Africa.

5.2.3.3 Detection of *Escherichia-Shigella*

To confirm the species of *Escherichia-Shigella* detected from *Rhipicephalus* ticks collected in this study, A PCR assay was conducted targeting the enzyme beta-glucuronidase (*uidA*) gene region using primer set uidA-F/uidA-R. A total volume of 25 µl of PCR assay contained 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water and 2 µl of template DNA. The PCR conditions used were that of the mastermix with initial denaturation step of 95°C for 10 minutes; followed by 35 cycles denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds and extension at 72°C for 60 seconds, and the final extension at 72°C for 7 minutes with a final hold at 4°C - ∞. The positive products of PCR were purified and sequenced in both direction by Inqaba Biotechnical Industries (Pty) Ltd in Pretoria, South Africa.

5.2.3.4 Detection of *Anaplasma* species

A nested PCR assay was conducted targeting the *16S rRNA* gene region for confirmation of the species of *Anaplasma* detected from *Rhipicephalus* ticks collected in this study. A total volume of 25 µl of PCR assay contained 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water and 2 µl of template DNA. The first round of PCR used external primer set EE-1/EE-2 (Barlough *et al*., 1996; Han *et al*., 2019, Yan *et al*., 2022). The PCR conditions used were that of the mastermix with initial denaturation step of 95°C for 10 minutes; followed by 35 cycles denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 60 seconds, and the final extension at 72°C for 7 minutes with a final hold at 4°C - ∞.

The second internal PCR was performed using a species-specific primerset (MSP45/MSP43) to amplify the for *Anaplasma ovis* target sequence. Additionally, a separate second PCR was conducted using the primer set SSAP2 F/SSAP2 R to amplify the *Anaplasma phygocytophilum* target sequence (Han *et al*., 2019; Yan *et al*., 2022). Each PCR reaction contained 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water and 2 µl of the 1st PCR product. The PCR conditions used were that of the mastermix with an annealing temperature of 60 ℃ for *A. ovis* and an annealing temperature of 55 ℃ for *A. phagocytophilum*. The positive products of the nested PCR were purified and sequenced in both direction by Inqaba Biotechnical Industries (Pty) Ltd in Pretoria, South Africa.

5.2.3.5 Detection of *Ehrlichia* species

To confirm the *Ehrlichia* species detected in *Rhipicephalus* ticks collected in this study, three conventional PCR assays were performed. The first PCR was conducted using the primer set Ehr 521/Ehr 747 (Welc-Faleciak *et al*., 2009, Mtshali *et al*., 2017) which was previously developed to amplify a variable region of the *16S rRNA* gene specific for *E. equi*, *E. phagocytophila* and the human Granulocytic ehrlichiosis (HGE) agent (Hodzic *et al*., 1998), An additional PCR targeted the V1 hypervariable region of the *16S SSU rRNA* gene using the primer set Ehr-F/Ehr-R biotin (Matjila *et al*., 2008). The last PCR conducted used species-specific primers Ec 16S forward/Ec 16S reverse (Mtshali *et al*., 2017) targeting the *E. canis 16S rRNA* gene region (Peleg *et al*., 2010. The above PCR assays were conducted in a total volume of 25 µl containing 125 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water and 4 µl

of template DNA. The PCR conditions used were that of the mastermix with initial denaturation step of 95°C for 10 minutes; followed by 40 cycles denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 60 seconds, and the final extension at 72°C for 7 minutes with a final hold at 4°C - ∞. The positive products of PCR were purified and sequenced in both direction by Inqaba Biotechnical Industries (Pty) Ltd in Pretoria, South Africa.

5.2.3.6 Detection of *Francisella* species

To confirm the species of *Francisella* detected from *Rhipicephalus* ticks collected in this study, conventional PCR assay was conducted targeting the *16S rRNA* gene region using the primer set Fr153F01 and Fr1281R01 (Duzlu *et al*., 2016, Ghoneim *et al*., 2017), An additional PCR was conducted using primerset *SdhF*/*SdhR* (Barns *et al*., 2005). A total volume of 25 µl was prepared for the different PCR reactions, each reaction contained 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water and 2 µl of template DNA. The PCR conditions used were that of the mastermix with initial denaturation step of 95°C for 10 minutes; Followed by 40 cycles denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 60 seconds, and the final extension at 72°C for 7 minutes with a final hold at 4°C - ∞. The annealing temperature for the 2nd primer set was 56°C. The positive products of PCR were purified and sequenced in both direction by Inqaba Biotechnical Industries (Pty) Ltd in Pretoria, South Africa.

5.2.3.7 Detection of *Staphylococcus* species

To confirm the species of *Staphylococcus* detected from *Rhipicephalus* ticks collected in this study, conventional PCR assay was conducted targeting the *16S rRNA* gene region using the *primerset 16S1* and *16S2* (Poulsen *et al*., 2003), an additional PCR was conducted using primer set SSU-bact-27f/SSU-bact-907r (Becker *et al*., 2004; Park *et al*., 2011). A total volume of 25 µl was prepared for the different PCR reactions, each reaction contained 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water and 2 µl of template DNA. The PCR conditions used were that of the mastermix with initial denaturation step of 95°C for 10 minutes; followed by 35 cycles denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 60 seconds, and the final extension at 72°C for 7 minutes with a final hold at 4°C - ∞. The annealing temperature for the 2nd primer set was 53°C. The positive products of PCR were purified and sequenced in both direction by Inqaba Biotechnical Industries (Pty) Ltd in Pretoria, South Africa.

5.2.3.8 Detection of *Clostridium* species

To confirm the species of *Clostridium* detected from *Rhipicephalus* ticks collected in this study, conventional PCR assay was conducted targeting the *triosephosphate isomerase* (*tpi*) gene using the primer set tpiA and tpiB (Dhalluin *et al.,* 2003), an additional PCR was conducted using primer set 16SA and 16SB (Dhalluin *et al*., 2003). A total volume of 25 µl was prepared for the different PCR reactions, each reaction contained 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water

and 2 µl of template DNA. The PCR conditions used were that of the mastermix with initial denaturation step of 95°C for 10 minutes; Followed by 40 cycles denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 60 seconds, and the final extension at 72°C for 7 minutes with a final hold at 4°C - ∞. The annealing temperature for the 2nd primer set was 55°C. The positive products of PCR were purified and sequenced in both direction by Inqaba Biotechnical Industries (Pty) Ltd in Pretoria, South Africa.

5.2.3.9 Detection of *Pseudomonas* species

To confirm the species of *Psuedomonas* detected from *Rhipicephalus* ticks collected in this study, conventional PCR assay was conducted targeting the *16S rRNA* gene region using the primer set 16S F and 16S R (Aguayo *et al.,* 2020). A total volume of 25 µl was prepared for the different PCR reactions, each reaction contained 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water and 2 µl of template DNA. The PCR conditions used were that of the mastermix with initial denaturation step of 95°C for 10 minutes; Followed by 40 cycles denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 60 seconds, and the final extension at 72°C for 7 minutes with a final hold at 4°C - ∞. The positive products of PCR were purified and sequenced in both direction by Inqaba Biotechnical Industries (Pty) Ltd in Pretoria, South Africa.

5.2.3.10 Detection of *Campylobacter* species

To confirm the *Camphylobacter* species detected in *Rhipicephalus* ticks collected in this study, two conventional PCR assay were performed. The first assay targeted the *16S rRNA* gene region using the primer set C412F/C1288R (Man *et al.,* 2010), while the second assay utilized the primer set MD16S1/MD16S2 (Danis *et al.,* 2001*;* Rahimi *et al.,* 2010). A total volume of 25 µl was prepared for the different PCR reactions, each reaction contained 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water and 2 µl of template DNA. The PCR conditions used were that of the mastermix with initial denaturation step of 95°C for 10 minutes; Followed by 40 cycles denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 60 seconds, and the final extension at 72°C for 7 minutes with a final hold at 4°C - ∞. The annealing temperature for the 2nd primer set was 59°C.

5.2.3.11 Detection of *Streptococcus* species

To confirm the *Streptococcus* species detected in *Rhipicephalus* ticks collected in this study, two conventional PCR assay were performed. The first assay targeted the *16S rRNA* gene region using the primer set Strep-F/Strep-R (Arafa *et al*., 2021), while the second assay utilized the primer set DysF/DysR *(*A total volume of 25 µl was prepared for the different PCR reactions, each reaction contained 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 8.5 µl of double distilled water and 2 µl of template DNA. The PCR conditions used for both reactions were that of the mastermix with initial denaturation step of 95°C for 10 minutes; Followed by 40 cycles denaturation at 95°C for 30 seconds, annealing at 57°C for 30 seconds and extension at 72°C for 60 seconds, and

the final extension at 72°C for 7 minutes with a final hold at 4°C - ∞.The sequences of all sets of primers used for gene amplification are presented in Table 5.

5.2.3.12 Sequence alignment and GenBank submission

Sequences were aligned using the Molecular Evolutionary Genetics Analysis (MEGA) software version 11 (Tamura *et al.,* 2021). The obtained sequences were compared with GenBank references using the Basic Local Alignment Search Tool (BLASTn) and all sequences meeting the NCBI requirements were deposited in the NCBI GenBank database.

Table 5.1: PCR Primers used for detection of tick-borne zoonotic pathogens

Pathogen		Primer sequences_ 5'-3'	Fragment size	Ref	Annealing temperature
Anaplasma phagocytophilum	*EHR-521*	TGT AGG CGG TTC GGT AAG TTA AAG	250	Welc-Faleciak *et al.,* (2009)	60°C
	EHR-747	GCA CTC ATC GTT TAC AGG GTG			
Ehrlichia/Anaplasma spp.	*Ehr- F*	GGA ATT CAG AGT TGG ATC MTG GYT CAG	460-520	Matjila *et al.,* (2008)	60°C
	Ehr-R biotin	CGGGATCCCGAGTTTGCCGGGACTTYTTCT			
Ehrlichia canis	*E.c 16S fwd*	TCGCTATTAGATGAGCCTA CGT	154	Peleg *et al*.; (2010)	60°C
	E.c 16S rev	GAGTCTGGACCGTATCTCAGT			
Coxiella	*Cox-16SF1*	CGTAGGAATCTACCTTRTAGWGG	1321–1429 1st PCR	Duron *et al.,* (2014); (2015), Kobayashi *et al.,* (2021)	
	Cox-16SR2	GCCTACCCGCTTCTGGTACAATT			52-56°C
	Cox-16SF1	CGTAGGAATCTACCTTRTAGWGG	719–826 2nd PCR Fragment 1		
	Cox-16SR2	ACTYYCCAACAGCTAGTTCTCA			
	Cox-16SF1	CGTAGGAATCTACCTTRTAGWGG	719–826 2nd PCR Fragment 2		
	Cox-16SR1	ACTYYCCAACAGCTAGTTCTCA			
	Cox-23SF1	GCCTGCGAWAAGCTTCGGGGAG	694–1188 1st PCR		56°C
	Cox-23SR2	CTCCTAKCCACASCTCATCCCC			
	Cox-23SF2	GATCCGGAGATWTCYGAATGGGG	583–867 2nd PCR		
	Cox-23SR1	TCGYTCGGTTTCGGGTCKACTC			
Coxiella burnetti	Trans *1*	TATGTATCCACCGTAGCCAGTC	687	Kilicoglu *et al*., (2020); Truong *et al*., (2012)	61°C
	Trans 2	CCCAACAACACCTCCTTATTC			
Escherichia coli	*uidAf*	AAAACGGCAAGAAAAAGCAG	147	Bej *et al.,* (1999); Shivakumaraswamy *et al.,* (2019)	60°C
	uidAr	ACGCGTGGTTACAGTCTTGCG			
Rickettsia	*Rr190.70p*	ATGGCGAA TATTTCTCCAAAA	631	Salomon *et al.,* (2022)	46°C
	Rr190.701n	GTTCCGTTAATG GCAGCATCT			
	Rp877p	GGGGACCTGCTCACGGCGG	381		48°C
	Rp1258n	ATTGCAAAAAGTACAGTGAACA			
Anaplasma spp.	*EE1*	TCCTGGCTCAGAACGAACGCTGGCGGC	1430	Yan *et al.,* (2022)	55°C
	EE2	GTCACTGACCCAACCTTAAATGGCTG			
Anaplasma ovis	*MSP45*	GGGAGCTCCTATGAATTACAGAGAATTGTTTAC	869	Yang *et al*., (2021)	55°C
	MSP43	CCGGATCCTTAGCTGAACAGAATCTTGC			
Anaplasma phagocytophilum	*SSAP2f*	GCTGAATGTGGGGATAATTTAT	641	Yan *et al.,* (2022)	55°C
	SSAP2r	ATGGCTGCTTCCTTTCGGTTA			

Pathogen		Primer sequences	Fragment size	Ref	Annealing temperature
Francisella	*Fr153F01*	GCCCATTTGAGGGGGATACC	1151	Duzlu *et al.,* 2016, Ghoneim *et al.,* (2017)	60°C
	Fr1281R01	GGACTAAGAGTACCTTTTTGAGT			
	SdhF	AAGATATATCAACGAGCKTTT	342	Barns *et al.,* (2005)	56°C
	SdhR	AAAGCAAGACCCATACCATC			
Staphylococcus	*16S1*	GTGCCAGCAGCCGCGGTAA	886	Poulsen *et al.,* (2003)	55°C
	16S2	AGACCCGGGAACGTATTCAC			
	SSU-bact-27f	AGA GTT TGA TCM TGG CTC AG	450	Becker *et al.,* (2004); Andrioti *et al*., (2011), Park *et al*., (2011)	53°C
	SSU-bact-907r	CCG TCA ATT CMT TTR AGT TT			
Clostridium	*tpiA*	GCWGGWAAYTGGAARATGMAYAA	501	Dhalluin *et al.,* (2003)	55°C
	tpiB	TTWCCWGTWCCDATWGCCCADAT			
	16SA	GAGAGTTTGATCCTGGCTCAG	800		
	16SB	GTGGACTACCAGGGTATCTAATCC			
Psuedomona	*16S F*	GAGTTTGATCCTGGCTCAG	550	Aguayo *et al.,* (2020) (This study)	60°C
	16S R	ATTACCGCGGCTGCTGG			
Camphylobacter	*C412F*	GGATGACACTTTTCGGAGC	817	Linton *et al.,* (1996)*;* Man *et al.,* (2010)	55°C
	C1288R	CATTGTAGCACGTGTGTC			
	MD16S1 N	ATC TAA TGG CTT AAC CAT TAA AC	857	Danis *et al.,* (2001); Rahimi *et al.,* (2010)	59°C
	MD16S2	GGA CGG TAA CTA GTT TAG TAT T			
Streptococcus	*Strep F*	CGGGGGATAACTATTGGAAACGATA	912	Arafa *et al*., (2021a)	57°C
	Strep R	ACCTGTCACCCGATGTACCGAAGTA			
	DysF	CGGGGGATAACTATTGGAAACGATA	401	Arafa *et al*., (2021a); Moriconi e*t al*., (2017)	57°C
	DysR	ATCTCTAGACCGGTCAGGA			

5.3 Results

The table below provides a summarized version of conventional PCR results obtained when confirming potential zoonotic pathogens detected using metabarcoding from *Rhipicephalus* ticks collected from domestic animals, vegetation, and laboratory reared ticks. Sequences generated in this study were submitted to the NCBI database, with the exception of sequences obtained for *E. coli* and *E. canis* using species-specific primers. The targeted gene region for these species was approximately 150 base pairs (Table 5.1). These sequences were rejected during the submission process as they were too short (<150 nucleotides) and did not meet the minimum length requirements set for the NCBI database.

Table 5.2: PCR confirmation of zoonotic pathogens detected using metabarcoding

Pathogen genera (detected by metabarcoding)	Targeted genes	PCR amplification	Sequence result quality	Confirmed species	comments
Rickettsia	*gltA*	positive	Good	*Rickettsia* spp. and *R. aeschlimannii*	BLASTn search results
	OmpA	positive	Good	*R. aeschlimannii*	BLASTn search results
Escherichia-Shigella	*uidA*	positive	Good	*Escherichia coli*	BLASTn search results
Coxiella	*16S rRNA*	positive	nd	*Coxiella* spp.	Presence of species confirmed by PCR amplification
	23S rRNA	positive	Good	*Coxiella*-like endosymbiont	BLASTn search results
	IS1111 transposon	positive	nd	*Coxiella burnetii*	Species confirmed using species-specific primers
Ehrlichia	*16S SSU rRNA*	positive	Good	*Ehrlichia canis*	BLASTn search results
	16*S rRNA*	positive	Good	*E. canis*	BLASTn search results
Anaplasma spp.	16*S rRNA*	positive	nd	-	-
	16*S rRNA*	positive	Bad	*Anaplasma phagocytophilum*	Presence of species not confirmed
	msp4	positive	Bad	*Anaplasma ovis*	Species confirmed using species-specific primers
Francisella	*16S rRNA*	positive	Good	*Francisella*-like endosymbiont	BLASTn search results
	(*sdhA*)	negative	-	-	-
Staphylococcus	*16S rDNA*	positive	Bad	*Staphylococcus* spp	Presence of species not confirmed
	16S rRNA	positive	Bad	*Staphylococcus* spp.	Presence of species not confirmed
Clostridium	*16S rRNA*	positive	Bad	*Clostridium* spp.	Presence of species not confirmed
	tpi	negative	-	-	-
Pseudomonas	*16S rRNA*	positive	Bad	*Pseudomonas* spp.	Presence of species not confirmed
Campylobacter	*16S rRNA*	positive	nd	*Campylobacter* spp.	Genus specific primers used
	16S rRNA	positive	nd	*Campylobacter* spp.	Presence of species not confirmed
Streptococcus	*16S rRNA*	positive	nd	*Streptococcus* spp.	Genus specific primers used
	DysF	positive	nd	*S. dysgalactiae* subspecies	Species confirmed using species-specific primers

nd= not done

5.3.1 *Coxiella* species

Conventional PCR targeting *23S rRNA* gene region of *Coxiella* spp. was conducted on species that showed presence of *Coxiella* spp using 16S in metabarcoding analysisBLASTn search results of sample IDs: DD4, Re12 and Smf3 of this study matched with *Coxiella*-like endosymbiont sequences GenBank accession numbers: LC635215.1, LC635196.1 and LC635204.1 (Appendix 8.5) on the NCBI database with query covers ranging between 96 and 97%, matching identities ranging between 98.52 and 100%, and E-values of 0.0. PCR amplification of the tagerted genes for PCR1 were not sequenced.

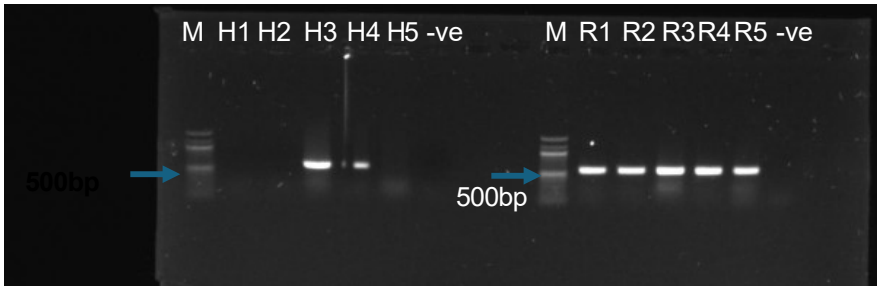


Figure 5.1: PCR amplicons for the 16S *Coxiella* gene with an expected product size of 719–826 bp and 23S *Coxiella* gene with an expected product size of 583–867 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane H3-4 and Lane R1-5 show samples with positive amplification. Lane R1-5 show positive presence of *Coxiella*-like endosymbiont DNA from *Rhipicephalu*s tick samples.

Furthermore, conventional PCR was conducted using species-specific primers targeting the *Coxiella IS1111 transposon* gene on species that showed presence of *Coxiella* spp using 16S in metabarcoding analysis. All PCR product that amplified at the targeted gene region were considered positive for *C. burnetii* species. Sample P1, 2 and 4 amplified at correct gene region, confirming the presence of *C. burnetii* in sampled ticks see, figure 5.2 below.

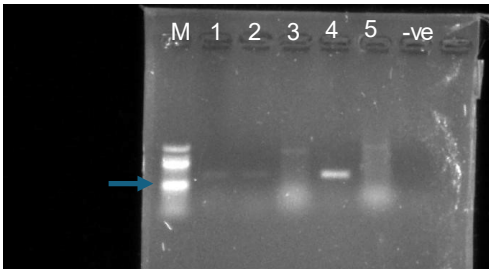


Figure 5.2: PCR amplicons for the *Coxiella burnetti* gene with an expected product size of 632 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane 1, 2 & 4 show samples positive for presence of *C. burnett*i DNA from *Rhipicephalus* tick samples.

5.3.2 *Rickettsia* species

Detection of *Rickettsia* sp. was conducted by PCR targeting the *gltA* and the *OmpA* gene region to confirm *Rickettsia* positive samples detected by 16S metabarcoding approach. BLASTn search results of *Rickettsia* targeting the *gltA gene* region (Sample IDs: h3 & h14) of this study were similar to *Rickettsia sp. and R. aeschlimannii* sequences (GenBank accession numbers: MW114885.1, MW114884.1 and MH267736.1) (Appendix 8.5) on the NCBI database with query covers ranging between 99 and 100%, matching identities ranging between 99.74 and 100%, and E-values of 0.0. Whilst BLASTn search results of *Rickettsia* targeting the *OmpA gene* (Sample IDs: h3 & h14) of this study were similar to *R. aeschlimannii* sequences (GenBank accession numbers: MG920561.1, MF379308.1and MF379306.1) on the

NCBI database with 100% query covers, 100% matching identities and E-values of 0.0.

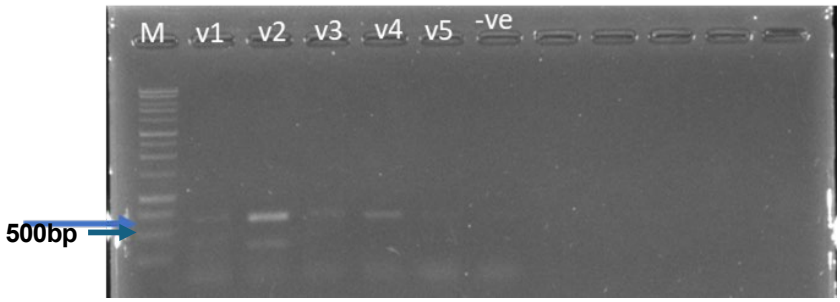


Figure 5.3: PCR amplicons for the *Rickettsia* gene (*OmpA*) with an expected product size of 632 bp. M is the molecular marker (1k bp), -ve is for the no template control. Lane v1-4 show samples positive for presence of *Rickettsia* DNA from *Rhipicephalus* tick samples

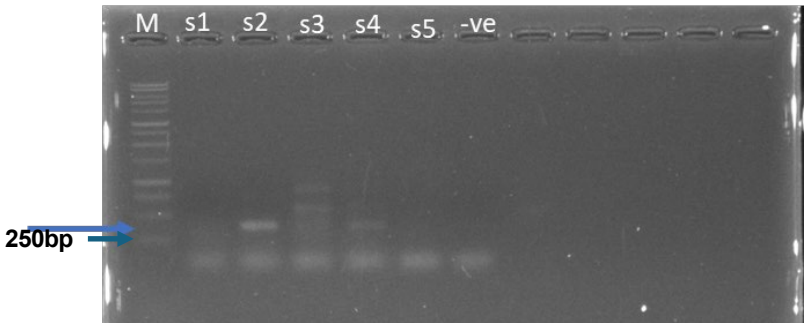


Figure 5.4: PCR amplicons for the *Rickettsia* (*gltA*) gene with an expected product size of 382 bp. M is the molecular marker (1kb bp), -ve is for the no template control. Lane s2 and s4 show samples positive for presence of *Rickettsia* DNA from *Rhipicephalus* tick samples.

5.3.3 *Escherichia-Shigella* species complex

Detection of *Escherichia coli* was conducted by PCR with species-specific primers targeting the *uidA* gene region to confirm *Escherichia Shigella* positive samples detected by 16S metabarcoding approach. BLASTn search results (Sample IDs: h1, Smf3 and gkm1) of this study were similar to *E. coli* sequences (GenBank accession numbers: CP054227.1; CP054224.1 and CP055259.1) (Appendix 8.5) on the NCBI

database with 100% query covers, 100% matching identities and E-values = 3e-66.0. Positive amplification of the targeted *E. coli* gene is shown below (Figure 5.5).

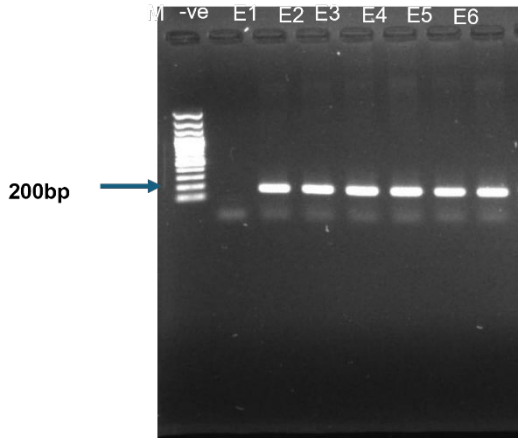


Figure 5.5: PCR amplicons for the *Escherichia coli* gene with an expected product size of 148 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane E1-E6 show samples positive for presence of *E. coli* DNA from *Rhipicephalus* tick samples.

5.3.4 *Ehrlichia* species

Detection of *Ehrlichia* was conducted by PCR using *Anaplasma/Ehrlichia* primers to confirm positive *Erhlichia* samples detected using the *16S rRNA* metabarcoding approach. Samples that amplified using *Anaplasma/Ehrlichia* primers are shown in figure 5.6. BLASTn search results (Sample IDs: gmm5, gkm1 and gmf7) of this study were similar to *E. cannis* sequences (GenBank accession numbers: CP025749.1; CP085275.1 and CP085276.1) (Appendix 8.5) on the NCBI database with 96% query covers, matching identities ranging from 98.94 to 99.15% and E-values of 0.0. Further species confirmation was done using species specific primers of *E. cannis*, and the amplified product below (Figure 5.7). The BLASTn search results (gmm5 and gmf7) were similar to *E. cannis* sequences (GenBank accession numbers: MH374122.1,

KY434111.1 and MF059355.1) on the NCBI database with 98% query covers, 100% matching identities and E-values of 8e-56.

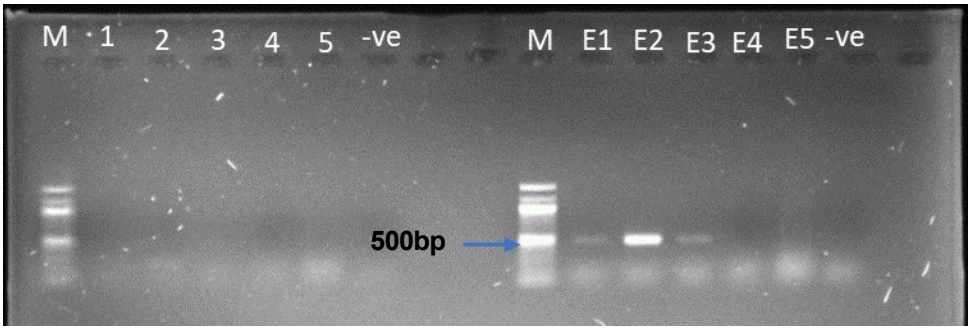


Figure 5.6: PCR amplicons for the *Anaplasma phagocytophilum* (*EHR-521/EHR-747*) gene with an expected product size of 250 bp and *Ehlichia/Anaplasma* (*Ehr-F/Ehr-R biotin*) gene with an expected product size of 460-520bp. M is the molecular marker (100 bp), -ve is for the template control. Lane E1-3 show samples positive for presence of *Ehlichia* DNA from *Rhipicephalus* tick samples.

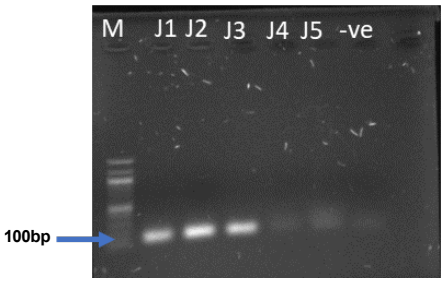


Figure 5.7: PCR amplicons for the *Ehrlichia canis* gene with an expected product size of 154 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane J1-3 show samples positive for presence of *Ehlichia* DNA from *Rhipicephalus* tick samples.

5.3.5 Francisella species

Detection of *Francisella* was conducted by PCR using Fr153F01/Fr1281R01 primers to confirm positive *Francisella* samples detected using the 16S metabarcoding approach. The amplified PCR product is shown in figure 5.8 below. BLASTn search results (Sample IDs: h14 and h40) of this study were similar to *Francisella*-like endosymbiont sequences (GenBank accession numbers: MH6452001.1;

MH645199.1 and MW287952.1) (Appendix 8.5) on the NCBI database with 99%-100% query covers, matching identities ranging from 99.72% and E-values of 0.0. Further species confirmation was done using species-specific primers *SdHF/SdHR* targeting the *putative succinate dehydrogenase locus (sdhA).* No amplification was achieved.

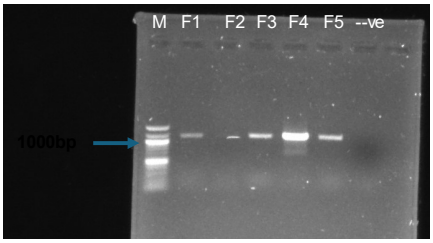


Figure 5.8: PCR amplicons for the *Francisella* gene with an expected product size of 1151 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane F1-5 show samples positive for presence of *Francisella like endosymbiont* DNA from *Rhipicephalus* tick samples.

5.3.6 *Anaplasma* species

Detection of *Anaplasma* was conducted by nested PCR using universal *Anaplasma* primers *EE1/EE2*, and species-specific primers *MSP43/MSP45* and *SSAP2F/SSAP2R*. Amplification was achieved for all primers (Figure 5.9) however no amplified samples produced good sequences for *A. ovis* and *phagocytophilum* primers.

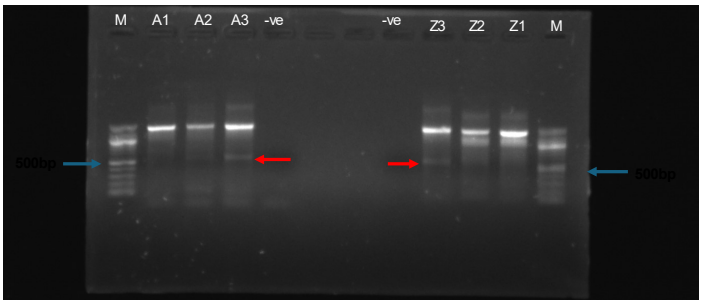


Figure 5.9: PCR amplicons for the *Anaplasma ovis* (MSP43/MSP45) gene with an expected product size of 869 bp and *Anaplasma phagocytophilum* (SSAP2f/SSAP2r) gene with an expected product size of 641-642 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane A1-3 show samples positive for presence of *A. ovis* DNA from *Rhipicephalus* tick samples.

5.3.7 *Staphylococcus* species

PCR-bases detection of *Staphylococcus* species was performed using *SSU-bact-27f/SSU-bact-901r* primers to confirm *Staphylococcus*-positive samples identified through the Metabarcoding approach. Sample clv1, D1 and gmm5 successfully amplified (Figure 5.10) and were submitted for sequencing. Further species confirmation was attempted using the *16S1/16S2* primers, which yielded successful amplification (Figure 5.10); however, high-quality sequence data could not be obtained.

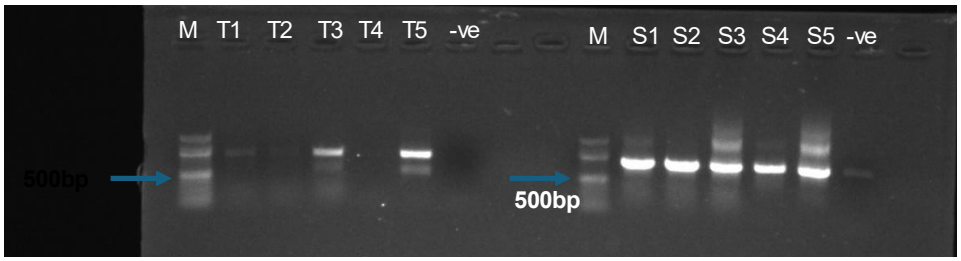


Figure 5.10: PCR amplicons for the *Staphylococcus* (16Sup-1/16Sup-2) gene with an expected product size of 886 bp and *Staphylococcus (*SSU-bact-27f / SSU-bact-907r) gene with an expected product size of 450 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane T1-3, T5 and S1-5 show samples with positive amplification.

5.3.8 *Pseudomonas* species

PCR-based detection of *Pseudomonas* spp. was performed by targeting the *16S ribosomal RNA* using *16S-F/16S-R* primers to confirm *Pseudomonas*-positive samples identified through the 16S metabarcoding approach. Sample P1-5 successfully amplified at the expected gene region (Figure 5.11); however, high-quality sequence data was not obtained.

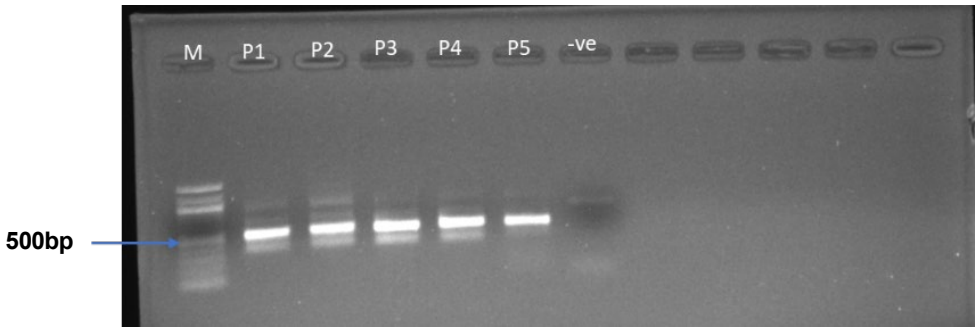


Figure 5.11: PCR amplicons for the *Pseudomonas* gene with an expected product size of +-550bp bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane P1-5 show samples with positive amplification.

5.3.9 *Clostridium* species

Conventional PCR targeting the *16S rRNA* gene and the species-specific *Triosephosphate Isomerase* (*tpi*) gene of *Clostridium* spp was conducted on tick species that showed presence of *Clostridium* spp. on 16S metabarcoding analysis. No amplification was achieved for all samples targeting the *tpi* gene, only samples targeting the *16S rRNA* gene amplified (Figure 5.12). Whilst a 100% amplification targeting the *16S rRNA* gene was achieved, no high-quality sequence data was obtained (Figure 5.12).

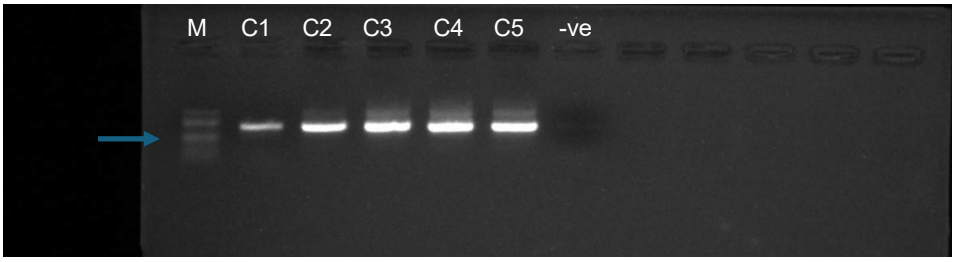


Figure 5.12: PCR amplicons for the *Clostridium* gene with an expected product size of 800 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane C1-5 show samples with positive amplification.

5.3.10 *Campylobacter* species

Conventional PCR targeting the *C412F/ C1288R* and *MD16S1 N/MD16S2* primers was performed to confirm *Campylobacter* spp. detected through 16S metabarcoding in the previous chapter. Amplification of the targeted gene regions was successful (Figure 5.13 and 5.14); however, high-quality sequence data was not obtained.

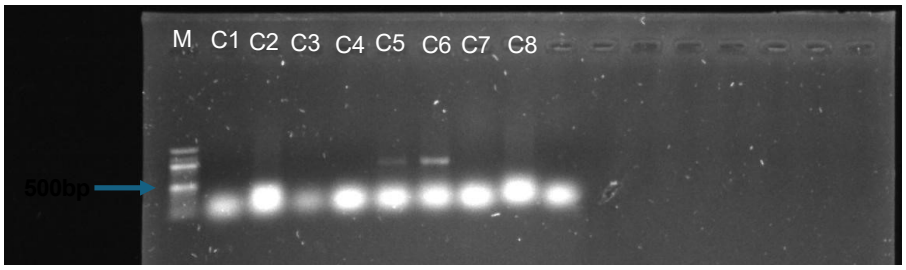


Figure 5.13: PCR amplicons for the *Camphylobacter* (C412F/C1288R) gene with an expected product size of 817 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane C5 and C6 show samples with positive amplification.

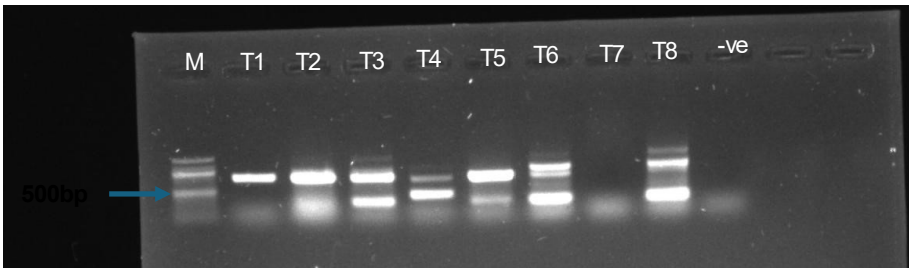


Figure 5.14: PCR amplicons for the *Camphylobacter* (MD16S1 N/MD16S2) gene with an expected product size of 857 bp. M is the molecular marker (100 bp), -ve is for the no template control. Lane T1-6 and T8 show samples with positive amplification.

5.3.11 *Streptococcus* species

PCR-based detection of *Streptococcus* species was performed using Strep-F/strep-R and StrepDysF/strepDysR primer sets to confirm *Streptococcus*-positive samples identified through the metabarcoding approach. Successful amplification at the expected band sizes was observed for both primer sets (Figures 5.15 and 5.16). Additionally, PCR amplification shown in figure 5.18 confirmed the presences of *Streptococcus dysgalactiae* subspecies using species-specific primers.

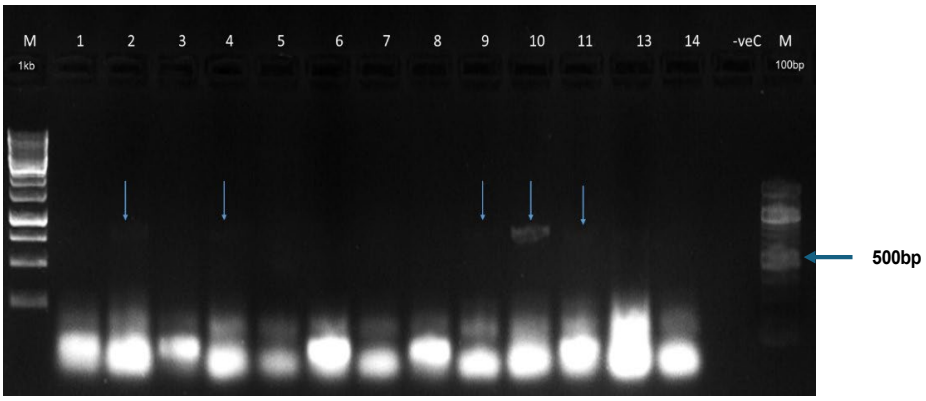


Figure 5.15: PCR amplicons for the *Streptococcus* (*Strep-F/strep-R*) gene with an expected product size of 912 bp. M (left) is the molecular marker (1Kb), M (right) is the molecular marker (100 bp), -ve is for the no template control. Lane 10 show positive amplification.

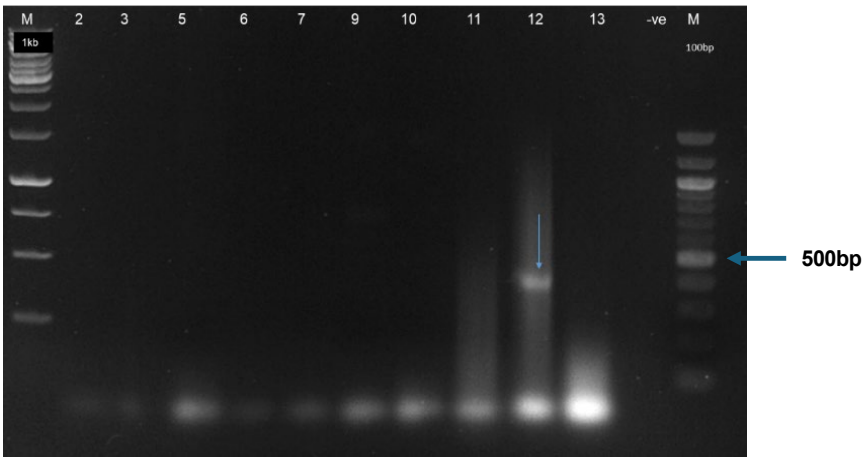


Figure 5.16: PCR amplicons for the *Streptococcus* (StrepDysF/strepDysR) gene with an expected product size of 401 bp. M (left) is the molecular marker (1Kb), M (right) is the molecular marker (100 bp), -ve is for the no template control. Lane12 show samples positive for presence of *Streptococcus dysgalactiae subspecies* DNA from *Rhipicephalus* tick samples.

5.4 Discussion

Ticks and tick-borne pathogens in domestic animals have been recorded over the years and across the world. These pathogens are known to cause zoonotic diseases impacting economies and the lifelyhood of small-scale farmers drastically. In many studies, ticks from the genus *Rhipicephalus* were always in the mix and have since been recorded as the most abundant genera from the family Ixodidae (hard ticks) contributing to tick-borne disease (Basu and Charles 2017; Ghafar *et al*., 2020; Tawana *et al*., 2022).

In the current study, eight different zoonotic pathogens have been detected by conventional PCR from *Rhipicephalus* ticks collected from domestic animals, confirming results generated by *16S rRNA* metabarcoding technique in chapter 4. Tick pools were respectively screened for detection of tick-borne pathogens using genera and species-specific primers. Subsequently, BLASTn search on the NCBI database identified species like *Ricketttsia aeschlimanni*, *Erlichia canis*, *Coxiella*-like endosymbionts, *Escherichia coli*, *Francisella*-like endosymbionts. *Coxiella burnetti, Streptococcus dysgalactiae subspecies* and *Anaplasma ovis* were identified based on the amplification of species-specific primers. *Camphylobacter* spp. and *Streptococcus* spp. were confirmed using genus-specific primers. PCR amplicons of *Staphylococcus* spp, *Anaplasma* spp., *A. ovis, Pseudomonas*, *A. phagocytophilum* and *Clostridium* spp. did not yield high-quality sequences. Due to exhaustion of funds, sequencing was not performed for *Coxiella burnetti*, *Campylobacter* spp. and *Streptococcus*.

In this study 73% (n=77) *Coxiella* sp. was detected by metarbarcoding in *Rhipicephalus* ticks (n=105) collected from livestock (sheep, goats, cattle, horses), dogs, laboratory reared, and vegetation as reported in Chapter 4. Further confirmation

was perforemed using PCR targeting the*23S rRNA* and *16S rRNA* markers, as well as species-specific marker (*IS111*). BLASTn analysis of the obtained sequences against the NCBI database identified the*23S rRNA* gene sequences as *Coxiella*-like endosymbionts (CLE's). The high prevalence of *Coxiella* spp. observed in this study likely reflect a mutualistic relationship between these bacteria and their tickhosts. *Coxiella* spp. are known to be a maternally inherited and play a crucial role tick molting and survival (Guizzo *et al*., 2017). This association explains their detection in both colony-reared ticks and those collected from vegetation in the present study. Previous research has shown that CLEs are closely related to the *C. burnetii* pathogen responsible for Q fever and are commonly linked to ixodid ticks (Rahal *et al*., 2020). In the aformention study conducted in Algeria, CLE*'s* were detected in 51.7% of ticks, representing 20% of the sampled tick species infesting cattle, the highest prevalence (70%) was reported in *Rhipicephalus* ticks. Simiarly, a study in Zambia detected CLEs in 45.7% of tick samples across six genera, namely, *Amblyomma*, *Argas*, *Haemaphysalis*, *Hyalomma*, *Ornithodorus* and *Rhipicephalus* (Kobayashi *et al*., 2021). *Coxiella* spp. have also been identified in ticks collected from dogs in North West, Free State, KwaZulu-Natal and Mpumalanga provinces of South Africa (Mtshali *et al*., 2017; Van Wyk *et al.,* 2022). Although the present study focused solely on *Rhipicephalus ticks*, endosymbionts are a significant component of the tick microbiota and warrantfurther investigation, particularly in ticks infesting livestock in South Africa. Endosymbionts have been implicated in pathogen maintenance and transmission in variousarthropods (Hosokawa *et al*., 2010; Cirimotich *et al*., 2011). However, the pathogenic potential of CLE's in ticks remain unclear (Machado-Ferreira *et al*., 2016). Notably, CLE's from *Rhipicephalus* ticks have been detected in both ticks and and skin samples from patients presenting eschars, raising concerns about their potential

pathogenicity (Angelakis *et al*., 2016). Further research is needed to elucidate the role of CLEs in tick-borne disease ecology and their potential impact on human and animal health

Furthermore, the current study showed the presence of *C. burnetii* IS1111 DNA in *Rhipicephalus* ticks infesting livestock. This is similar to findings from a study done in Xinjiang areas of China were *C. burnetii* DNA was detected in 973 (973/1507) DNA samples of ticks from different genera collected from livestock (Ni *et al*., 2020). From the same study, ticks from the genus *Rhipicephalus* showed an infection rate of 74.56%, which was the highest among the four genera sampled. *Coxiella burnetii* is known as an agent of Q-fever causing delivery of weak offspring, infertility, postpartum metritis, still-birth and coxiellosis (arbortion) in animals (Derrick *et al*., 1983; Cabrera Orrego *et al*., 2020; Menadi *et al.,* 2020; Mangena *et al*., 2021; Magadu and Thompson 2023). Whilst *C. burnetii* infection in humans may cause vomiting, pneumonia, fever, headache hepatitis, endocarditis, and intravascular infections (Wildman *et al.,* 2002; Porter *et al.,* 2011, Vanderburg *et al.*, 2014; Njeru *et al*., 2016). This pathogen is transmitted through direct contact with milk, urine, feces or semen from infected animals, as well as inhalation of aerosolized particles from animal placentas, parturient fluids, aborted fetuses and environmental dust (Tissot-Dupont and Raoult 2008; Vanderburg *et al*., 2014). There are limited studies on the extent of *C. burnetii* infections in livestock in South Africa. Mangena *et al*., (2021) reported 9.4% prevalence of Q-fever in cattle, followed by 4.3% in sheep and 0.9% seroprevalence in pigs where random sera samples were collected during slaughter in selected red meat abattoirs in Gauteng Province, South Africa. Most recently, another study was conducted in South Africa documenting the presence of *C. burnetii* in goats showing an estimated animal-level and herd-level seroprevalences of 18.4% and 51%

respectively (Magadu and Thompson 2023). Infections with *C. burnetii* causes significant impact on small scale farmers livestock and zoonotic risk to the workers exposed to livestock (Mangena *et al*., 2021; Gomes *et al*., 2022; Roughan *et al*., 2023). According to the Animal Diseases Act 35 of 1984 of South Africa, Q fever is not classified as a controlled or notifiable disease (Maurin and Raoult, 1999; Mangena *et al*., 2021), unlike in several other countries where it is subjected to regulatory control (Gomes *et al*., 2022; Roughan *et al*., 2023). Given the potential implications of this disease, further research is needed to assess its impact on both animal and human health in South Africa. The detection of the *C. burnetii* pathogen the present study suggests the potential for disease maintenance within tick populations infesting livestock. This finding highlights the possible risk of transmission to livestock and humans in close contact with infected animals, underscoring the need for enhanced surveillance and risk assessment.

In this study, *Rickettsia* spp were detected using conventional PCR targeting *OmpA* and *gltA* genes (Roux *et al.*, 1997; Salomon *et al*., 2022). The PCR amplicons were sequenced and subjected to BLASTn search on the NCBI database which identified these species as *R. aeschlimanni.* A recognized species of one of the major groups of *Rickettsia* called the spotted fever group (SFG) consisting of many species that are exclusively transmitted to humans through tick bites (Iweriebor *et al*., 2020). Examples of three medically impontant *Rickettsia* species in sub-saharan Africa include *R. africae* which causes tick-bite fever and *R. conorrii* causing Mediterranean spotted fever and finally *R. aeschachlimannii* causing eschar in humans (Pretorius and Birtles 2002; Ndip *et al*., 2005; Rutherford *et al.,* 2004; Parola *et al*., 2005; Althaus *et al*., 2010; Mahlobo-Shwabede *et al.,* 2021). This is the first report in which *R. aeschlimannii* was detected in *R. e. evertsi* ticks collected in horses in South Africa.

The first demonstration of the presence of *R. aeschlimannii* in South Africa was from a tourist patient attached *with R. appendiculatus* tick on his right thigh and an eschar around the attachment site (Pretorius and Birtles 2002). The sequence analysis of the *R. aeschlimannii OmpA* gene in this study indicate that the sequences shared 100% identity to *R. aeschlimannii* sequence (MG920561) from unfed larvae obtained from engorged female *Hyalomma marginatum* ticks from domestic animals in Turkey (Orkun, 2018), proving transovarial transmission of this pathogen in ticks. Again 100% pathogen sequence similarities (MF379308; MF79306) were recovered from *H. marginatum* collected from wild animals (wild boar, hare and fox) in Turkey for the *OmpA* gene (Orkun and Cakmak 2019). In the same light, sequence analysis of the *gltA* showed 100% similarity to *R. aeschlimannii* strain (MH267736) from *H. marginatum* ticks collected from livestock in China (Yin *et al.,* 2018). The above confirms the presence of *Rickettsia* spp. detected using metabarcoding in 8 of the 105 tick DNA pools from ticks collected from hosts and vegetation (This study, Chapter 4). Of the six different hosts, only ticks collected from horses were detected with *R. aeschlimannii.* Which was not the case in Eastern Cape, were *Rickettsia* spp were absent in *Rhipicephalus* ticks collected from horses, but present in ticks collected from sheep, goats and cattle targeting the *gltA*, *ompA* and *ompB* genes (Iweriebor *et al*., 2020). Previous studies done to detect *Rickettsia* pathogens in South African livestock/domestic animals resulted in the recovery of *R. africae* in ticks from the *Rhipicephalus* genus (Mtshali *et al*., 2016; Guo *et al*., 2019; Iweriebor *et al*., 2020). In Africa, *R. aeschlimannii* has been reported in *Hyalomma excavatum*, *H marginatum*; *Amblyomma variegatum* collected from livestock (Reye *et al*., 2012; Abdelkadir *et al*., 2019 Chitanga *et al*., 2021). Human infections caused by *R. aeschlimannii* have been documented in several cases (Raoult *et al*., 2002; Parola *et al*., 2005). Parola *et al*.

(2005) reported a case in a patient who was bitten in Morocco and subsequently developed an inoculation eschar on his ankle, accompanied by fever and maculopapular rash. Additionally, an autochthonous case was reported in Greece, where a male patient presented with Mediterranean spotted fever-like symmtoms (Germanakis *et al*., 2013). Another case was documentd in Italy, where a patient exhibited liver dysfunction and significant hyperaminotransferasemia (Tosoni *et al*., 2016). These reports highlight the clinical variability of *R. aeschlimannii* infections and underscore the need for further research on its epidemiology and impact on human health.

Escherichia coli was detected in this study using conventional PCR targeting the *uidA* genes (Bej *et al.,* 1999 and Shivakumaraswamy *et al.,* 2019). The above was done to confirm the presence of species from the genus *Eschericha-shigella* that were detected using metabarcoding in the previous chapter. *E. coli* is a gram-negative bacteria classified in the family Enterobacteriaceae alongside other potential pathogenic species in the genera *Shigella*, found mainly in normal gut flora of humans and animals, and may be widely distributed in soil and water (Ergunay *et al*., 2022). In the previous chapter, the bacterial abundance of members of the genus *Eschericia-shigella was reported* at 28.57% in *Rhipicephalus* ticks collected from livestock using metabarcoding. Tekin *et al*., (2017) reported a 4 % bacterial abundance of *E. coli* and 2% of *Shigella* in Turkish *Rhipicephalus* ticks using pyrosequencing. A 48.03% *E coli* prevalence was also reported from *Rhipicephalus* and *Hyalomma* ticks collected from buffalos in Iraq (Khalaf *et al*., 2018). The studies above confirm the potential ticks from the genus *Rhipicephalus* have in haboruing *E. coli*, a pathogen that can be transmitted to livestock through tick bite, and to humans through consumption of infected meat (Stein and Katz 2017). This pathogen is also known as a foodborne pathogen that

causes gastrointestinal illnesses in humans (El-Sayed and Kamel 2020). The presence of *E coli* in ticks has not been fully explored in south Africa, especially in *Rhipicephalus* ticks infesting livestock. However, a study that was conducted in Gauteng province reported a 7 % abundance of *Escherichia* in *Amblyoma* tick species infesting Nguni cattle (Chigwada *et al*., 2022). The same study further identified members of the genus *Escherichia* pathogens to species- level as *E. coli similar to the present study*. The presence of members of the genus *Escherichia-Shigella* in ticks warrants further investigation because this bacterium is identified as central in tick microbial communities (Maitre *et al*., 2022) and is of public and medical importance.

The presence of *Ehrlichia* spp. was detected by PCR targeting *Ehrlichia*/*Anaplasma* spp (*Ehr-F/Ehr-R biotin*) and species-specific primers *E. canis* PCR assay. These results confirmed findings detected in the previous chapter using metabarcoding were members of the *Ehrlichia* genus were detected in *Rhipicephalus* tick samples collected from cattle, sheep and goats. The presence of *E. canis* in *Rhipiciphelus* ticks were previously reported by Iweriebor *et al*., (2017); Mtshali *et al*., (2017), Adelabu *et al*., (2020); Van Wyk *et al*., (2022) in South Africa. *Ehrlichia canis* is known to cause canine monocytic ehrlichiosis in dogs (Rene-martellet *et al*., 2015). The *E canis* bacteria can cause human granulocytic ehrlichiosis *(*HGE) and human monocytic ehrlichiosis *(*HME) (Andrić 2014). Therefore, understanding the importance of this pathogen is important inorder to come up with strategies to prevent disease transmission. The present study reports the presence of *E. canis* in *Rhipicephalus* ticks collected from ruminants, similar to a study conducted in the Estern Cape where *E. canis* was detected in ticks collected from goats and sheep (Adelabu *et al*., 2020). This is not unsual, questing ticks can attach to different host, including goats, sheep, cattle which could possibly cause infection of *E. canis*. Ruminants can also be infested with ticks

infected with *E. canis* through host switching if these ruminants live in proximity with companion animals like dogs.

The present study attempted to identify *Anaplasma* spp. at species level using nested PCR targeting the *16S rRNA* gene. This was performed with outer primers (EE1/EE2) and inner primers specific for *A. ovis* (MSP43/MSP45) and *A. phygocytophilum* (SSAP2F/SSAP2R) -) to confirm metabarcoding results fromthe previous chapter. Species identification yielded positive results for *A. ovis.* These findings are consistent with a study by Han *et al*. (2019), in which similar primers were used to detect *A. ovis* from three genera of questing ixodid ticks from Qinghai-Tibet Plateau. The same primers were successfully used in nested PCR to detect *A. phygocytophilium* in ruminants (goats and sheep) and *Haemaphysalis longicornis* ticks in china (Yan *et al*., 2022). However, in the current study, *A. phygocytophilium* was not detected using these primers. This discrepancy may be attributed to PCR amplification errors, the presence of chimeric sequences, or variations in DNA quality. The detection of *A*. ovis from sampled *Rhipicephalus* ticks is noteworthy, these findings remain significant, as *A. ovis* is the cause of ovine anaplasmosis in sheep (Friedhoff 1997; Kolo 2023). In Africa, *A. ovis* has frequently been detected in *Rhipicephalus* ticks, further supporting the results of the present study (Oundo *et al*., 2020; Sadeddine *et al*., 2020; Getange *et al*., 2021; Kolo 2023). Given that eight known *Anaplasm*a species are obligate intracellular pathogens capable of causing anaplasmosis in both humans and animals (Hove *et al*., 2018; Yan *et al*., 2022), the detection of *Anaplasma* spp. in ticks collected from sheep warrants further investigation. This highlights the need for continued surveillance and molecular characterization to better understand the epidemiology and potential zoonotic risk associated with these bacteria.

In this study, *Francisella*–like endosymbionts were detected using conventional PCR assays targeting the *16S rRNA* gene region. The PCR was performed to confirm the 8.6% (n=9) infection prevalence of members from the genus *Francisella* detected using metabarcoding in the previous chapters. The PCR amplicons were sequenced and subjected to BLASTn search on the NCBI database, the results confirmed that sequences from this study were similar to *Francisella*–like endosymbionts. The *Francisella*-like endosymbionts were detected from *Rhipicephalus e. evertsi* ticks collected from horses and cattle in the current study. A 4.7 % infection prevalence of *Francisella* spp was reported in *Hyalomma dromedarii* ticks collected from camels in Egypt (Ghoneim *et al*., 2017). Sequences from the above study were closely related to *Francisella*-like endosymbiots showing similar findings to the present study. *Francisella*-like endosymbionts are maternally inherited and identified as obligate nutritional mutualist in *Ornithodoros moubata* ticks (Duron *et al*., 2018). *Francisella tularensis* could not be identified among all *16S rRNA* metarbarcoding *Francisella* spp ‘s positive samples. In the current study, only *Francisella*-like endosymbionts were detected using broad range primers. A study conducted in the United Arab Emirates investigating tick-borne pathogens in livestock found a 5.81% infection prevalence *Francisella* spp. in camels. Sequences from the above study were very similar to *Francisella-like* endosymbiont based on DNA similarity records from Genbank (Perveen *et al*., 2021). All the above studies used primers targeting the *16S rRNA* gene region, for more species-specific results. This is the 1st study to report on the presence of *Francisella*-like endosymbionts in *R. e. evertsi* ticks infecting horses and cattle in South Africa. A species-specific PCR targeting a putative succinate dehydrogenase locus (*sdhA*) was conducted using primers SdhF/ SdhR, however, no DNA amplification was successful.

In the previous chapter, *Staphylococcus* spp. was detected in ticks collected from livestock and vegetation; however, their presence could not be conclusively confirmed using PCR. Although amplification at the expected band sizes was achieved, the sequencing results yielded low-quality sequences, making the fidings unreliable. This highlights the need for further research to identify *Staphylococcus* species at the species level in ticks infesting livestock to better understand their ecological role and potential as vectors. Staphylococci are colonizers and common opportunistic pathogens of mammals and birds (Lindsay, 2019). *Staphylococcus aureus* is clinically the most significant or frequently sequenced genera due to its virulence and antimicrobial resistance (Hauschild and Stepanovic 2008). The *S. aureus* was not detected in the present study. Andreotti *et al*., (2011); reported 18% of *Staphylococcus* spp. in *R. microplus* ticks collected from cattle. A 31.79% infection prevalence of *Staphylococcus* was reported in dogs from Mnisi community, South Africa (Ackermann *et al*., 2019). Al-Rubaie *et al*., (2019) reported 23.88% infection of *S. aureus* in *Rhipicephalus* spp. collected from cattle and sheep in Baghdad city. All the above studies showed lower infection prevalence of *Staphylococcus* as compared to the high (78.1%) infection prevalence detected in the current study (Chapter 4). The presence of *Staphylococcus* species in *Rhipicephalus* dog ticks was supported in a systematic review study conducted on *R. sangueneus* and *Heamaphysalis elliptica* dog ticks. *Staphylococcus* spp. are the primary bacteria consistently identified in mastitic milk affecting the diary farming sector (Dabele *et al*., 2021). Which is why, further studies on *Staphylococcus* in livestock and their associated ticks are important. There are limited studies on the presence of *Staphylococcus* in ticks collected from livestock.

In the previous chapter, the prevalence of *Clostridium* spp was determined to be 19.05% in ticks collected from livestock and vegetation. Species-identification could not be confirmed using species-specific primers. Even so, the presence of this bacterium remains significant and warrants further investigation. Clostridium species are commonly associated with meat spoilage (Burgess *et al*., 2022), which can lead to substantial economic losses, particularly for small-scale farmers who rely on livestock for their livelihoods. Given the potential implications of *Clostridium* spp. in both animal health and food safety, further investigations are warranted. Future studies should focus on identifying *Clostridium* species at the species level in ticks infesting livestock and assess their potential role as vectors. The bacterial genera of *Clostridium* have been detected in various athropods (Makhahlela *et al*., 2022; Mwaki *et al.,* 2022). Karim *et al*., (2017) found that *Clostridium* species were the most prevalent microbial species, accounting for over 80%, in *Haemaphysali*s ticks collected from goats in Pakistan.

In the current study 8.57% (n=9) *Campylobacter* spp. was detected by metarbarcoding in *Rhipicephalus* ticks (n=105) collected from livestock (sheep, goats, cattle, horses), dogs, laboratory reared and vegetation (previous chapter). In this chapter, these samples were further confirmed using PCR, targeting the *16S rRNA* gene using genera-specific primers. The PCR amplification yielded positive results for the different markers at correct band sizes, confirming the presence of *Campylobacter* species in some tick samples collected from sheep, goats, cattle, and horses. A study done in South Africa in 2023 suggested that campylobacteriosisis is environmentally mediated fuelled by conatiminated rivers (Nnadozie, 2023). Members of this genus are known to be a leading cause of foodborne gastrointestinal illness (Komba *et al*., 2015). From the same study, an overall prevalence of *Campylobacter* infection of 11.4 % was

reported from sampled human subjects (n= 1195). The presence of these pathogens in ticks infesting livestock and humans cannot be ignored. Furthermore, In Botswana phylogeny results showed that the *Campylobacter* spp. Isolated from different poultry and human sources were highly related, suggesting that zoonotic transmission has likely occurred (de Vries *et al*., 2018). Besides chicken consumption, ticks could also be a contributing factor to the above transmission and these warrants further investigaton. Future studies should focus on identifying *Campylobacter* species at the species level in ticks infesting livestock and assess their potential role as vectors.

In the previous chapter, *Pseudomonas* spp. were detected in ticks collected from livestock and vegetation; however, their presence could not be conclusively confirmed using PCR. Although amplification at the expected band sizes was achieved, the sequencing results yielded low-quality sequences, making the fidings unreliable. Despite this limitation, a high *Pseudomonas* spp. prevalence of 61.09% was reported in *Rhipiciphalus* ticks (Chapter 4). This highlights the need for further research to identify *Pseudomonas* species at the species level in ticks infesting livestock to better understand their ecological role and potential as vectors. Members of the *Pseudomonas* genus are of particular concern due to their high resistance to commonly used antibiotics in livestock treatment (Falodun and Musa 2020). Several studies have frequently detected *Pseudomonas* spp. in microbial analyses of ticks, with reported prevalence rates of 61%; 67.5%; and 35% in ixodes tick samples (Moreno *et al*., 2006; Tveten *et al.,* 2013; Tahulending *et al*., 2022. Moreover, *Pseudomonas* spp. are known pathogens implicated in bovine mastitis (Aguayo *et al*., 2020), which can negatively impact livestock health and productivity. Beyond veterinary significance, *Pseudomonas* species are also recognized as opportunistic human pathogens, causing a wide range of infections, including osteomyelitis, arthritis,

bacteremia, endocarditis, endophthalmitis, pneumonia, empyema, urinary tract infections, and meningitis are human infections caused by *Pseudomonas* species (Tahulending *et al*., 2022). Given their prevalence and potential impact on both animal and human health, further investigation is warranted to determine their role in tick microbiota and assess their potential for disease transmission.

The presence of *Streptococcus* spp. was detected by conventional PCR using genera and species-specific primers. The correct amplification of the PCR products at the correct band size confirms the presence of *Streptococcus* in tick samples collected from livestock and vegetation in this study. The above was done to confirm the presence of species from the genus *Streptococcus* that were detected using *16S rRNA* metabarcoding in the previous chapter. The presence of *Streptococcus* was detected in relatively moderate abundance in this study at 28.57% using metabarcoding (previous Chapter), This is similar to a previous study where *Streptococcus* relative abundance was ranging between 6-22% in *Hyalomma anatolicum* ticks collected from livestock. Andreotti *et al.,* (2011), reported the presence of *Streptococcus* in relatively low percentages of 2.9% in *R. microplus* tick eggs and 1% in adult males through tag-encoded pyrosequencing. Although the presence of *Streptococcus* was not in relative high abundance in the current study, this finding cannot be ignored. Members of this genus are known to form part of the variety of pathogens that cause Upper respiratory tract infections commonly found in horses (Arafa *et al.,* 2021a). Members of the genus *Streptococcus* detected in the present study were from ticks collected from a variety of livestock including horses. *Streptococcus dysgalactiae subspecies* was detected using species-specific primers. *Streptococcus dysgalactiae subsp. dysgalactiae* has been known as a strict animal pathogen (Alves-Barroco et al., 2021), however recent reports of human infections have been reported *(He et al., 2023).* Members of this

species are known as one of the most prevalent pathogens *causing bovine mastitis* (*Xu et al., 2021*). The epidemiology of *Streptococcus dysgalactiae* bacterium has been described in dairy herds (Cameron *et al*., 2016; Horpiencharoen *et al*., 2019). This is the first study to report the presence of *Streptococcus dysgalactiae* subsp. *dysgalactiae* in South African *R. evertsi evertsi* ticks collected from horsed. This warrants further investigations where all samples with positive amplification for the targeted *Streptococcus* gene are sequenced successfully for species identification. In South Africa, the presence of *Streptococcus* in *R. sanguneaus* ticks collected from dogs was reported at a prevalence of 44.58% (Ackerman, 2019) and again in relatively lower abundance in a study conducted in *R. sanguineus* collected from dog samples in North West Province (Van Wyk, 2019).

CHAPTER 6:

General conclusion and recommendations

6.1 Introduction

This section summarizes all the major findings from Chapters 3 to 5 and addresses any observed shortcomings. Subsequently, the major findings are linked to the study's aims, conclusions are drawn, and recommendations for future research are suggested. The study aimed to characterize and compare the microbial populations of field-derived and colony-reared *Rhipicephalus* tick species, focusing on their symbiotic, medical, veterinary, and environmental significance.

6.2.1 Characterization of *Rhipichepalus* ticks collected from domestic animals and/or vegetation in four provinces in South Africa

The objective was to identify *Rhipicephalus* tick species morphologically using microscopy and by amplification and sequencing of *CO1*, *16S rRNA*, *ITS2* and *18S rRNA* genes and phylogenetic analysis. Field-derieved ticks were morphologically identified as *R. e. evertsi*, *R. appendiculatus* and *R. sanguineus;* whilst colony reared ticks were identified as *R. e. evertsi*, *R. appendiculatus*, *R. decoloratus* and *R. microplus.*

Molecular identification was conducted to confirm morphological findings. The sequencing quality of ribosomal *16 rRNA* was highest among the four markers in the current study. Followed by the mitochondrial *CO1* gene known as the universal standard barcoding marker. The later could not produce quality sequences for some *Rhipicephalus* species, this could be due to DNA quality or that the samples were

pooled and produced chimeras. The *18S rRNA* gene could not provide a clear separation of species but resolved relationships at higher taxa, this corresponds with previous findings (Lv *et al*., 2014a; Burger *et al*., 2014; Low *et al*., 2015, Mahlobo-Shwabede *et al*., 2022). The *ITS2* fragment yielded more poor-quality sequences compared to the *CO1*, *18S rRNA*, and *16S rRNA* fragments, corroborating Lv *et al*. (2014a). In the current study, *R. e. evertsi* was the most abundant species collected from the different hosts (Horses, sheep, goats, and cattle).

All ticks collected from KwaZulu-Natal province on vegetation were identified as *R. appendiculatus*, these corresponds to findings made by Mahlobo-Shwabede *et al*., 2022 in Lesotho. This isn't surprising, as this tick species typically favors woodlands and vegetation (Walker *et al*., 2014). In this study, *R. sanguineus* ticks were found on dogs, aligning with previous research (Walker *et al*., 2004) that led to the tick being commonly referred to as "a dog tick." Sequences from this study matched the morphological determinations on the NCBI database and clustered with relevant species on the Maximum Likelyhood phylogenetic tree. The discrepancy found in the current study was with the identification of laboratory reared *R. (B) decoloratus* and *R. (B) microplus* nymphs. These were morphologicaly described as separate species but molecularly identified as *R. (B) microplus* using NCBI BLASTn search results. Identifying closely related species at early life stages using morphology is challenging (Olson *et al.,* 1991), due to equivocal traits, underdeveloped features, even seasoned taxonomist can make a mistake.

6.2.2. Recommendations

Further research is necessary to obtain a comprehensive picture on the relative abundance of *Rhipicephalus* ticks in livestock and vegetation from the sampled areas and across the country. Sampling should be conducted seasonally to have an idea of which *Rhipicephalus* tick species are abundant at which season from the different hosts and vegetation. Sample collection should be consistent for each host in the four different provinces. Morphological identification of tick species must be accompanied with molecular identification to avoid errors. The use of at least two reliable identification markers is encouraged. This study sucessfully identified *Rhipicephalus* ticks (*R. sanguineus*, *R. e. evertsi*, *R. appendiculatus* and *R. microplus*) based on *16S*, *CO1*, *ITS2 markers.* The *CO1* marker should be the primary choice for tick species identification (standard DNA barcode), while *16S rRNA* and *ITS2* can be used to support *CO1* data or in cases where *CO1* yields unreliable results. The *18S rRNA* marker can be used to resolve higher tick taxon.

6.3.1 Microbiota in *Rhipicephalus* ticks from four provinces of South Africa

The objective was to investigate the bacterial communities harbored by *Rhipicephalus* ticks (*R. sanguineus*, *R. appendiculatus*, *R. e. evertsi* and *R. (B) decolaratus* and *R. (B) microplus*) by the *16S rRNA* v3-v4 hypervariable region amplicon sequencing approach utilizing Illumina next generation sequencing platform. The most abundant phyla included Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. The remaining 23 phyla contributed 22.2% of the overall ASV sequences in low abundance. The most dominant families detected were Coxiellaceae, Pseudomadaceae, Enterobacteriaceae, Staphylococceae and Maraxelleae. The

metabarcoding method used in the present study was able to detect bacteria down to genus level (or higher taxonomic levels), species detection was not recorded. Genera that may include potential pathogenic species and environmentally important genera detected include *Sphingobium*, *Achromobacter*, *Roalstonia*, *Neisseria*, *Proteus*, *Legionella*, *Haemophilus*, *Paracoccus*, *Acinetobacter*, *Moraxella*, *Pseudomonas* and *Escherichia-Shigella, Enterococcus, Lactobacillus, Streptococcus, Aerococcus, Clostridium sensu stricto 1,13,18, and 8, Staphylococcus, Corynebacterium, Micrococcus, Rhodococcus and Streptomyces, Cloacibacterium.* Members of the genera *Rickettsiae, Coxiella, Francisella, Ehrlichia* and *Anaplasma* detected in the present study are known to contain potential tick-borne zoonotic pathogens. Furthermore, endosymbionts genera detected were *Coxiella*, *Rickettsia*, and *Francisella.* Endosymbionts play different roles in the survival of athropods, they provide important benefits to host affecting its development, nutrition, chemical defense and reproduction (Machado-Ferreira *et al*., 2016). This should be investigated further as this information could assist when formulating tick control strategies. Many veterinary, environmental and medically important bacteria genera detected in field derived *Rhipicephalu*s ticks were not detected in colony reared ticks using metabarcoding (*Appendix 8.6*).

6.3.2 Recommendations

Future metagenomic research on *Rhipicephalus* tick species should focus on identifying bacterial communities at various developmental stages. This approach may help explain bacterial diversity throughout the tick's life cycle and aid in tracing bacterial transmission from egg to adult. Avoid using whole ticks in metabarcoding analysis, this does not allow one to define the tissue specific microbiome. When whole

ticks are assessed, one cannot discern exoskeleton-associated environmental contaminants from bona-fide tick tissue-associated bacteria. However, exoskeleton-associated bacteria should not be overlooked, as they might offer additional barrier surveillance strategies similar to those observed in the mammalian skin microbiome. Bacteria composition in male ticks should also be compared to that of females. Again, spatio-stemporal scale (the scale within the tick at organ level) or the context in which the study has been performed (environmental parameters) (Pollet *et al*., 2020) may be considered in microbial communitie studies.

6.4.1 Molecular detection of zoonotic pathogens from collected *Rhipicephalus* ticks

The objective of this chapter was to conduct molecular detection of zoonotic pathogens from *Rhipicephalus* ticks collected from the environment (vegetation), livestock and domestic animals using conventional PCR. Zoonotic pathogens were investigated using conventional PCR with the intetion of resolving species of genera generated by *16S rRNA* metabarcoding technique in chapter 4. Genus and species-specific PCR assays were used, BLASTn search on the NCBI database identified species like *Ricketttsia aeschlimanni*, *Erlichia canis*, *Escherichia coli*, *Coxiella-like endosymbionts*, and *Francisella-like endosymbionts*. The following pathogens were amplified and sequenced several times (*Staphylococcus* spp, *Anaplasma* spp. and *Clostridium* spp.) however, the sequences yielded no quality results. The presence of *Coxiella burnetti* and *Streptococcus dysgalactiae* subspecies was confirmed by PCR amplification using species-specific primers. Lastly, the presence of *Pseudomonas* spp. and *Campylobacter* spp., were confirmed by PCR amplification using genus-specific primers, species names for these pathogens were not resolved. Sequencing

could not be conducted for all pathogens detected, the funding for the project was exhausted. Most PCR protocols for species like *Clostridium*, *Staphylococcus*, *Streptococcus*, *Campylobacter*, *Pseudomonas* was previously developed and evaluated using isolated bacterial colonies (Manson *et al*., 2001; Ivanova *et al*., 2014; Komba *et al*., 2015, Moriconi *et al*., 2017; Falodun and Musa 2020; Arafa *et al*., 2021a; Burgess *et al*., 2022; Elbehiry *et al*., 2022; Feyissa *et al*., 2023). This was not the case in the present study and could have been a limiting factor in retrieving high-quality DNA for sequencing and BLASTn search on the NCBI database for species identification.

6.4.2 Recommendations

Further detection of tick-borne pathogens must be conducted using species-specific primers for all bacteria of veterinary, environmental, and medical importance in *Rhipicephalus* tick species infesting domestic animals and vegetation. Where suitable primers are missing, primer design studies are encouraged. This will speed up diagnosis inturn, providing information that can be used in formulating control strategies of potential pathogens to both animals and humans.

Results from this study indicates that ticks from the genus *Rhipicephalus* are widely distributed in South Africa and are potential reseviors of important bacterial pathogens of zoonotic potential. Identified tick simbionts such as *Coxiella*-like and *Francisella*-like, require additional research as they play a large role in the survival of ticks and transmission of pathogens. In the present study ethanol was used as a surface treatment to remove microbe contaminants present on tick cuticle. However, a study done by Binetruy *et al*., (2019) suggest bleach as a better method for surface

sterilization when investigating internal microbial diversity in ticks. This should be considered in future studies.

7. REFERENCES

Abbas, R.Z., Zaman, M.A., Colwell, D.D., Gilleard, J. and Iqbal, Z., (2014). Acaricide resistance in cattle ticks and approaches to its management: The state of play. *Veterinary Parasitology.* 203:6-20

Abdel-Shafy, S. (2018). Is the cattle tick *Rhipicephalus annulatus* Say, 1821 reared on the rabbit? *Journal of Parasite Diseases.* 42(2): 297-302.

Ackermann, R. (2019). Bacterial of *Rhipicephalus sanguineus* ticks collected from dogs in the Mnisi community, South Africa (master's dissertation, University of Pretoria (South Africa).

Adehan, S.B., Biguezoton, A., Adakal, H., Assogba, M.N., Gbaguidi, A.M. and Tonouhewa, A., (2016). Acaricide resistance of *Rhipicephalus microplus* ticks in Benin. *African Journal of Agricultural Research.* 11: 1199-1208.

Adelabu, O.A., Iweriebor, B.C., Okoh, A.I. and Obi, L.C. (2020). Phylogenetic profiling for zoonotic *Ehrlichia* spp. from ixodid ticks in the Eastern Cape, South Africa. *Transboundary and Emerging Diseases*. 67(3): 1247-1256.

Aguayo, P.R., Larenas, T.B., Godoy, C.A., Rivas, B.C., González-Casanova, J., Rojas-Gómez, D. and Fuentes, N.C. (2020). Antimicrobial and Antibiofilm capacity of chitosan nanoparticles against wild type strain of *Pseudomonas sp*. isolated from milk of cows diagnosed with *Bovine mastitis*. *Antibiotics.* 9: 551

Ahantarig, A., Trinachartvanit, W., Baimai, V. and Grubhoffer, L. (2013) Hard ticks and their bacterial endosymbionts (or would be pathogens). *Folia Microbiologica* (Praha). 58: 419–428.

Ahmad, M., Hassan, M., Khalid, A., Tariq, I., Asad, M.H.H.B., Abdul Samad, A., Qaisar Mahmood, Q., and Murtaza, G., (2016). Prevalence of extended spectrum β-lactamase and antimicrobial susceptibility pattern of clinical isolates of

Pseudomonas from patients of Khyber Pakhtunkhwa, Pakistan. *BioMed Research International*, Article ID 6068429.

Aivelo, T., Lemoine, M. and Tschirren, B. (2022). Elevational changes in bacterial microbiota structure and diversity in an arthropod-disease Vector. *Microbial Ecology*. 84(3): 868-878.

Alim, M.A., Das, S., Roy, K., Masuduzzaman, M., Sikder, S., Hassan, M.M., Siddiki, A.Z. and Hossain, M.A. (2012). Prevalence of hemoprotozoan diseases in cattle population of Chittagong division, Bangladesh. *Pakistan Veterinary Journal.* 32: 221–224.

Al-Rubaie, E.M.M., Al-Maaly, N.M.H. and Al-Rubaie, H.M.A. (2019). Isolation and characterization of *Klebsiella* spp. and *Staphylococcus aureus* from engorged adult females of *Rhipicephalus* spp. *Journal of Pure Applied Microbiology.* 13(3): 1763-1767

Althaus, F.; Greub, G.; Raoult, D. and Genton, B. (2010). African tick-bite fever: A new entity in the differential diagnosis of multiple eschars in travelers. Description of five cases imported from South Africa to Switzerland. *International Journal of Infectious Diseases*. 14S: e274–e276.

Alves-Barroco, C., Caço, J., Roma-Rodrigues, C., Fernandes, A.R., Bexiga, R., Oliveira, M., Chambel, L., Tenreiro, R., Mato, R. and Santos-Sanches, I. (2021). New insights on *Streptococcus dysgalactiae* subsp. *Dysgalactiae* Isolates. *Frontiers in Microbiology*.12: 686413

Anacker, R.L., List, R.H., Mann, R.E, and Wiedbrauk, D.L. (1986). Antigenic Heterogeneity in High- and Low-virulence strains of *Rickettsia rickettsii* revealed by monoclonal antibodies. *Infection and Immunity*. c 51(2): 653-660

Anderson, J.F. and Magnorelli, L.A. (2008). Biology of ticks. *Infectious Disease Clinics of North America.* 22: 195-215.

Andreotti, R., Pérez de León, A.A., Dowd, S.E., Guerrero, F.D., Bendele, K.G. and Scoles, G.A. (2011). Assessment of bacterial diversity in the cattle tick *Rhipicephalus (Boophilus) microplus* through tag-encoded pyrosequencing. *BMC Microbiology*. 11(1): 6.

Andrić, B. (2014) Diagnostic evaluation of *Ehrlichia canis* human infections. *Open Journal of Medical Microbiology*. 4: 132-139.

Angelakis, E., Mediannikov, O., Jos, S., Berenger, J., Parola, P. and Raoult, D. (2016) Candidatus Coxiella massiliensis infection. *Emerging Infectious Diseases*. 22: 285-288.

Aouadi, A., Leulmi, H., Boucheikhchoukh, M., Benakhla, A., Raoult, D. and Parola, P. (2017). Molecular evidence of tick-borne hemoprotozoan-parasites (*Theileria ovis* and *Babesia ovis*) and bacteria in ticks and blood from small ruminants in Northern Algeria. *Comparative immunology, Microbiology and Infectious Diseases.* 50: 34-39.

Arafa, A.A., Hedia, R.H., Ata, N.S. and Ibrahim, E.S. (2021a). Vancomycin resistant *Streptococcus equi* subsp*. Equi* isolated from equines suffering from respiratory manifestation in Egypt. *Veterinary World*. 14(7):1 808-1814.

Arafa, W.M., Aboelhadid, S.M., Moawad, A., Shokeir, K.M., Ahmed, O. and de Leon, A.A.P., (2021b). Control of *Rhipicephalus annulatus* resistant to deltamethrin by spraying infested cattle with synergistic eucalyptus essential oil-thymol-deltamethrin combination. *Veterinary Parasitology*. 290:

Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H-P., Clément, C., Ouhdouch, Y. and van Wezel, G.P. (2016). Taxonomy, physiology,

and natural products of *Actinobacteria*. *Microbiology and Molecular Biology Reviews*. 80: 1-43.

Barker, S.C. (1998). Distinguishing species and populations of rhipicephaline ticks with *ITS 2 ribosomal RNA*. *Journal of Parasitology.* 84: 887-892.

Barker, S.C. and Walker, A.R. (2014). Ticks of Australia. The species that infest domestic animals and humans. *Zootaxa.* 3816 (1): 1-144

Barlough, J.E., Madigan, J.E., DeRock, E. Bigornia, L. (1996). Nested polymerase chain reaction for detection of *Ehrlichia equi* genomic DNA in horses and ticks (*Ixodes pacificus*). *Veterinary Parasitology.* 63 (3-4): 319-329.

Barns, S. M., Grow, C. C., Okinaka, R. T., Keim, P. and Kuske, C. R. (2005). Detection of diverse new *Francisella-like bacteria* in environmental samples. *Applied and Environmental Microbiology*. 71(9): *5494–5500*.

Basu, A.K. and Charles, R. (2017) Chapter 1—A general account of ticks. In ticks of Trinidad and Tobago—An overview; Academic press: Cambridge, MA, USA, 2017: 1-33.

Bay, V., Griffiths, B., Carter, S., Evans, N.J., Lenzi, L., Bicalho, R.C. and Oikonomou, G. (2018) *16S rRNA* amplicon sequencing reveals a polymicrobial nature of complicated claw horn disruption lesions and interdigital phlegmon in dairy cattle. *Scietific Reports*. 8(1):15529.

Becker, K., Harmsen, D., Mellmann, A., Meier, C., Schumann, P., Peters, G. and von Eiff, C. (2004). Development and evaluation of a quality-controlled ribosomal sequence database for 16S ribosomal DNA-based identification of *Staphylococcus* species. *Journal of Clinical Microbiology*. 42(11): 4988-95.

Bedouhene, A., Kelanemer, R., Medrouh, B., Kernif, T., Saidi, F., Tail, G. and Ziam, H. (2022). Seasonal dynamics and predilection sites of ticks (Acari: Ixodidae)

feeding on cows in the western parts of the Djurdjura, Algeria. *Frontiers in Tropical Diseases*. 3: 856179.

Bej, A.K., Patterson, D.P., Brasher, C.W., Vickery, M.C.L., Jones, D.D. and Kaysner, C.A., (1999). Detection of total and hemolysin-producing vibrio parahaemolyticus in shellfishusing multiplex PCR amplification of *tl*, *tdh* and *trh*. *Journal of Microbiological Methods* 36: 215-225.

Berrada, Z.L. and Telford 3rd., S.R. (2009). Burden of tick-borne infections on American companion animals. *Topics in Companion Animal Medicine*. 24: 175-181.

Binetruy, F., Dupraz, M. and Buysse, M. (2019). Duron, O. Surface sterilization methods impact measures of internal microbial diversity in ticks. *Parasites & Vectors.* 12(1): 268.

Black, W.C. 4th. and Piesman, J. (1994). Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proceedings of the National Academy of the Sciences of the United States of America*. 91(21): 10034-8.

Bochniarz, M., Dąbrowski, R., Kocki, T., Błaszczyk, P., Szczubiał, M., Brodzki, P., Krakowski, L.and Tursk, W.A. (2021). Content of tryptophan and kynurenines in serum and milk of dairy cows with mastitis caused by *Streptococcus spp*. *Reproduction in Domestic Animals*. 57: 277-283.

Bonnet, S., Michelet, L., Moutailler, S., Cheval, J., Hebert, C., Vayssier-Taussat, M. and Eliot, M. (2014). Identification of parasitic communities within European ticks using next-generation sequencing. *PLOS Neglected Tropical Diseases.* 8 (3): e2753.

Bonnet, S.I. and Pollet, T. (2021). Update on the intricate tango between tick microbiomes and tick-borne pathogens. *Parasite Immunology*. 43(5): e12813.

Bonnet, S.I., Binetruy, F., Hernández-Jarguín, A.M. and Duron, O. (2017) The Tick Microbiome: Why non-pathogenic microorganisms matter in tick biology and pathogen transmission. *Frontiers in Cellular Infection Microbiology*. 7: 236.

Boucher, F., Moutroifi, Y., Peba, B., Ali, M., Moindjie, Y., Ruget, A.S., Abdouroihamane, S., Madi Kassim, A., Soulé, M., Charafouddine, O., Cêtre-Sossah, C. and Cardinale, E. (2020). Tick-borne diseases in the Union of the Comoros are a hindrance to livestock development: Circulation and associated risk factors. *Ticks and Tick-borne Diseases*. 11(1): 101283.

Brahma, R.K., Dixit, V., Sangwan, A.k. and Doley, R. (2014). Identification and characterization of *Rhipicephalus* (*Boophilus*) *microplus* and *Haemaphysalis bispinosa* ticks (Acari: Ixodidae) of North East India by ITS2 and 16S rDNA sequences and morphological analysis. *Experimental and Applied Acarology.* 62 (2): 253-265.

Brianti, E., Otranto, D., Dantas-Torres, F., Weigl, S., Latrofa, M.S., Gaglio, G., Napoli, E., Brucato, G., Cauquil, L., Giannetto, S. and Bain, O. (2012). *Rhipicephalus sanguineus* (Ixodida, Ixodidae) as intermediate host of a canine neglected filarial species with dermal microfilariae. *Veterinary Parasitology.* 183: 330-337.

Brites-Neto, J., Duarte K.M.R., and Martins T.F. (2015). Tick-borne infections in human and animal population worldwide. *Veterinary World.* 8 (3): 301-315.

Brook, I. (2015). Fusobacterial head and neck infections in children. *International Journal of Pediatric Otorhinolaryngology*,79(7): 953-958, ISSN 0165-5876

Brown, W.M. (1985). The mitochondrial genome of animals. In *Molecular evolutionary genetics*, MacIntyre RJ. New York, NY: Springer.

Brown, W.M., Prager, E.M., Wang, A., and Wilson, A.C. (1982). Mitochondrial DNA sequences of primates: tempo and mode of evolution. *Journal of Molecular Evolution*. 18(4): 225-39.

Burgdorfer, W., Barbour, A.G., Hayes, S.F., Benach, J.L., Grunwaldt, E and Davis, J.P. (1982) Lyme disease-a tick-borne spirochetosis? *Science* 216: 1317-1319

Burgdorfer, W., Brinton, L.P. and Hughes, L.E. (1973). Isolation and characterization of symbiotes from the rocky mountain wood tick *Dermacentor andersoni*. *Journal of Invertebrates Pathology.* 22: 424-434.

Burger, T.D., Shao, R. and Barker, S.C. (2013). Phylogenetic analysis of the mitochondrial genomes and nuclear rRNA genes of ticks reveals a deep phylogenetic structure within the genus *Haemaphysalis* and further elucidates the polyphyly of the genus Amblyomma with respect to Amblyomma sphenodonti and Amblyomma elaphense. *Ticks Tick-Borne Disease*. 4: 265-274.

Burger, T.D., Shao, R., Labruna M.B. and Barker, S.C. (2014). Molecular phylogeny of soft ticks (Ixodida: Argasidae) inferred from mitochondrial genome and nuclear rRNA sequences. *Ticks Tick-Borne Disease*. 5: 195-207.

Burgess, S.A., Palevich, F.P., Gardner, A., Mills, J., Brightwell, G. and Palevich, N. (2022). Occurrence of genes encoding spore germination in *Clostridium* species that cause meat spoilage. *Microbial Genomics*. 8(2): 000767.

Bush, L.M. and Vazquez-Pertejo, M.T. (2018). Tick borne illness-Lyme disease. *Disease-a-Month*. 64(5): 195-212.

Cabezas-Cruz, A., Pollet, T., Estrada-Peña, A., Allain, E., I. Bonnet, S. and Moutailler, S. (2018). Handling the microbial complexity associated to ticks. *IntechOpen*. doi: 10.5772/intechopen.80511

Cabrera Orrego, R., Ríos-Osorio, L.A., Keynan, Y., Rueda, Z.V. and Gutiérrez, L.A. (2020). Molecular detection of *Coxiella burnetii* in livestock farmers and cattle from Magdalena Medio in Antioquia, Colombia. *PLoS One*. 15(6): e0234360.

Cameron, M., Saab, M., Heider, L., McClure, J. T., Rodriguez-Lecompte, J. C. and Sanchez, J. (2016). Antimicrobial susceptibility patterns of environmental *Streptococci* recovered from bovine milk samples in the maritime provinces of Canada. *Frontiers in Veterinary Science* .3: 79

Campbell, A. G., Schwientek, P., Vishnivetskaya, T., Woyke, T., Levy, S., Beall, C. J. and Podar, M. (2014). Diversity and genomic insights into the uncultured *Chloroflexi* from the human microbiota. *Environmental Microbiology,* 16(9): 2635-2643.

Caporale, D.A., Rich, S.M., Spielman, A., Telford, S.R. and Kocher, T.D. (1995). Discriminating between Ixodes ticks by means of mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution.* 4(4): 361-365.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., … and Huttley, G.A. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods.* 7(5): 335-336.

Cardenas-Cadena, S.A., Castañeda-Lopez, M.E., Mollinedo-Montaño, F.E., Vazquez-Reyes, S., Lara-Arias, J., Marino-Martinez, I.A., Rodriguez-Sanchez, I.P., Garza-Veloz, I. and Martinez-Fierro, M.L. (2023). Tick-borne pathogens screening using a Multiplex real-time polymerase hain reaction-based method. *Acta Parasitology.* 68(3): 705-710.

Carlsson, F. and Brown, E.J. (2006). Actin-based motility of intracellular bacteria, and polarized surface distribution of the bacterial effector molecules. *Journal of Cellular Physiology.* 209: 288–296.

Carmichael, J.R. and Fuerst, P.A. A. (2006). Rickettsial mixed infection in a *Dermacentor variabilis* tick from Ohio. *Annals of New York Academy.of Science.* 1078:334–337.

Carpi, G., Cagnacci, F., Wittekindt, N.E., Zhao, F., Qi, J.I., Tomsho, L.P., Drautz, D.I., Rizzoli, A. and Schuster, S. (2011). Metagenomic profile of the bacterial communities associated with *Ixodes ricinus* ticks. *PLoS ONE* 6(10): e25604

Caudata: Hynobiidae). *Molecular Ecology Resources*. 12(1): 48-56.

Chandra, S., Harvey, E., Emery, D., Holmes, E.C. and Šlapeta, J. (2021) Unbiased characterization of the microbiome and virome of questing ticks. *Frontiers in Microbiology*. 12: 627327.

Chen X, Y.u.Z., Guo, L., Li, L., Meng, H., Wang, D., Liu, R. and Liu, J. (2012). Life cycle of haemaphysalis doenitzi (acari: ixodidae) under laboratory conditions and its phylogeny based on mitochondrial *16S rDNA*. *Experimental and Applied Acarology.* 56:143-50.

Chicana, B., Couper, L.I., Kwan, J.Y., Tahiraj, E. and Swei, A. (2019). Comparative microbiome profiles of sympatric tick species from the Far-Western United States. *Insects*. 10(10): 353

Chigwada, A.D., Mapholi, N.O., Ogola, H.J.O., Mbizeni, S. and Masebe, T.M. (2022). Pathogenic and endosymbiotic bacteria and their associated antibiotic resistance biomarkers in *Amblyomma* and *Hyalomma* ticks nfesting Nguni Cattle (*Bos* spp.). *Pathogens.*11(4): 432.

Chitanga, S., Chibesa, K., Sichibalo, K., Mubemba, B., Nalubamba, K.S., Muleya, W., Changula, K. and Simulundu, E. (2021). Molecular detection and characterization of *Rickettsia* species in *Ixodid* ticks collected from cattle in Southern Zambia. *Frontiers in Veterinary Science.* 8: 684487.

Chitimia-Dobler, L.; Langguth, J.; Pfeffer, M.; Kattner, S.; Küpper, T.; Friese, D.; Dobler, G.; Guglielmone, A.A. and Nava, S. (2017). Genetic analysis of *Rhipicephalus sanguineus* sensu lato ticks parasites of dogs in Africa North of the sahara based on mitochondrial DNA sequences. *Veterinary Parasitology. 239*: 1-6.

Chomel B. (2011). Tick-borne infections in dogs-an emerging infectious threat. *Veterinary Parasitology.* 179: 294-301.

Cirimotich, C.M., Dong, Y., Clayton, A.M., Sandiford, S.L., Souza-Neto, J.A., Mulenga, M. and Dimopoulos, G. (2011). Natural microbe-mediated refractoriness to Plasmodium infection in *Anopheles gambiae*. *Science*. 332(6031): 855-8.

Clay, K., Klyachko, O., Grindle, N., Civitello, D., Oleske, D. and Fuqua, C. (2008) Microbial community ecology of ticks: prokaryotic diversity, distribution and interactions in the lone star tick, *Amblyomma americanum*. *Molecular Ecology.* 17: 4371-4381.

Clifford, C.M., Anastos, G., and Elbi, A. (1961). The larval ixodid ticks of the Eastern United States (Acarina-Ixodidae). *Miscellaneous Publications of the Entomological Society of America*. 2: 213-237.

Cockell, C. S., Kelly, L. C., and Marteinsson, V. (2013). Actinobacteria - an ancient phylum active in volcanic rock weathering. *Geomicrobiology Journal.* 30: 706-720

Coetzer, J.A.W. and Tustin R.C. (2004). Infectious diseases of livestock: 2nd edition Oxford University Press Southern Africa, Cape Town.

Colombo, V.C., Montani, M.E., Pavé, R., Antoniazzi, L.R., Gamboa, M.D., Fasano, A.A., Félix, M.L., Nava, S., and Venzal, J.M. (2020) First detection of "*Candidatus Rickettsia wissemanii*" in *Ornithodoros hasei* (Schulze, 1935) (Acari: Argasidae) from Argentina. *Ticks Tick Borne Diseases*. 11(4):101442.

communities. *Ticks Tick Borne Diseases*. 9:563–572.

Cook, T.M., Protheroe, R.T. and Handel, J.M. (2001) Tetanus: A review of the literature. *British Journal of Anaesthesia.* 87(3): 477-487.

Cooper A, Hedlefs R, McGowan M, Ketheesan N, Govan B. (2011). Serological evidence of *Coxiella burnetii* infection in beef cattle in Queensland. *Australian Veterinary Journal*. 89(**7**):260-4.

Cortés, M.G.M., Fernández-García, J.L. and Martínez-Estéllez, M.A.H. (2017). Seroprevalence of *Theileria equi* and *Babesia caballi* in horses in Spain, *Parasite* 24: 14

Costa, D. and Iraola, G. (2019). Pathogenomics of emerging *Campylobacter* species. *Clinical Microbiology Reviews*. 32(4): e00072-18.

Couper, L.I., Kwan, J.Y., Ma, J. and Swei, A. (2019). Drivers and patterns of microbial community assembly in a Lyme disease vector. *Ecology and Evolution* 9: 7768-7779.

Cowdry, E.V. (1925). A group of micro-organisms transmitted hereditarily in ticks and apparently unassociated with disease. *Journal of Experiemental Medicine.* 41: 817-830.

Cutler, S., Abdissab, A., Adamub, H., Tadele Tolosac, T. and Gashawc, A. (2012) Borrelia in Ethiopian ticks. *Ticks and Tick-borne Diseases.* 3: 14-17

Dabele, D.T., Borena, B.M., Snr P.A. and Gebremedhin, E.Z. (2021). Marami, L.M. Prevalence and risk factors of mastitis and isolation, identification and antibiogram of *Staphylococcus* species from mastitis positive zebu cows in toke kutaye, cheliya, and dendi districts, West Shewa zone, Oromia, Ethiopia. *Infection and Drug Resistance.*14: 987.

Dalis, J.S., Kazeem, H. M, Kwaga, J. K. P and Kwanashie, C. N. (2014). An outbreak of ringworm caused by Trichophyton verrucosum in a group of calves in Vom, Nigeria, *African Journal of Microbiology Research.* 8(8): 783-787.

Daly, J.A. (1985). *Haemophilus ducreyi*. *Infection Control*. 6(5): 203-5

Dantas-Torres F. (2008). The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): from taxonomy to control. *Veterinary Parasitology* 152: 173-85.

Dantas-Torres, F. (2010). Biology and ecology of the brown dog tick, *Rhipicephalus sanguineus*. *Parasites Vectors.* 3: 26.

Dantas-Torres, F., Chomel, B.B and Otranto, D. (2012) Ticks and tick-borne diseases: a One Health perspective, *Trends in Parasitology.* 10: 437-46.

Dantas-Torres, F., Latrofa, M.S., Annoscia, G., Giannelli, A., Parisi, A. and Otranto, D. (2013). Morphological and genetic diversity of *Rhipicephalus sanguineus* sensu lato from the New and Old Worlds. *Parasite & Vectors*. 6 :213.

Davitt, C., Huggins, L.G., Pfeffer, M., Batchimeg, L., Jones, M., Battur, B., Wiethoelter, A.K. and Traub, R. (2024). Next-generation sequencing metabarcoding assays reveal diverse bacterial vector-borne pathogens of Mongolian dogs. *Current Research in Parasitology and Vector Borne Diseases*. 5: 100173. doi

Day, M.J. (2011). One Health: the importance of companion animal vector-borne diseases. *Parasites & Vectors.* 4: 49.

de la Fuente, J., Estrada-Pena, A., Venzal, J.M., Kocan, K.M. and Sonenshine, D.E. (2008). Overview: Ticks as vectors of pathogens that cause disease in humans and animals. *Frontiers in Bioscience-Landmark*. 13: 6938-46.

de Vries, S.P.W., Vurayai, M., Holmes, M., Gupta, S., Bateman, M., Goldfarb, D., Maskell, D.J., Matsheka, M.I., and Grant, A.J. (2018) Phylogenetic analyses and

antimicrobial resistance profiles of *Campylobacter* spp. from diarrhoeal patients and chickens in Botswana. *PLoS ONE* 13 (3): e0194481.

De Waal D.T. and Potgieter, F.T. (1987). The transstadial transmission of *Babesia Caballi* by *Rhipicephalus evertsi evertsi*. *Onderstepoort Journal of veterinary Research* 54: 655-656.

De Wall, D.T. (2000). Anaplasmosis control and diagnosis in South Africa. *Annals of the New York Academy of Sciences*. 916: 474-83.

Demma, L.J., Traeger, M.S, Nicholson, W.L., Paddock, C.D., Blau, D.M., Eremeeva, M.E. and McQuiston, J.H. (2005). Rocky mountain spotted fever from an unexpected tick vector in Arizona. *The New England journal of medicine.* 353: 587-94.

Derrick, E.H. (1983). "Q" fever, a new fever entity: clinical features, diagnosis and laboratory investigation. *Reviews of Infectious Diseases.* 5: 790–800.

Dhalluin, A., Lemée, L., Pestel-Caron, M., Mory, F., Leluan, G., Lemeland, J.F, Pons, J.L (2003). Genotypic differentiation of twelve *Clostridium* species by polymorphism analysis of the triosephosphate isomerase (*tpi*) gene. *Systematic and Applied Microbiology*. (1): 90-6.

Dhariwal, A.; Chong, J.; Habib, S.; King, I.L.; Agellon, L.B. and Xia, J. (2017). MicrobiomeAnalyst: A web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. *Nucleic Acids Research.* 45: W180–W188.

Dinkisa G. (2018). Review on control of cowdriosis in ruminants. *International Journal of Veterinary Science & Technology.* 2(1): 013-019

Dobson, S.J. and Barker, S.C. (1999). Phylogeny of the hard ticks (Ixodidae) inferred from 18S rRNA indicates that the genus Aponomma is paraphyletic. *Molecular Ecology Resources.* 11(2): 288-295.

Duan, D and Cheng, T. (2016). Determination of the microbial community features of Haemaphysalis flava in differenpot developmental stages by high-throughput sequencing. *Journal of Basic Microbiology.* 57: 302-308.

Duron, O., Jourdain, E., and McCoy, K. D. (2014). Diversity and global distribution of the *Coxiella* intracellular bacterium in seabird ticks. *Ticks and Tick-Borne Diseases*. 5(5), 557-563.

Duron, O.; Noël, V.; McCoy, K.D.; Bonazzi, M.; Sidi-Boumedine, K.; Morel, O.; Vavre, F.; Zenner, L.; Jourdain, E.; Durand, P.; Arnathau, C., Renaud, F., Trape, J.F., Biguezoton, A.S., Cremaschi, J., Dietrich, M., Léger, E., Appelgren, A., Dupraz, M., Gómez-Díaz, E., Diatta, G., Dayo, G.K., Adakal, H., Zoungrana, S., Vial, L. and Chevillon, C. (2015). The recent evolution of a maternally-inherited endosymbiont of ticks led to the emergence of the Q fever pathogen*, Coxiella burnetii*. PLoS Pathogens. 11: e1004892.

Duron, O., Morel, O., Noël, V., Buysse, M., Binetruy, F., Lancelot, R., Loire, E., Ménard, C., Bouchez, O., Vavre, F. and Vial, L. (2018). Tick-bacteria mutualism depends on B vitamin synthesis pathways. *Current Biology*. 28(12): 1896-1902.e5.

Duzlu, O., Yildirim, A., Inci, A., Gumussoy, K.S., Ciloglu, A. and Onder, Z. (2016). Molecular investigation of *Francisella-like endosymbiont* in ticks and *Francisella tularensis* in Ixodid ticks and mosquitoes in Turkey. *Vector-borne and zoonotic diseases.* 16(1): DOI: 10.1089/vbz.2015.1818.

Dzemo, W.D., Thekisoe, O. and Vudriko, P. (2022). Development of acaricide resistance in tick populations of cattle: A systematic review and meta-analysis. Heliyon. 8(1): e08718.

Ebani, V.V. (2019). Serological evidence of *Anaplasma phagocytophilum* and spotted fever group *Rickettsi* spp. exposure in horses from Central Italy. *Pathogens*. 8(3): 88.

Egan, S.L., Taylor, C.L., Banks, P.B., Northover, A.S., Ahlstrom, L.A., Ryan, U.M., Irwin, P.J., Oskam, C.L. (2021). The bacterial biome of ticks and their wildlife hosts at the urban-wildland interface. *Microbial Genomics*. 7(12):000730.

Elbehiry, A., Marzouk, E., Aldubaib, M., Moussa, I., Abalkhail, A., Ibrahem, M., Hamada, M., Sindi, W., Alzaben, F., Almuzaini, A.M., Algammal, A.M. and Rawway, M. (2022). *Pseudomonas* species prevalence, protein analysis, and antibiotic resistance: an evolving public health challenge. *AMB Express.* 12(1): 53.

El-Sayed, A. and Kamel, M. (2020). Climatic changes and their role in emergence and re-emergence of diseases. *Environmental Science and Pollution Research International.* 27(18): 22336-22352.

Emmerson, A.M., Perinpanayagam, R.M. and Barnado, D.E. (1981). Haemophilus endocarditis. *Postgraduate Medical Journal*. 57(664): 117-9

Ergunay, K., Mutinda, M., Bourke, B., Justi, S.A., Caicedo-Quiroga, L., Kamau, J., Mutura, S., Akunda, I.K., Cook, E., Gakuya, F., Omondi, P., Murray, S., Zimmerman, D. and Linton, Y-M. (2022). Metagenomic investigation of ticks from Kenyan wildlife reveals diverse microbial pathogens and new country pathogen records. *Frontiers in Microbiology.* 13: 932224.

Estrada-Peña, A. and Salman, M. (2013). Current Limitations in the Control and Spread of Ticks that Affect Livestock: A Review. *Agriculture*, *3*(2), 221-235.

Ezekiel, C.N., Ayeni, K.I., Ezeokoll, O.T., Sulyok, M., van Wyk, D.A.B. Oyedele, O.A. and Krska, R. (2019). High-throughput sequence analyses of bacterial communities

and multi-mycotoxin profiling during processing of different formulations of Kunu, a traditional fermented beverage. *Frontiers in Microbiology.* 9: 3282.

Falodun, O.I. and Musa, I.B. (2020) *Pseudomonas* species from cattle dung producing extended spectrum and metallo beta-lactamases. *European Journal of Biological Research* 210(1): 1-10

Fard, S.R.N. and Khalili, M. (2011). PCR-Detection of *Coxiella burnetii* in ticks collected from sheep and goats in South East Iran, *Iranian Journal of Arthropod-Borne Diseases.* 5(1): 1-6.

Feng, Y., Li, Q., Kong, L. and Zheng, X. (2011). DNA barcoding and phylogenetic analysis of pectinidae (Mmollusca: bivalviaBivalvia) based onmitochondrial coi and 16S rRNA genes. *Molecular Biology Reports.* 38: 291–9.

Feyissa, N., Alemu, T., Jirata Birri, D., Desalegn, A., Sombo, M., Abera, S. (2023). Isolation and determination of antibacterial sensitivity characteristics of *Staphylococcus aureus* from lactating cows in West Shewa zone, Ethiopia. *Veterinary Medicine International*. 2023: 3142231.

Finney, C.A.M., Kamhawi, S. and Wasmuth J.D. (2015). Does the arthropod microbiota impact the establishment of vector-borne diseases in mammalian host? *PLOS Pathogens.* 11(4): e1004646.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology.* 3(5): 294-299.

Foster, T. (1996) Chapter 12: *Staphylococcus Medical Microbiology*. 4th edition Baron S, editor. Galveston (TX):The University of Texas medical branch at Galveston Bookshelf ID: NBK8448PMID: 21413338

Foster. A.P. (2012). Staphylococcal skin disease in livestock. *Veterinary Dermatology* 23: 342–e63.

Friedhoff, K.T. (1997). Tick-borne diseases of sheep and goats caused by *Babesia*, *Theileria* or *Anaplasma* spp. *Parassitologia*. 39(2): 99-109.

Galay, R.L., Manalo, A.A.L., Dolores, S.L.D., Aguilar I.P.M., Sandalo, K.A.C., Cruz, K.B., Divina, B.P., Andoh, M., Masatani, T. and Tanaka T. (2018). Molecular detection of tick-borne pathogens in canine population and *Rhipicephalus sanguineus* (sensu lato) ticks from southern Metro Manila and Laguna, Philippines. *Parasites & Vectors.* 11: 643.

Galtier, N., Nabholz, B., Glémin, S. and Hurst, G.D. (2009). *Mitochondrial DNA* as a marker of molecular diversity: a reappraisal. *Molecular Ecology*. 18(22): 4541-50.

García, F.M., Guerrero, S.I.B., la Peña, C.G., Gutiérrez, D.R.A, Rodríguez, Q.K.S., Herrera, C.A.M., Paniagua, F.V., Velásquez, C.D., Montoya, A.C., Núñez, L.M.V. (2024). Bacteria in the blood of healthy stray dogs infested by ticks in northern Mexico. *Journal of Advanced Veterinary and Animal Research*. 11(1): 132-138.

García-De la Peña, C.; Zamudio-López, A.; Barraza-Guerrero, S.I.; Martínez-Aranda, E.; De la Cruz-Ramos, J.M.; Acosta-Ayala, A.; Siller-Rodríguez, Q.K.; Torres-Delgado, M.G.; Ávila-Rodríguez, V.; Vásquez-Arroyo, J.; Valenzuela-Núñez, L.M. and Herrera-Salazar, J.C. (2024). Bacterial microbiota of the brown dog tick (*Rhipicephalus sanguineus*), a broad starting point to establish potential pathogens in Northern Mexico. *Microbiological Research*. 15: 2507–2521.

Gay, N.R., Fleming, E. and Oh, J. (2016). Draft genome sequence of Cloacibacterium normanense NRS-1 Isolated from municipal wastewater. *Genome Announcement*s. 4(6): e01397-16.

Gerhart, J.G., Moses, A.S and Raghavan, R. (2016). A *Francisella-like endosymbiont* in the Gulf Coast tick evolved from a mammalian pathogen *Scientific Reports* 6:33670.

Germanakis, A., Chochlakis, D., Angelakis, E., Tselentis, Y. and Psaroulaki, A. (2013). *Rickettsia aeschlimannii* infection in a man, Greece. *Emerging Infectious Disease*. 19(7): 1176-7.

Getange D., Bargul J.L., Kanduma E., Collins M., Bodha B., Denge D., Chiuya T., Githaka N., Younan M., Fèvre E.M., Bell-Sakyi L and Villinger J (2021). Ticks and Tick-borne pathogens associated with Dromedary camels (*Camelus dromedarius*) in Northern Kenya. *Microorganisms*. 9(7): 1414.

Ghafar, A., Abbas, T., Rehman, A., Sandhu, Z.U., Cabezas-Cruz, A. and Jabbar, A. (2020). Systematic review of ticks and tick-borne pathogens of small ruminants in Pakistan. *Pathogens*. 9(11): 937.

Ghalehnoo, Z.R. (2018). Diseases caused by *Staphylococcus aureus*. *International Journal of Medical and Health Research.* 4(11): 65-67.

Ghannam, M.G. and Varacallo, M. (2023). Biochemistry, polymerase chain reaction. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 30571074.

Ghoneim, N. H.; Abdel-Moein, Khaled A. and Zaher, H.M. (2017). Molecular detection of *Francisella spp*. among ticks attached to camels in Egypt. *Vector-Borne and Zoonotic Diseases. vbz.2016.2100–.* doi: 10.1089/vbz.2016.2100.

Ghosh. S. and Nagar G. (2014). Problem of ticks and tick-borne diseases in India with special emphasis on progress in tick control research: A review. *Journal of Vector Borne Disease.* 51: 259-2710.

Githaka, N.W., Kanduma, E.G., Wieland, B., Mohamed A. Darghouth, M.A., and Bishop, R.P. (2022). Acaricide resistance in livestock ticks infesting cattle in Africa: current status and potential mitigation strategies. *Current Research in Parasitology & Vector-Borne Diseases.* 2: 100090.

Gomes, R., Carlos, P., Faísca, A.R. and Vicente, L. Q. (2022). Fever spondylodiscitis: A rare manifestation of a challenging disease. *European Journal of Case Reports Internal Medicine*. 9(12): 003703.

Gou, H., Xue, H., Yin, H., Luo, J. and Sun, X. (2018). Molecular characterization of hard ticks by Cytochrome c Oxidase Subunit 1 sequences. *Korean Journal Parasitology* 56(6): 583–588.

Greay, T.L., Gofton, A.W., Paparini, A., Ryan, U.M., Oskam, C.L. and Irwin, P.J. (2018) Recent insights into the tick microbiome gained through next-generation sequencing. *Parasites & Vectors*. 11(1): 12.

Green, M.R. and Sambrook, J. (2018). The basic polymerase chain reaction (PCR). Cold Spring Harb Protocol. 2018(5).

Green, M.R. and Sambrook, J. (2019). Nested polymerase chain reaction (PCR). *Cold Spring Harb Protocol*. 2019(2).

Guglielmone, A.A., Robbins, R.G., Apanaskevich, D.A., D.A., Petney, Estrada-Peña, A., Horak, I.G.; Shao, R. and Barker, S.C. (2010). The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid species names. *Zootaxa* 2528(1): 1.

Guglielmone, A.A., Robbins, R.G., Apanaskevich, D.A., Petney, T.N., Estrada-Peña, A. and Horak, I.G.; (2009). Comments on controversial tick (Acari: Ixodida) species names and species described or resurrected from 2003 to 2008. *Experimental Applied Acarology.* 48: 311–327.

Guglielmone, A.A., Venzal, J.M., Gonza´lez-Acun, D., Nava, S., Hinojosa, A., and Mangold, A.J. (2006). The phylogenetic position of Ixodes stilesi Neumann, 1911 (Acari: Ixodidae): morphological and preliminary molecular evidence from 16S rDNA sequences. *Systematic Parasitology.* 65: 1–11 DOI 10.1007/s11230-005-9024-4.

Guizzo, M.G., Neupane, S., Kucera, M., Perner, J., Frantova, H., da Silva Vaz, I., de Oliveira, P.L., Kopacek, P. and Zurek, L. (2020). Poor unstable midgut microbiome of hard ticks contrasts with abundant and stable monospecific microbiome in ovaries. *Frontiers Cellular Infectious Microbiology.* 10: 211

Guizzo, M.G., Parizi, L.F., Nunes, R.D., Schama, R., Albano, R.M., Tirloni, L., Oldiges, D.P., Vieira, R.P., Oliveira, W.H.C., Leite, M.S., Gonzales, S.A., Farber, M., Martins, O., Vaz, I.D.S. Jr. and Oliveira, P.L. (2017). A *Coxiella* mutualist symbiont is essential to the development of *Rhipicephalus microplus*. *Scientific Report*. 7(1): 17554.

Gulia-Nuss, M., Nuss, A.B., Meyer, J.M., Soneshine, D.E., Roe, R.M., Waterhouse, R.M. and Hill, C.A. (2016). Genomic insights into the *Ixodes scapularis* tick vector of lime disease. *Nature Communications.* 7: 10507.

Guo, H., Adjou Moumouni, P.F., Thekisoe, O., Gao, Y., Liu, M., Li, J., Galon, E.M., Efstratiou, A., Wang, G., Jirapattharasate, C., Ringo, A.E., Mtshali, K., Inoue, N., Suzuki, H., and Xuan, X (2019). Genetic characterization of tick-borne pathogens in ticks infesting cattle and sheep from three South African provinces. *Ticks and Tick Borne Diseases*. 10(4): 875-882.

Han, R., Yang, J.F., Mukhtar, M.U., Chen, Z., Niu, Q.L., Lin, Y.Q., Liu, G.Y., Luo, J.X., Yin, H. and Liu, Z.J. (2019). Molecular detection of *Anaplasma* infections in ixodid ticks from the Qinghai-Tibet Plateau. *Infectious Diseases of Poverty*. 8(1): 12.

Han, R., Yang, J.F., Mukhtar, M.U., Chen, Z., Niu, Q.L., Lin, Y.Q., Liu, G.Y., Luo, J.X., Yin, H., Liu, Z.J. (2019). Molecular detection of *Anaplasma* infections in *ixodid* ticks from the Qinghai-Tibet Plateau. *Infectious Diseases of Poverty*. 8(1): 12.

Han, R., Yang, J-F., Mukhtar, M.U., Chen, Z., Niu, Q-L., Lin, Y-Q., Liu G-U., Luo, J-X., Yin, H. and Liu, Z-J. (2019). Molecular detection of Anaplasma infections in ixodid ticks from the Qinghai-Tibet Plateau. *Infectious Diseases of Poverty.* 8: 12

Harrisa, E.K., Verhoevea, V.I., Banajeea, K.H., Macalusob, J.A., Azadb, A.F. and Macaluso, K.R (2017). Comparative vertical transmission of *Rickettsia* by *Dermacentor variabilis* and *Amblyomma maculatum. Ticks and Tick-borne Diseases.* 8: 598-604

Hauschild, T. and Stepanovic, S. (2008). Identification of *Staphylococcus* spp. by PCR-restriction fragment length polymorphism analysis of dnaJ gene. *Journal of Clinical Microbiology.* 46: 3875-3879

He, C.H., Feng, S.F., Chen, S.X., Han, D.K., He, T.R., Cao, J.W. and Mai, H.Q. (2023). *Streptococcus dysgalactiae* subsp. *dysgalactiae* presents with progressive weakness in limbs: a case report and literature review. *BMC Infectious Diseases*. 23(1): 192.

Hebert, P.D., Cywinska, A., Ball, S.L. and deWaard, J.R., (2003). Biological identifications through DNA barcodes. *Proceeding Biological Sciences.* 270(1512): 313-21. doi: 10.1098/rspb.2002.2218.

Henning, T.C., Orr, J.M., Smith, J.D., Arias, J.R., Rasgon, J.L and Norris, D.E. (2016). Discovery of filarial nematode DNA in *Amblyomma americanum* in Northern Virginia. *Ticks and Tick Borne Diseases*. 7(2): 315-8.

Hernández-Jarguín, A., Díaz-Sánchez, S., Villar, M., and de la Fuente, J. (2018). Integrated metatranscriptomics and metaproteomics for the characterization of bacterial microbiota in unfed *Ixodes ricinus. Ticks and Tick-Borne Diseases*. 9(5).

Hestetun, J.T., Bye-Ingebrigtsen, E., Nilsson, R.H., Glover, A.G., Johansen, P-O., Thomas, G. and Dahlgren, T.G. (2020). Significant taxon sampling gaps in DNA databases limit the operational use of marine macrofauna metabarcoding. *Marine Biodiversity.* 50: 70.

Hodzic, E., Fish, D., Maretzki, C.M., De Silva, A.M., Feng, S. and Barthold, S.W. (1998). Acquisition and transmission of the agent of human granulocytic ehrlichiosis by Ixodes scapularis ticks. *Journal of Clinical. Microbiology.* 36: 3574–3578.

Hoffmann, A.; Müller, T.; Fingerle, V. and Noll, M. (2021). Presence of human pathogens of the *Borrelia burgdorferi* sensu lato complex shifts the sequence read abundances of tick microbiomes in two German locations. *Microorganisms*. 9(9): 1814.

Hoogstraal, H., Wasseff, H.Y. and Buttiker, W. (1981). Ticks (Acarina) of Saudia Arabia farm. Argasidae Ixodidae. *Fauna Saudi Arab.* 32: 25–110.

Horak, I.G, Heyne, H., Williams, R., Gallivan ,G.J, Spickett, A.M, Bezuidenhout. JD and Estrada-Peña A (2018). The ticks (Acari: Ixodidae) of Southern Africa. Basel, Switzerland: Springer International Publishing AG.

Horak, I.G., Biggs, H.C., Hanssen, T.S. and Hanssen, R.E. (1983). The prevalence of helminth and arthropod parasites of warthog, *Phacochoerus aethiopicus*, in South West Africa/Namibia. *Onderstepoort Journal of Veterinary Research.* 50(2): 145-8.

Horak, I.G., Fourie, L.J., Heyne, H., Walker, J.N. and Needham, G.R. (2002). Ixodid ticks feeding on humans in South Africa: with notes on preferred host, geographical

distribution, seasonal occurrence and transmission of pathogens. *Experimental and Applied Acarology.* 27: 113-136.

Horak, I.G., Heyne, H. and Donkin, E.F. (2017). Parasites of domestic and wild animals in South Africa. XLVIII. Ticks (Acari: Ixodidae) infesting domestic cats and wild felids in southern Africa. *Onderstepoort Journal of Veterinary Research.* 77 (1*):* 1-7.

Horak, I.G., Heyne, H., Halajian, A., Booysen, S. and Smit, W.J. (2017). Parasites of domestic and wild animals in South Africa. L. Ixodid ticks infesting horses and donkeys. *Onderstepoort Journal of Veterinary Research*. 84(1): e1-e6.

Horak, I.G., Jordaan, A.J., Nel, P.J., van Heerden, J., Heyne, H., and van Dalen, E.M. (2015). Distribution of endemic and introduced tick species in Free State Province, South Africa. *Journal of South African Veterinary Association.* 86: 1-9.

Horak, L.G and Knight, M.M. (1986). A comparison of the tick burdens of wild animals in a nature reserve and on an adjacent farm where tick control is practiced. *Journal of the South African Veterinary Association.* 57 (4): 199-203.

Horpiencharoen, W., Thongratsakul, S. and Poolkhet, C. (2019). Risk factors of clinical mastitis and antimicrobial susceptibility test results of mastitis milk from dairy cattle in western Thailand: bayesian network analysis. *Preventive Veterinary Medicine*. 164: 49–55.

Hosokawa, T., Koga, R., Kikuchi, Y., Meng, X.Y. and Fukatsu, T. (2010). Wolbachia as a bacteriocyte-associated nutritional mutualist. *Proceedings of the National Academy of Sciences of the United States of America*. 107: 769‑774.

Houpikian, P. and, Raoult, D. (2002). Traditional and molecular techniques for the study of emerging bacterial diseases: one laboratory's perspective. *Emerging Infectious Diseases.* 8(2): 122-31.

Hove, P., Khumalo, Z.T.H., Chaisi, M.E., Oosthuizen, M.C., Brayton, K.A. and Collins, N.E. (2018). Detection and characterisation of *Anaplasma marginale* and *A. centrale* in South Africa. *Veterinary Science*. 5(1): 26.

Hove, T., Mukandi, R., Bere M., Horak, I.G. and Latif A.A (2008). Ixodid ticks infesting domestic goats in communal land areas of Zimbabwe. *Journal of the South African Veterinary Association.* 79(3): 116-120 https:// doi.org/10.1002/ece3.5361.

Illumina. (2013). 16S Metagenomic sequencing library preparation. Retrieved from http://www.science.smith.edu/cmbs/wp-content/uploads/sites/36/2015/09/16s metagenomics-library-prep-guide-1504422-b.pdf.

Ioannou, P., Alexakis, K., Baliou, S. and Kofteridis, D.P. (2022). Infective endocarditis by *Moraxella* species: A systematic review. *Journal of Clinical Medicine*. 11(7): 1854.

Irwin, P.J and Jefferies, R. (2004). Arthropod-transmitted diseases of companion animals in Southeast Asia. *TRENDS in Parasitology.* 20 (1): 1471-4922.

Isler, B., Kidd, T.J., Stewart, A.G., Harris, P.and Paterson, D.L. (2020). *Achromobacter* infections and treatment options. *Antimicrobial Agents and Chemotherapy*. 64(11): e01025-20.

Ivanova, M., Singh, R., Dharmasena, M., Gong, C., Krastanov, A. and Jiang, X. (2014). Rapid identification of *Campylobacter jejuni* from poultry carcasses and slaughtering environment samples by real-time PCR. *Poultry Science*. 93(6): 1587-97.

Iweriebor, B.C., Mmbaga, E.J., Adegborioye, A., Igwaran, A, Obi, L.C. and Okoh, A.I. (2017). Genetic profiling for *Anaplasma* and *Ehrlichia* species in ticks collected in the Eastern Cape Province of South Africa. *BMC Microbiology.* 17(1): 45.

Iweriebor, B.C., Nqoro, A. and Obi, C.L. (2020). *Rickettsia africae* an agent of African tick bite fever in ticks collected from domestic animals in Eastern Cape, South Africa. *Pathogens*. 9(8) :631.

Jongejan, F and Uilenberg G. (2004). Global importance of ticks. *Parasitology.* 129: S3-S14

Kamani, J., (2021). Molecular identification and genetic analysis of *Rhipicephalus sanguineus* sensu lato of dogs in Nigeria, West Africa. *Experimental and Applied Acarology. 85*(2): 277-289.

Karim, S., Budachetri, K., Mukherjee, N., Williams, J., Kausar, A., Hassan, M.J., Adamson, S., Dowd, S.E., Apanskevich, D., Arijo, A., Sindhu, Z.U., Kakar, M.A., Khan, R.M.D., Shafiq Ullah, S., Sajid, M.S., Ali, A., and Iqbal, Z (2017). A study of ticks and tick-borne livestock pathogens in Pakistan. *PLoS Neglected Tropical Disease.* 11(6): e0005681.

Keirans, J.E. and Clifford, C.M., (1978). The genus Ixodes in the United States: a scanning electron microscope study and key to the adults. *Journal Medical Entomology Supplement.* 2:1-149

Kirecci, E., Salih, W.M., Metin Uğuz, M.T., Mohammed, B.A., Namıq, O.A. and Rızgar Mahmood Kareem, R.M. (2015). Isolation and identification of tick-borne bacterial pathogens in Turkey and Iraq. *African Journal of Microbiology Research.* 9(24): 1608-1612.

Khalaf, J.M., Mohammed, I.A. and Karim, A.J. (2018). The epidemiology of tick in transmission of *Enterobacteriaceae* bacteria in buffaloes in marshes of the south of Iraq. *Veterinary World*. 11(12):1677-1681.

Khehra, N., Padda, I.S. and Swift, C.J. (2003). Polymerase Chain Reaction (PCR). 2023 Mar 6. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 36943981.

Khoo, J-K., Chen, F., Kho, K.L., Shanizza, A.I.A., Lim, F-S., Tan, K-K., Chang, L-Y. and Sazaly, A. (2016). Bacterial community in *Haemaphysalis* ticks of domesticated animals from the Orang Asli communities I Malaysia. *Ticks and Tick-Borne Disease.* 7: 929-937.

Kim, Y.S., Kim, J. and Park, S.J. (2015). High-throughput 16S rRNA gene sequencing reveals alterations of mouse intestinal microbiota after radiotherapy. *Anaerobe*. 33: 1-7.

Kimemia, B.B., Musila, L., Langat, S., Odoyo, E., Cinkovich, S., Abuom, D, Yalwala, S., Khamadi, S., Johnson, J., Garges, E., Ojwang E. and Eyase, F. (2024) Detection of pathogenic bacteria in ticks from Isiolo and Kwale counties of Kenya using metagenomics. *PLoS ONE*. 19(4): e0296597.

Kirecci, E., Wasan, M., Metin, T., Bawar, A., Omer, A. and Rızgar, M. (2015). Isolation andidentification of tick-borne bacterial pathogens in Turkey and Iraq. *African Journal of Microbiology Research*. 9(24): 1608-1612.

Kivaria, F.M., (2006). Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania. *Tropical Animal Health and Production.* 38: 291-299.

Kleinjan, J.E. and Lane, R.S. (2008). Larval keys to the genera of Ixodidae (Acari) and species of Ixodes (Latreille) ticks established in California. *Pan-Pacific Entomologist.* 84(2): 121–142. doi:10.3956/2007-38.1.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., and Glöckner, F.O. (2013). Evaluation of general 16S ribosomal RNA gene PCR

primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research.* 41(1): e1.

Klompen, H., Lekveishvili, M. and Black. WCT. (2007). Phylogeny of parasitiform mites (acari) based on rrna. *Molecular Phylogenetics and Evolution.* 43: 936-51.

Knuth, R.M., Woodruff, K.L., Hummel, G.L., Williams, J.D., Austin, K.J., Stewart, W.C., Cunningham-Hollinger, H.C. and Bisha, B. (2022). Effects of management strategies during early lactation and weaning on etiological agents of ovine subclinical mastitis and antimicrobial susceptibility of milk-derived bacterial isolates. *Journal of Animal Science*. 100(6): skac171.

Kobayashi, T., Chatanga, E, Qiu, Y., Simuunza, M., Kajihara, M., Hang'ombe B.M., Eto, Y., Saasa, N., Mori-Kajihara, A., Simulundu, E., Takada, A., Sawa, H., Katakura, K., Nonaka, N. and Nakao, R. (2021). Molecular detection and genotyping of *Coxiella*-like endosymbionts in ticks collected from animals and vegetation in Zambia. *Pathogens*. 10(6): 779.

Kocan, K.M., de la Fuente, J., Blouin, E.F., Coetzee, J.F. and Ewing, S.A. (2010). The natural history of *Anaplasma marginale*. *Veterinary Parasitology*. 167(2-4): 95-107.

Kolo, A. (2023). *Anaplasma* species in Africa-a century of discovery: A review on molecular epidemiology, Genetic Diversity, and Control. *Pathogens*. 12(5): 702.

Komba, E. V. G., Mdegela, R. H., Msoffe, P. L. M., Nielsen, L. N., and Ingmer, H. (2015). Prevalence, Antimicrobial Resistance and Risk Factors for Thermophilic *Campylobacter* Infections in symptomatic and asymptomatic humans in Tanzania. *Zoonoses and Public Health*. 62: 557-568.

Krieger, M., AbdelRahman, Y.M., Choi, D., Palmer, E.A., Yoo, A., McGuire, S., Kreth, J. and Merritt, J. (2023). The prevalence of *Fusobacterium nucleatum* subspecies

in the oral cavity stratifies by local health status. bioRxiv [Preprint]. 26:2023.10.25.563997. Update in: *Cell Host Microbe*. 32(4): 479-488.e4.

Kubista, M., Andrade, J.M., Bengtsson, M., Forootan, A., Jonák, J., Lind, K., Sindelka, R., Sjöback, R., Sjögreen, B., Strömbom. L., Ståhlberg, A. and Zoric, N. (2006). The real-time polymerase chain reaction. *Molecular Aspects of Medicine*. 27(2-3): 95-125

Kueneman, J.G., Esser, H.J., Weiss, S.J., Jansen, P.A. and Foley, J.E. (2021). Tick microbiomes in Neotropical Forest fragments are best explained by tick-associated and environmental factors rather than host blood source. *Applied and Environmental Microbiology.* 87(7): e02668-20.

Lalzar, I., Harrus, S., Mumcuoglu, K.Y. and Gottlieb, Y. (2012). Composition and seasonal variation of *Rhipicephalus turanicus* and *Rhipicephalus sanguineus* bacterial communities. *Applied Environmental Microbiology*. 78(12): 4110-6.

Lane, R.S., Steinlein, D.B and Mun, J. (2004). Human behaviors elevating exposure to *Ixodes pacificus* (Acari: Ixodidae) nymphs and their associated bacterial zoonotic agents in a Hardwood Forest. *Journal of Medical Entomology*. 41(2): 239-248.

Latif, A., Putteriff, J.F., De Kerk, D.G., Pienaar, R., and Mans, B.J. (2012). *Nuttalliella Namaqua* (Ixodoidea: Nuttalliellidae): first description of the male, immature stages and re-description of the female. *PLoSOne.* 7(7): e41651-e41660.

Latif, A.A. (2013). Illustrated guide to identification of African tick species: Ticks and tick-borne Diseases Monograph 2, Johannesburg: AGRI Connect (Pty) Ltd., South Africa, p. 79.

Latif, A.A. and Hovec, T. (2011). History and critical review of *Theileria parva* (Boleni), the vaccine stock against Zimbabwean cattle theileriosis. *Ticks and Tick-borne Diseases.* 2: 163- 167.

Latrofa, M.S., Dantas-Torres, F., Alessio Giannelli, A.and Otrantoa, D. (2014). Molecular detection of tick-borne pathogens in *Rhipicephalus sanguineus* group ticks. *Ticks and Tick-borne Diseases.* 5: 943-946.

Lilak, A.A., Pecor, D.B., Matulis, G., Potter, A.M., Wofford, R.N., Kearney, M.F., Mitchell, S., Jaradat, F., Kano, A., Zimmerman, D.M., Hassell, J.M., Kumsa, B., Kamau, M., Linton, Y.M. and von Fricken, M.E. (2024). Data release: targeted systematic literature search for tick and tick-borne pathogen distributions in six countries in sub-Saharan Africa from 1901 to 2020. *Parasite Vectors*. 17(1): 84.

Lin, X., Stur, E. and Ekrem, T. (2015). Exploring Genetic Divergence in a Species-Rich Insect Genus Using 2790 DNA Barcodes. *PLoS ONE.* 10(9): e0138993.

Lindsay, J.A. (2019). *Staphylococci*: evolving genomes. *Microbiology Spectrum.* 7(6): GPP3-0071-2019.

Livanova, N.N., Tikunov, A.Y., Kurilshikov, A.M., Livanov, S.G., Fomenko, N.V., Taranenko, D.E., Kvashnina, A.E. and Tikunova, N.V. (2015). Genetic diversity of *Ixodes pavlovskyi* and *I. persulcatus* (Acari: Ixodidae) from the sympatric zone in the south of Western Siberia and Kazakhstan. *Experimental and Applied Acarology*. 67(3): 441-56.

Low, V.L., Tay, S.T., Kho, K.L., Koh, F.X., Tan, T.K., Lim, Y.A., Ong, B.L., Panchadcharam, C., Norma-Rashid, Y. and Sofian-Azirun, M. (2015). Molecular characterisation of the tick *Rhipicephalus microplus* in Malaysia: new insights into the cryptic diversity and distinct genetic assemblages throughout the world. *Parasites and Vectors*. 8: 341.

Lu, X., Linb, X.D., Wanga, J.B., Qina, X.C., Tianc, J.H., Guoa, W.P., Fan, F.N., Shaoe, R., Xua, J., and Zhanga, Y.Z. (2013). Molecular survey of hard ticks in endemic areas of tick-borne diseases in China. *Ticks and Tick-Borne Diseases.* 4: 288-296.

Lutomiah, J., Musila, L., MAKIO, A., Ochieng, C., Koka, H., Chepkorir, E., Mutisya, M., Mulwa, F., Khamadi, S., Miller, B.R., Bast, J., Schnabel, D., Wurapa, E.K. and Sang, R., (2014). Ticks and tick-borne viruses from livestock host in arid and semiarid regions of the Eastern and Northeastern parts of Kenya. *Vector-borne diseases, surveillance, prevention journal of medical entomology.* 51(1): 271-277.

Lv, J., Wu, S., Zhang, Y., Chen, Y., Feng, C., Yuan, X., Jia, G., Deng, J., Wang, C., Wang, Q., Mei, L. and Lin, X. (2014a). Assessment of four DNA segments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). *Parasite Vectors.* 7 (1): 93.

Lv, J., Wu, S., Zhang, Y., Zhang, T., Feng, C., Jia, G and Lin, X. (2014b). Development of a DNA barcoding system for the Ixodida (Acari: Ixodida). *Mitochondrial DNA.* 25(2): 142-9.

Lynen, G., Zeman, P., Bakuname, C., Di Giulio, G., Mtui, P., Sanka, P.and Jongejan, F. (2008). Shifts in the distributional ranges of *Boophilus* ticks in Tanzania: evidence that a parapatric boundary between *Boophilus microplus* and *B. decoloratus* follows climate gradients. *Experimental Applied Acarology*. 42: 147-164.

Machado-Ferreira, E., Vizzoni, V.F., Balsemão-Pires, E., Moerbeck, L., Gazeta, G.S., Piesman, J., Voloch, C.M. and Soares, C.A. (2016). *Coxiella* symbionts are widespread into hard ticks. *Parasitology Research*. 115(12): 4691-4699.

Madder, M., Horak, I. and Stoltsz, H. (2013). Ticks: Tick identification. Creative Commons Attribution license, South Africa.

Madder, M., Thys, E., Geysen, D., Baudoux, C. and Horak, I. (2007). *Boophilus microplus* ticks found in West Africa. *Experimental and Applied Acarology*. 43(3): 233-4.

Magadu, R. and Thompson, P.N. (2023). Seroprevalence and factors associated with *Coxiella burnetii* exposure in goats in Moretele. *Onderstepoort Journal of Veterinary Research*. 90(1): a2071.

Mahlobo-Shwabede, S.I.C., Zishiri, O.T., Thekisoe, O.M.M. and Makalo, M.J.R. (2021). Molecular detection of *Coxiella burnetii*, *Rickettsia africae* and *Anaplasma* species in ticks from domestic animals in Lesotho. *Pathogens.* 10(9): 1186

Mahlobo-Shwabede, S.I.C., Zishiri, O.T., Thekisoe, O.M.M., Bakkes, D., Bohloa, L., Molomo, M., Makalo, M.J.R., Mahloane, G.R. and Mtshali, M.S. (2022). Ticks of domestic animals in Lesotho: Morphological and molecular characterization. *Veterinary Parasitology: Regional Studies and Reports.* 29: 100691.

Maitre, A., Wu-Chuang, A., Aželytė, J., Palinauskas, V., Mateos-Hernández, L., Obregon, D., Hodžić, A., Valiente Moro, C., Estrada-Peña, A., Paoli, J.C., Falchi, A. and Cabezas-Cruz, A. (2022). Vector microbiota manipulation by host antibodies: the forgotten strategy to develop transmission-blocking vaccines. *Parasite Vectors*. 15(1): 4.

Makhahlela, N.B., Liebenberg, D., van Hamburg, H., Taioe, M.O., Onyiche, T., Ramatla, T. and Thekisoe, O.M.M. (2022). Detection of pathogens of veterinary importance harboured by *Stomoxys calcitrans* in South African feedlots. *Scientific African*. 15: e01112.

Makwarela, T.G.; Nyangiwe, N.; Masebe, T.; Mbizeni, S.; Nesengani, L.T.; Djikeng, A. and Mapholi, N.O. (2023). Tick diversity and distribution of hard (Ixodidae) cattle ticks in South Africa. *Microbiology Research*. 14: 42-59.

Man, S.M., Zhang, L., Day, A.S., Leach, S.T., Lemberg, D.A. and Mitchell, H. (2010). *Campylobacter* concisus and other *Campylobacter* species in children with newly diagnosed Crohn's disease. *Inflammatory Bowel Disease*. 16(6): 1008-16.

Mangena, M., Gcebe, N., Pierneef, R., Thompson, P.N. and Adesiyun, A.A. (2021). Q fever: seroprevalence, risk factors in slaughter livestock and genotypes of *Coxiella burnetii* in South Africa. *Pathogens*. 10(3): 258.

Manjunathachar, H. V., Saravanan, B. C., Kesavan, M., Karthik, K.., Rathod, P., Gopi, M., Tamilmahan, P. and Balaraju, B. L. (2014). Economic importance of ticks and their effective control strategies. *Asian Pacific Journal of Tropical Disease*. 4(2): 770-779.

Mans, B.J., de Klerk, D., Pienaar, R. and Latif, A.A. (2011). *Nuttalliella namaqua*: A living fossil and closest relative to the ancestral tick lineage: Implications for the evolution of blood-feeding in ticks. *Plos one*. 6: e23675.

Mans, B.J., Gothe, R. and Neitz A.W.H. (2004). Biochemical perspectives on paralysis and other forms of toxicoses caused by ticks. *Parasitology.* 129: pp S1-S17.

Mapholi, N.O., Marufu, M.C., Maiwashe, A., Banga, C.B., Muchenje, V., MacNeil, M.D., Chimonyo, M. and Dzama, K. (2014). Towards a genomics approach to tick (Acari: Ixodidae) control in cattle: a review. *Ticks and Tick-Borne Diseases.* 5:475-483.

Mapholi, N.O.; Maiwashe, A.; Matika, O.; Riggio, V.; Bishop, S.; MacNeil, M.; Banga, C., Taylor, J. and Dzama, K. (2016). Genome-wide association study of tick resistance in South African Nguni cattle*. Ticks and Tick-Borne Diseases*. 7: 487-497.

Markoulatos, P., Siafakas, N. and Moncany, M. (2002). Multiplex polymerase chain reaction: a practical approach. *Journal of Clinical Laboratory Analysis*. 16(1): 47-51.

Martínez, M. A. (2011). Retrato microbiológico*. Journal of Microbiology Immunology and Infection*. 44(1): 289-295 40.

Mashiane, R.A., Ezeokoli, O.T., Adeleke, R.A. and Bezuidenhout, C.C. (2017). Metagenomic analyses of bacterial endophytes associated with the phyllosphere of a Bt maize cultivar and its isogenic parental line from South Africa. *World Journal of Microbiology and Biotechnology.* 33(4): 80.

Mason, W.J., Blevins, J.S., Beenken, K., Wibowo, N., Ojha, N. and Smeltzer, M.S. (2001). Multiplex PCR protocol for the diagnosis of *staphylococcal* infection. *Journal of Clinical Microbiology*. 39(9): 3332-8.

Matjila, P.T., Leisewitz, A.L., Jongejan, F. and Penzhorn, B.L. (2008). Molecular detection of tick-borne protozoal and ehrlichial infections in domestic dogs in South Africa. *Veterinary Parasitology.* 155: 152-157.

Maurin, M. and Raoult, D. (1999). Q fever. *Clinical Microbiology Reviews*. 12(4): 518-53

Mbati, P.A., Hlatshwayo, M., Mtshali, M.S., Mogaswane, K.R., De Waal, T.D. and Dipeolu, O.O. (2002). Ticks and tick-borne diseases of livestock belonging to resource-poor farmers in the eastern Free State of South Africa. *Experimental and Applied Acarology*. 28(1-4): 217-24.

Mbizeni, S., Potgieter, F.T., Troskie, C., Ben J. Mans, B.J., Penzhornb, B.L. and Latif, A.A. (2013). Field and laboratory studies on Corridor disease (*Theileria parva* infection) in cattle population at the livestock/game interface of uPhongolo-Mkuze area, South Africa. *Ticks and Tick-borne Diseases.* 4: 227-234.

McCoy, K.D, Léger, E., and Dietrich, M. (2013). Host specialization in ticks and transmission of tick-borne diseases: a review. *Frontiers in Cellular and Infection Microbiology.* 57(3).

Mekonnen, S., Bryson, N.R., Fourie, L.J., Peter, R.J., Spickett, A.M., Taylor, R.J., Strydom, T. and Horak, I.G. (2002). Acaricide resistance profiles of single-and multi-

host ticks from communal and commercial farming areas in the Eastern Cape and North-West Provinces of South Africa. *Onderstepoort Journal of Vetenary Research.* 69: 99-105.

Menadi, S.E., Mura, A., Santucciu, C., Ghalmi, F., Hafsi, F. and Masala, G. (2020). Seroprevalence and risk factors of *Coxiella burnetii* infection in cattle in northeast Algeria. *Tropical Animal Health Production*. 52(3): 935-942.

Miller S.E. (2007). DNA barcoding and the renaissance of taxonomy. *Proceedings of the National Academy of Sciences.* 104(12): 4775–4776.

Mofokeng, L.S., Smit, N.J., and Cook, C.A. (2021). Molecular screening of ticks of the genus *Amblyomma* (Acari: Ixodidae) infesting South African reptiles with comments on their potential to act as vectors for *Hepatozoon fitzsimonsi* (Dias, 1953) (Adeleorina: Hepatozoidae). *International Journal for Parasitology: Parasites and Wildlife*. 16: 163-167.

Montes Cortés, M.G., Fernández-García, J.L. and Habela Martínez-Estéllez, M.Á. (2017). Seroprevalence of *Theileria equi* and *Babesia caballi* in horses in Spain. *Parasite*. 24: 14.

Moreno, C.X., Moy, F., Daniels, T.J., Godfrey, H.P., and Felipe C. Cabello, F.C. (2006). Molecular analysis of microbial communities identified in different developmental stages of Ixodes scapularis ticks from Westchester and Dutchess Counties, New York. *Environmental Microbiology.* 8(5): 761-772.

Moreno-Forero, S.K. and van der Meer, J.R. (2015). Genome-wide analysis of *Sphingomonas wittichii* RW1 behaviour during inoculation and growth in contaminated sand. *ISME Journal*. 9(1): 150-65.

Moriconi, M., Acke, E., Petrelli, D. and Preziuso, S. (2017). Multiplex PCR-based identification of *Streptococcus canis*, *Streptococcus zooepidemicus* and

Streptococcus dysgalactiae subspecies from dogs. *Comparative Immunology. Microbiology and Infectious Diseases.* 50: 48-53.

Moyo, B. and Masika, P.J. (2009). Tick control methods used by resource-limited farmers and the effect of ticks on cattle in rural areas of the Eastern Cape Province, South Africa. *Tropical Animal Health and Production*. 41(4): 517-23.

Msimang, V., Weyer, J., le Roux, C., Kemp, A., Burt, F.J., Tempia, S., Grobbelaar, A., Moolla, N., Rostal, M.K., Bagge, W., Cordel, C., Karesh, W.B., Paweska, J.T. and Thompson P.T. (2021). Risk factors associated with exposure to Crimean-Congo haemorrhagic fever virus in animal workers and cattle, and molecular detection in ticks, South Africa. *PLoS Neglected Tropical Diseases.* 15(5): e0009384.

Mtshali, K., Khumalo, Z., Nakao, R., Grab, D.J., Sugimoto, C. and Thekisoe, O. (2016). Molecular detection of zoonotic tick-borne pathogens from ticks collected from ruminants in four South African provinces. *Journal of Veteterinary Medical Science*. 77(12): 1573-9.

Mtshali, K., Nakao, R., Sugimoto, C. and Thekisoe, O. (2017). Occurrence of *Coxiella burnetii*, *Ehrlichia canis*, *Rickettsia* species and *Anaplasma phagocytophilum*-like bacterium in ticks collected from dogs and cats in South Africa. *Journal of the South African Veterinary Association.* 88(0): a1390.

Muhanguzi, D., Byaruhanga, J., Amanyire, W., Ndekezi, C., Ochwo, S., Nkamwesiga, J., Mwiine, F.N., Tweyongyere, R., Fourie, J., Madder, M., Schetters, T., Horak, I. Juleff, N. and Jongejan, F. (2020). Invasive cattle ticks in East Africa: morphological and molecular confirmation of the presence of Rhipicephalus microplus in south-eastern Uganda. *Parasites Vectors.* 13: 165.

Mukhacheva, T.A., and Kovalev, S.Y. (2017). Bacteria of the family '*Candidatus Midichloriaceae*' in sympatric zones of the Ixodes ticks: Genetic Evidence for Vertical Transmission. *Microbial Ecology*. 74(1): 185-193.

Murrell, A. and Barker, S. C. (2003). *Synonymy of Boophilus Curtice, 1891 with Rhipicephalus Koch, 1844 (Acari: Ixodidae). Systematic Parasitology.* 56(3): 169-172.

Mutai, B., Njaa nake, K., Gathii, K., Estambale, B.B. and Waitumbi, J.N. (2022). Bacteriome in ticks collected from domestic livestock in Kenya. *Advances in Microbiology*. 12: 67-82. https://doi.org/10.4236/aim.2022.122006

Mwaki, D.M., Kidambasi, K.O., Kinyua, J., Ogila, K., Kigen, C., Getange, D., Villinger, J., Masiga, D.K., Carrington, M. and Bargul, J.L. (2022). Molecular detection of novel *Anaplasma* sp. and zoonotic hemopathogens in livestock and their hematophagous biting keds (genus *Hippobosca*) from Laisamis, northern Kenya. *Open Research Africa.* 5: 23.

Mysak, J., Podzimek, S., Sommerova, P., Lyuya-Mi, Y., Bartova, J., Janatova, T., Prochazkova, J. and Duskova, J. (2014). *Porphyromonas gingivalis*: major periodontopathic pathogen overview. *Journal of Immunology Research*. 2014: 476068.

Mysterud, A., Jore, S., Østerås, O. and Viljugrein, H. (2017). Emergence of tick-borne diseases at northern latitudes in Europe: a comparative approach. *Science Report* 7: 16316.

Nakayima, J., Magona, J.W. and Sugimoto, C. (2015). Molecular detection of tick-borne pathogens in ticks from Uganda. *Open Access Research.* 1: 767.

Narasimhan, S. and Fikrig, E. (2015). Tick microbiome: the force within. *Trends Parasitology.* 31(7): 315-23.

Narasimhan, S., Rajeevan, N., Liu, L., Zhao, Y.O., Heisig, J., Pan, J., Eppler-Epstein, R., Deponte, K., Fish, D. and Fikrig, E. (2014). Gut microbiota of the tick vector *Ixodes scapularis* modulate colonization of the Lyme disease spirochete. *Cell Host Microbe.*15: 58-71.

Ndip, L.M., Ndip, R.N., Esemu, S.N., Dickmu, V.L., Fokam, E.B., Walker, D.H. and McBride, J.W. (2005). Ehrlichial infection in Cameroonian canines by *Ehrlichia canis* and *Ehrlichia ewingii*. *Veterinary Microbiology*. 111(1-2): 59-66.

Neelakanta, G. and Sultana, H. (2016). Viral receptors of the gut: vector-borne viruses of medical importance. *Current Opinion in Insects Science.* 16: 44-50.

Nguyen, N.P., Warnow, T., Pop, M. and White, B. A. (2016). perspective on 16S rRNA operational taxonomic unit clustering using sequence similarity. *NPJ Biofilms and Microbiomes*. 2:16004.

Nimo-Paintsil, S.C., Mosore, M., Addo, S.O., Lura, T., Tagoe, J., Ladzekpo, D., Addae, C., Bentil, R.E., Behene, E., Dafeamekpor, C., Asoala, V., Fox, A., Watters, C.M., Koehler, J.W., Schoepp, R.J., Arimoto, H., Dadzie, S., Letizia, A. and Diclaro, J.W., (2022). Ticks and prevalence of tick-borne pathogens from domestic animals in Ghana. *Parasite and Vectors*.15(1): 86.

Niyogi, S.K. (2005). Shigellosis. *The Journal of Microbiology.* 43(20): 133-143.

Njeru, J., Henning, K., Pletz, M.W., Heller, R. and Neubauer, H. (2016). Q fever is an old and neglected zoonotic disease in Kenya: a systematic review. *BMC Public Health*. 16: 297.

Nnadozie C.F. (2023). Averting campylobacteriosis through management of environmental Health: End route for reducing the environmentally mediated campylobacteriosis in South Africa. *BMJ Global Health* (suppl 10): A1-A131.

Noh, Y., Lee, Y.S., Kim, H.C., Chong, S.T., Klein, T.A., Jiang, J., Richards, A.L., Lee, H.K. and Kim, S.Y. (2017). Molecular detection of *Rickettsia* species in ticks collected from the southwestern provinces of the Republic of Korea. *Parasites & Vectors*. 10(1): 20.

Nyangiwe, N., Harrison, A. and Horak, I.G. (2013). Displacement of *Rhipicephalus decoloratus* by *Rhipicephalus microplus* (Acari: Ixodidae) in the Eastern Cape Province, South Africa. *Experimental Applied Acarology*. 61(3): 371-82.

Nyangiwe, N., Horak, I.G., Van der Mescht, L., and Matthee, S. (2017). Range expansion of the economically important Asiatic blue tick, *Rhipicephalus microplus*, in South Africa. *Journal of the South African Veterinary Associatiom.* 88(0): e1-e7.

Obara, I., Githaka, N., Nijhof, A., Krücken, J., Nanteza, A., Odongo, D., Lubembe, D., Atimnedi, P., Mijele, D., Njeri, A., Mwaura, S., Owido, G., Ahmed, J., Clausen, P.H. and Bishop, R.P. (2020). The *Rhipicephalus appendiculatus* tick vector of *Theileria parva* is absent from cape buffalo (*Syncerus caffer*) populations and associated ecosystems in northern Uganda. *Parasitology Research.* 119(7): 2363-2367.

Ogden, N.H., Beard, C.B., Ginsberg, H.S., Tsao, J.I. (2021). Possible effects of climate change on Ixodid ticks and the pathogens they transmit predictions and observations. *Journal of Medical Entomology*. 58(4): 1536–45.

Olson, R. R., Runstadler, J. A. and Kocher, T. D. (1991). Whose larvae?. *Nature*. 351: 357-358.

Onyiche, T.E., Suganuma, K., Igarashi, I., Yokoyama, N., Xuan, X. and Thekisoe, O. A. (2019). Review on *Equine piroplasmosis*: epidemiology, vector ecology, risk factors, host immunity, diagnosis and control. *International Journal of Environmental Research and Public Health*. 16(10): 1736

Orkun, Ö, and Çakmak, A. (2019). Molecular identification of tick-borne bacteria in wild animals and their ticks in Central Anatolia, Turkey. *Comparative Immunology, Microbiology & Infectious Diseases*. 63: 58-65.

Orkun, Ö. (2018). Molecular Characterization Based on *16S rDNA* Phylogeny of Some Ixodid Ticks in Turkey. *Turkiye Parazitoloji Dergisi*. 42(2): 121-129.

Otranto, D., Brianti, E., Latrofa, M.S., Annoscia, G., Weigl, S., Lia, R.P., Gaglio, G., Napoli, E., Giannetto, S., Papadopoulos, E., Miro, G., Dantas-Torres, F and Bain, O. (2012). On a *Cercopithifilaria* sp. transmitted by *Rhipicephalus sanguineus*: a neglected, but widespread filarioid of dogs. *Parasites & Vectors* 5: 1.

Oundo, J.W., Villinger, J., Jeneby, M., Ong'amo, G., Otiende, M.Y., Makhulu, E.E., Musa, A.A., Ouso, D.O. and Wambua L. (2020). Pathogens, endosymbionts, and blood-meal sources of host-seeking ticks in the fast-changing Maasai Mara wildlife ecosystem. *PLoS One*. 15(8): e0228366.

Paddock, C.D. and Goddard, J. (2015). The evolving medical and veterinary importance of the Gulf Coast tick (Acari: Ixodidae). *Journal Medical Entomology.* 52(2): 230-252.

Paddock, C.D., Sumner, J.W., Comer, J.A., Zaki, S.R., Goldsmith, C.S., Goddard, J., McLellan S.L., Tamminga, C.L, and Ohl, C.A. (2004). *Rickettsia parkeri*: a newly recognized cause of spotted fever rickettsiosis in the United States. *Clinical Infectious Diseases.* 38(6): 805-11.

Papa, A., Tsioka, K., Daskou, M.A., Minti, F., Papadopoulou, E. and Melidou, A. (2020). Giadinis, N. Application of 16S rRNA next generation sequencing in ticks in Greece. *Heliyon*. 6(7): e04542.

Park, J.Y., Fox, L.K., Seo, K.S., McGuire, M.A., Park, Y.H., Rurangirwa, F.R., Sischo, W.M. and Bohach, G.A. (2011) Comparison of phenotypic and genotypic methods

for the species identification of coagulase-negative staphylococcal isolates from bovine intramammary infections. *Veterinary Microbiology*. 147(1-2): 142-8.

Parker, R.R, Pickens E.G., Lackman, D.B., Bell, E.J. and Thraikill L.B. (1951). Isolation and Characterization of Rocky Mountain Spotted Fever Rickettsiae from the Rabbit Tick *Haemaphysalis leporis-palustris* Packard. *Public Health Reports.* 66: 455-486.

Parola, P., Paddock, C.D. and Raoult, D. (2005). Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clinical Microbiology Reviews*. 18(4): 719-56.

Parola, P., Paddock, C.D., Socolovschi, C., Labruna, M.B., Mediannikov, O., Kernif, T., Abdad, M.Y., Stenos, J., Bitam, I., Fournier, P.E., and Raoult, D. (2013). Update on tick-borne rickettsioses around the world: a geographic approach. *Clinical Microbiology Reviews*. 26(4): 657-702.

Peleg, O., Baneth, G., Eyal, O., Inbar, J. and Harrus, S. (2010). 'Multiplex real-time qPCR for the detection of *Ehrlichia canis* and *Babesia canis vogeli*', *Veterinary Parasitology.* 173: 292-299.

Pérez de León, A.A., Strickman, D.A., Knowles, D.P., Fish, D., Thacker, E., de la Fuente, J., Krause, P.J., Wikel, S.K., Miller, R.S., Wagner, G.G., Almazán, C., Hillman, R., Messenger, M.T., Ugstad, P.O., Duhaime, R.A., Teel, P.D., Ortega-Santos, A., Hewitt, D.G., Bowers, E.J., Bent, S.J., Cochran, M.H., McElwain, T.F., Scoles, G.A., Suarez, C.E., Davey, R., Howell Freeman, J.M., Lohmeyer, K., Li, A.Y., Guerrero, F.D., Kammlah, D.M., Phillips, P. and Pound, J.M. (2010). Group for emerging Babesioses and One Health Research and development in the U.S. One Health approach to identify research needs in bovine and human babesioses: workshop report. *Parasite & Vectors*. 3(1): 36.

Perry, B and Grace, D. (2009). The impacts of livestock diseases and their control on growth and development processes that are pro-poor. *Philosophical Transactions of the Royal Society.* 364: 2643-2655.

Perveen, N., Muzaffar, S.B. and Al-Deeb, M.A. (2021). Four tick-borne microorganisms and their prevalence in *Hyalomma* ticks collected from livestock in United Arab Emirates. *Pathogens.* 10: 1005.

Petersen, J.M., Mead, P.S. and Schriefer, M.E. (2009). *Francisella tularensis*: an arthropod-borne pathogen. *Veterinary research.* 40(2):1.

Philip, R.N., Casper, E.A., Burgdorfer, W., Gerloff, R.K., Hughes, L.E and Bell, E.J. (1978). Serologic typing of *Rickettsiae* of the spotted fever group by microimmunofluorescence. *The Journal of Immunology.* 121(5): 1961-8

Pollet, T., Sprong, H., Lejal, E., Krawczyk, A.I., Moutailler, S., Cosson, J.F., Vayssier-Taussat, M. and Estrada-Peña, A. (2020). The scale affects our view on the identification and distribution of microbial communities in ticks. *Parasites & Vectors*. 13(1): 36.

Ponnusamy L, Gonzalez, A., Van Treuren, W., Weiss, S., Parobek, C.M., Juliano, J.J., Knight, R., Roe, R.M., Apperson, C.S. and Meshnick, S.R. (2014). Diversity of Rickettsiales in the microbiome of the lone star tick, *Amblyomma americanum*. *Applied and environmental microbiology.* 80: 354–359.

Porter, S.R., Czaplicki, G., Mainil, J., Guattéo, R. and Saegerman, C. (2011). Q Fever: current state of knowledge and perspectives of research of a neglected zoonosis. *International Journal of Microbiology*. 2011: 248418.

Potgieter F.T., De Waal, D.T. and Posnett E.S. (1992). Transmission and Diagnosis of Equine Babesiosis in South Africa. *Memórias do Instituto Oswaldo Cruz.* 87: 139-142

Poulsen, A.B., Skov, R. and Pallesen, L.V. (2003). Detection of methicillin resistance in coagulase-negative *staphylococci* and in *staphylococci* directly from simulated blood cultures using the EVIGENE MRSA detection kit. *Journal of Antimicrobial Chemotherapy*. 51(2): 419-21.

Pretorius, A.M. and Birtles, R.J. (2002). *Rickettsia aeschlimannii:* A new pathogenic spotted fever group *rickettsia*, South Africa. *Emerging Infectious Diseases.* 8(8): 874.

Rach, J., Bergmann, T., Paknia, O., DeSalle, R., Schierwater, B. and Hadrys, H. (2017). The markerchoice: unexpected resolving power of an unexplored *CO1* region for layered DNA barcoding approaches. *PLoS ONE.* 12(4): e0174842.

Rahal, M., Medkour, H., Diarra, A.Z., Bitam, I., Parola, P. and Mediannikov, O. (2020). Molecular identification and evaluation of *Coxiella-like endosymbionts genetic diversity carried by cattle ticks in Algeria*. *Ticks and Tick-Borne Diseases.* 11(5): 101493.

Rahimi, E., Momtaz, H., Ameri, M., Ghasemian-Safaei, H. and Ali-Kasemi, M. (2010). Prevalence and antimicrobial resistance of *Campylobacter* species isolated from chicken carcasses during processing in Iran. *Poultry Science*. 89(5): 1015-20.

Randela, R. (2007) Socio-economic impact analysis of livestock disease controlprogrammes with special reference to ticks and tick-borne diseases. In Anandajayasekeram P, Rukuni M, Babu S, Liebenberg F and Keswani CL (eds). Impact of science on African agriculture and food security, Oxfordshire, UK: *CABI International*, 12: 221-235.

Raoult, D., Fournier, P.E., Abboud, P. and Caron, F. (2002) First documented human *Rickettsia aeschlimannii* infection. *Emerging Infectious Diseases.* 8: 748-749.

Rawat, S., and Johri, B. N. (2013). “Role of thermophilic microflora in composting,” in thermophilic microbes in environmental and industrial biotechnology, eds T. Satyanarayana, J. Littlechild, and Y. Kawarabayasi (Heidelberg; Dordrecht; New York, NY; London: Springer). 137-169.

Redondo, L.M., Farber, M., Venzano, A., Jost, B.H., Parma, Y.R, and Fernandez-Miyakawa, M.E. (2013). Sudden death syndrome in adult cows associated with *Clostridium perfringens* type E. *Anaerobe*. 20: 1-4.

Reye, A.L, Arinola, O.G., Hübschen, J.M. and Muller, C.P. (2012). Pathogen prevalence in ticks collected from the vegetation and livestock in Nigeria. *Applied and Environmental Microbiology*.78(8): 2562-8.

Richmond-Coggan, L.; (2014). Compararive abundance and ranging behaviour of brown hyaena (Parahyena brunnea) inside and outside protected areas in South Africa, Doctoral Thesis, Nottingham Trent University.

Rizzatti, G., Lopetuso, L.R., Gibiino, G., Binda, C. and Gasbarrini, A. (2017). Proteobacteria: a common factor in human diseases. *Biomedical Research International.* 2017: 9351507.

RMRD SA, (2012). Research and development plan for the large and small stock meat industries in South Africa. 2012-2013. www.rmrdsa.co.za

Robbertse, L., Baron, S., van der Merwe, N.A., Madder, M., Stoltsz, W.H. and MaritzOlivier, C. (2016). Genetic diversity, acaricide resistance status and evolutionary potential of a *Rhipicephalus microplus* population from a disease-controlled cattle farming area in South Africa. *Ticks and Tick-Borne Diseases*. 7: 595-603.

Rocha, S.P., Elias, W.P., Cianciarullo, A.M., Menezes, M.A., Nara, J.M., Piazza, R.M., Silva, M.R., Moreira, C.G., and Pelayo, J.S. (2007). Aggregative adherence of

uropathogenic *Proteus mirabilis* to cultured epithelial cells. *FEMS Immunology and Medical Microbiology.* 51: 319-326.

Rojas-Jaimes, J., Lindo-Seminario, D., Correa-Núñez, G. and Diringer, B. (2021). Characterization of the bacterial microbiome of *Rhipicephalus (Boophilus) microplus* collected from Pecari tajacu "Sajino" Madre de Dios, Peru. *Scientific Reports*. 11(1): 6661.

Roughan, M., Hodge, E., Khan, A. and Chor, J. (2023). Evolving epidemiology of Q fever in Wide Bay. *Communicable Diseases Intelligence* (2018). 26: 47.

Roux, V., Rydkina, E., Eremeeva, M. and Raoult, D. (1997). Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. International. *Journal of Systematic Bacteriology.* 47(2): 252-61.

Rutherford, J.S.; Macaluso, K.R.; Smith, N.; Zaki, S.R.; Paddock, C.D.; Davis, J.; Peterson, N.; Azad, A.F. and Rosenburg, R. (2004). Fatal spotted fever Rickettsiosis, Kenya. *Emerging Infectious Disease*. 10: 910-913.

Ryan, M.P. and Adley, C.C. (2014). *Ralstonia* spp.: emerging global opportunistic pathogens. *European Journal of Clinical Microbiology and Infectious Diseases*. 33(3): 291-304.

Sadeddine R., Diarra A.Z., Laroche M., Mediannikov O., Righi S., Benakhla A., Dahmana H., Raoult D. and Parola P. (2020). Molecular identification of protozoal and bacterial organisms in domestic animals and their infesting ticks from north-eastern Algeria. *Ticks and Tick-Borne Disease*. 11: 101330.

Sahin, O., Yaeger, M., Wu, Z., and Zhang, Q. (2017). *Campylobacter*-associated diseases in animals. *Annual Review Animal Biosciences.* 5:21-42.

Salomon, J., Fernandez Santos, N.A., Zecca, I.B., Estrada-Franco, J.G., Davila, E., Hamer, G.L, Rodriguez Perez, M.A. and Hamer, S.A. (2022). Brown dog tick

(*Rhipicephalus sanguineus* Sensu Lato) infection with endosymbiont and human pathogenic *Rickettsia* spp., in Northeastern México. *International Journal of Environmental Research and Public Health*. 19(10): 6249.

Sambado, S., Sparkman, A., Swei, A., MacDonald, A.J., Young, H.S., Salomon, J., Crews, A., Ring, K., Copeland, S. and Briggs, C.J. (2024). Climate-driven variation in the phenology of juvenile *Ixodes pacificus* on lizard hosts. *Research Square* [Preprint]. rs.3.rs-5671938.

Santajit, S. and Indrawattana, N. (2016). Mechanisms of antimicrobial resistance in ESKAPE pathogens. *Biomed Research International*. 2016: 2475067.

Sassera, D., Beninati, T., Bandi, C., Bouman, E.A.P., Sacchi, L., Fabbi, M. and Lo, N. (2006). *Candidatus* Midichloria mitochondrii', an endosymbiont of the *tick Ixodes ricinus* with a unique intramitochondrial lifestyle. *International Journal of Systematic and Evolutionary Microbiology. 56*: 2535-2540.

Scarpulla, M., Barlozzari, G., Marcario, A., Salvato, L., Blanda, V., De liberato, C., D'Agostini, C., Torina, A. and Macri, G. (2016). Molecular detection and characterization of spotted fever group rickettsiae in ticks from central Italy. *Ticks and Tick-borne Disease.* 7: 1053-1056.

Schindel, D.E. and Miller, S.E. (2005). DNA barcoding a useful tool for taxonomists. *Nature*. 435(7038): 17.

Schorderet-Weber, S., Noack, S., Selzer, P.M. and Kaminsky, R. (2017). Blocking transmission of vector-borne disease. *International Journal for parasitology: Drugs and Drug Resistance.* 7: 90-109.

Schroder, B. and Reilly, B.K. (2013). A comparison between tick species collected in a controlled and control free area on a game ranch in South Africa. *Journal of the South African Veterinary Association*. 84(1): E1-5.

Schulz, A., Kargerb, A., Bettinb, B., Eisenbartha, A., Sasa, M.A., Silaghic, C. and Groschupa, M.H. (2020). Molecular discrimination of Hyalomma tick species serving as reservoirs and vectors for Crimean-Congo hemorrhagic fever virus in sub-Saharan Africa. *Ticks and Tick-borne Diseases.* 11 (3): 101382.

Scoles, G. (2004). Phylogenetic analysis of the *Francisella*-like endosymbionts of *Dermacentor* ticks. *Journal of Medical Entomolology*. 41: 277-286.

Scott, J.D, Foley, J.E., Anderson, J.F., Clark, K.L, and Durden L.A. (2017). Detection of Lyme Disease Bacterium, *Borrelia burgdorferi sensu lato*, in Blacklegged ticks collected in the Grand River Valley, Ontario, Canada. *International Journal of Medical Sciences*. 14(2): 150-158.

Seyboldt, C., Discher, S., Jordan, E., Neubauer, H., Jensen, K.C., Campe, A., Kreienbrock, L., Scheu, T., Wichern, A, Gundling, F., DoDuc, P., Fohler, S., Abdulmawjood, A., Klein, G., and Hoedemaker, M. (2015). Occurrence *of Clostridium botulinum* neurotoxin in chronic disease of dairy cows. *Veterinary Microbiology.* 177(3-4):398-402.

Shange, N., Gouws, P. and Hoffman, L.C. (2019). *Campylobacter* and *Arcobacter* species in food-producing animals: prevalence at primary production and during slaughter. *World Journal of Microbiology and Biotechnology*. 35(9):146.

Sharaby, Y., Nitzan, O., Brettar, I., Höfle, M.G., Peretz, A. and Halpern, M. (2019). Antimicrobial agent susceptibilities of *Legionella pneumophila* MLVA-8 genotypes. *Scientific Report*. 9(1): 6138.

Sheppard, S.K., Dallas, J.F., MacRae, M., McCarthy, N.D., Sproston, E.L., Gormley, F.J., Strachan, N.J., Ogden, I.D., Maiden, M.C., Forbes, K.J. (2009). *Campylobacter* genotypes from food animals, environmental sources and clinical

disease in Scotland 2005/6. *International Journey of Food Microbiology*. 134(1-2): 96-103.

Shirai, A., Bozeman, M., Perri, S., Humphries, J.W. and Fuller, H.S. (1961). Ecology of Rocky Mountain spotted fever. I. *Rickettsia rickettsii* recovered from a cottontail rabbit from Virginia. *Proceedings of the Society for Experimental Biology and Medicine.* 107: 211–214.

Shivakumaraswamy, S.K., Deekshit, V.K., Vittal, R., Akhila, D.S, Mundanda, D.M., Mohan Raj J.R., Chakraborty, A.and Karunasagar, I. (2019) Phenotypic & genotypic study of antimicrobial profile of bacteria isolates from environmental samples*. Indian Journal Medical Research*. 149(2): 232-239.

Shivlata, L. and Satyanarayana, T. (2015). Thermophilic and alkaliphilic Actinobacteria: biology and potential applications*. Frontiers Microbiology.* 6: 1014*.*

Silatsa, B.A., Kuiate, J.R., Njiokou, F., Simo, G., Feussom, J.M.K., Tunrayo, A., Amzati, G.S., Bett, B., Bishop, R., Githaka, N., Opiyoi, S.O., Djikeng, A. and Pellea, R. (2019). A countrywide molecular survey leads to a seminal identification of the invasive cattle tick *Rhipicephalu*s (*Boophilus*) *microplus* in Cameroon, a decade after it was reported in Cote d'Ivoire. *Ticks and Tick-Borne Diseases*. 10: 585-593.

Simpson, V.C. (2019). Ticks and Tick-borne pathogens of horses in Potchefstroom. Honours Project, North-West University (Potchefstroom campus), South Africa.

Skotarczak, B., (2018). The role of companion animals in the environmental circulation of tick-borne bacterial pathogens. *Annals of Agricultural and Environmental Medicine*. 25 (3): 473-480.

Smith, R.D. Brener, J., Osorno, M., and Ristic, M. (1978). Pathobiology of *Borrelia theileri* in the tropical cattle tick, *Boophilus microplus*. *Journal of Invertebrate Pathology*. 32: 182-190.

Smith, T.A., Driscoll, T., Gillespie, J.J., Raghavan, R. A. (2015). *Coxiella*-like endosymbiont is a potential vitamin source for the Lone Star tick. *Genome Biology and Evolution*. 7(3): 831-8.

Soneshine, D.E., (1991). Biology of ticks, *Volume 1*. Oxford University Press. 472 pages

Song, S., Shao, R., Atwell, R., Barker, S. and Vankan, D. (2011). Phylogenetic and phylogeographic relationships in Ixodes holocyclus and Ixodes cornuatus (Acari: Ixodidae) inferred from COX1 and ITS2 sequences. *International Journal for Parasitology.* 41 (8): 871-80.

Speirs, L.B.M., Rice, D.T.F., Petrovski, S. and Seviour, R.J., (2019). The phylogeny, biodiversity, and ecology of the *Chloroflexi* in activated sludge. *Frontier Microbiology*. 10: 2015.

Spellman, F. R. and Stoudt, M. L. (2013). *The handbook of environmental health* (1st ed.). Scarecrow Press, Inc. pg425

Spickett, A.M., Heyne, I.H. and Williams, R. (2011). Survey of the livestock ticks of the North West province, South Africa. *Onderstepoort Journal of Veterinary Research*. 78(1): 305.

Stanley, and Jones (2003). Cattle and sheep farms as reservoirs of *Campylobacter*. *Applied Microbiology International.* 94: 104S-113S.

Stein, R.A., and Katz, D.E. (2017). *Escherichia coli*, cattle and the propagation of disease. *FEMS Microbiology Letters*. 364(6): fnx050.

Steinbrink. A., Brugger, K., Margos, G., Kraiczy, P. and Klimpel, S. (2022). The evolving story of *Borrelia burgdorferi* sensu lato transmission in Europe. *Parasitology Research.* 121(3) :781-803.

Swerczek, T.W. (2019). Exacerbation of *Streptococcus equi* (strangles) by overly nutritious diets in horses: a model for infectious bacterial diseases of horses and other livestock. *Animal and Veterinary Sciences*. 7(1): 18-23.

Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., Vermat, T., Corthier, G., Brochmann, C. and Willerslev, E. (2007). Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. *Nucleic Acids Research*. 35(3): e14.

Tahulending, J., Tulung, M., Pelealu, J. and Doda, D.V. (2022). Molecular detection of pathogenic bacteria in *Rhipicephalus sanguineus* (sensu lato) ticks from Bitung, North Sulawesi, Indonesia. *Biodiversitas.* 23 (12): 6164-6170.

Taioe, M.O. (2017). Molecular characterization of horse flies (Diptera: Tabanidae) and determination of their role in transmission of haemoparasites in South Africa. Doctoral Thesis, North-West University (Potchefstroom campus), South Africa.

Tamura, K., Stecher, G. and Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*. 38(7): 3022-3027.

Tan, L.P., Hamdan, R.H., Hassan, B.N.H., Reduan, M.F.H., Okene, I.A.- A., Loong, S.K., Khoo, J.J., Samsuddin, A.S. and Lee, S.H. (2021). *Rhipicephalus* tick: a contextual review for Southeast Asia. *Pathogens* 10: 821.'

Tawana, M., Onyiche, T.E., Ramatla, T., Mtshali, S. and Thekisoe, O. (2022). Epidemiology of ticks and tick-borne pathogens in domestic ruminants across Southern African Development Community (SADC) region from 1980 until 2021: A systematic review and meta-analysis. *Pathogens*. 11(8) :929.

Tekin, S., Dowd, S.E., Davinic, M., Bursali, A, Keskin, A. (2017). Pyrosequencing based assessment of bacterial diversity in Turkish *Rhipicephalus annulatus* and

Dermacentor marginatus ticks (Acari: Ixodidae). *Parasitology Research*. 116(3): 1055-1061.

Teshale, S., Kumsa, B, Menandro, M.L. Cassini, R. and Martini, M. (2016). *Anaplasma*, *Ehrlichia* and *Rickettsial* pathogens in ixodid ticks infesting cattle and sheep in western Oromia, Ethiopia. *Experimental and Applied Acarology.* 70: 231–237.

Thapa, S., Zhang, Y. and Allen, M.S. (2019). Effects of temperature on bacterial microbiome composition in *Ixodes scapularis* ticks. *MicrobiologyOpen*. 8(5): e00719.

Thomas, T., Gilbert, J. and Meyer, F. (2012). Metagenomics: A guide from sampling to data analysis. *Microbial Informatics and Experimentation.* 2: 3.

Thompson, I.M and Dahl, G.E. (2012). Dry-period seasonal effects on the subsequent lactation. *The Professional Animal Scientist* 28(6): 628-631.

Tissot-Dupont, H. and Raoult, D. (2008). Q fever. *Infectious Disease Clinics of North America*. 22(3): 505-14, ix.

Tomassone, L., De Meneghi, D., Adakal, H., Rodighiero, P., Pressi, G. and Grego, E. (2016). Detection of *Rickettsia aeschlimannii* and *Rickettsia africae* in ixodid ticks from Burkina Faso and Somali Region of Ethiopia by new real-time PCR assays. *Ticks and Tick Borne Diseases*. 7(6): 1082-1088.

Tosoni, A., Mirijello, A., Ciervo, A., Mancini, F., Rezza, G., Damiano, F., Cauda, R., Gasbarrini, A. and Addolorato, G. (2016). Internal medicine sepsis study group. Human *Rickettsia aeschlimannii* infection: first case with acute hepatitis and review of the literature. *European Review for Medical and Pharmacologgical Sciences*. 20(12): 2630-3.

Trout Fryxell, R.T. and DeBruyn, J.M. (2016). The microbiome of *Ehrlichia*-infected and uninfected Lone Star ticks (*Amblyomma americanum*). *PLoS One*. 11(1): e0146651.

Truong, A.T., Yun, B.R., Lim, J., Min, S., Yoo, M.S., Yoon, S.S., Yun, Y.M., Kim, J.T. and Cho, Y.S. (2021). Real-time PCR biochip for on-site detection of *Coxiella burnetii* in ticks. *Parasite & Vectors*. 14(1): 239.

Trzebny, A., Liberska, J., Slodkowicz-Kowalska, A. and Dabert, M. (2022). Metabarcoding reveals low prevalence of microsporidian infections in castor bean tick (*Ixodes ricinus*). *Parasite & Vectors*. 15(1): 26.

Tveten, A-K., Andreas Riborg, A., and Hanne Tjelle Vadseth, H.T. (2013) DGGE dentification of microorganisms associated with Borrelia burgdorferi Sensu Lato- or Anaplasma phagocytophilum-infected Ixodes ricinus ticks from Northwest Norway. *International Journal of Microbiology*. 2013: 805456.

Uilenberg, G. (1992). Veterinary significance of ticks and tick-borne diseases. In: B. Fivaz, T. Petney and I. Horak (Editors). Tick Vector Biology. Medical and Veterinary Aspects. Springer, Berlin/ Heidelberg, pp. 23-33.

Uni, S., Bain, O., Fujita, H., Matsubayashi, M., Fukuda, M. and Takaoka, H. (2013) Infective larvae of *Cercopithifilaria* spp. (Nematoda: Onchocercidae) from hard ticks (Ixodidae) recovered from the Japanese serow (Bovidae). *Parasite*. 20: 1.

van den Heever, M.J.J. Lombard, W.A., Bahta, Y.T. and Marè F.A. (2022). The economic impact of heartwater on the South African livestock industry and the need for a new vaccine. *Preventive Veterinary Medicine.* 203: 105634.

van Wyk, C. (2019). Exploration of tick-borne pathogens and microbiota of dog ticks collected at Potchefstroom Animal Welfare Society, Masters Dissertation, North-West University (Potchefstroom campus), South Africa.

van Wyk, C.V., Mtshali, K., Taioe, M.O., Terera, S., Bakkes, D., Ramatla, T., Xuan, X. and Thekisoe O. (2022). Detection of ticks and tick-borne pathogens of urban stray dogs in South Africa. *Pathogens.* 11(8): 862.

van Wyk, D.A.B., Adeleke, R., Rhode, O.H.J., Bezuidenhout, C.C., and Mienie, C. (2017). Ecological guild and enzyme activities of rhizosphere soil microbial communities associated with Bt-maize cultivation under field conditions in North West province of South Africa. *Journal of Basic Microbiology.* 57(9): 781-792.

Vanderburg, S., Rubach, M.P., Halliday, J.E., Cleaveland, S., Reddy, E.A. and Crump, J.A. (2014). Epidemiology of *Coxiella burnetii* infection in Africa: A One Health systematic review. *PLoS Neglected Tropical Diseases*. 8(4): e2787.

Varela-Stokes, A.S., Park, S.H., Kim, S.A. and Ricke, S.C. (2017.) Microbial communities in North American *Ixodid* ticks of veterinary and medical importance. *Frontiers Veterinary Science.* 4: 179.

Vayssier-Taussat, M., Moutailler, S., Michelet, L., Devillers, E., Bonnet, S., Cheval, J., He´bert, C and Eloit, M. (2013). Next generation sequencing uncovers unexpected bacterial pathogens in ticks in Western Europe. *PLoS ONE.* 8(11): e81439.

Vilcins, I. M., Old, J. M. and Deane, E. (2009). Molecular detection of *Rickettsia*, *Coxiella* and *Rickettsiella* DNA in three native Australian tick species. *Experimental and Applied Acarology*. 49: 229-242.

Virji, M. (2009). Pathogenic neisseriae: surface modulation, pathogenesis and infection control. *Nature Reviews Microbiol*ology. 7: 274-286.

Vudriko, P., Okwee-Acai, J., Tayebwa, D.S., Byaruhanga, J., Kakooza, S., Wampande, E. Omara, R., Muhindo, J.B., Tweyongyere, R., Owiny, D.O., Hatta, T., Tsuji, N., Umemiya-Shirafuji, R., Xuan, X., Kanameda, M., Fujisaki, K., Suzuki,

H. (2016). Emergence of multi-acaricide resistant *Rhipicephalus* ticks and its implication on chemical tick control in Uganda. *Parasites & Vectors.* 9: 4.

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Pena, A., Horak, I.G., Latif, A.A., Pegram, R.G. and Preston, P.M., (2003). Ticks of domestic animals in Africa, A guide to identification of species. *Bioscience* Reports. U.K, pp.86-214.

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Pena, A., Horak, I.G., Latif, A., Pegram, R.G. and Preston, P.M. (2014). Ticks of domestic animals in Africa, A guide to identification of species. Edinburgh: *Bioscience* Reports. U.K, pp.86-214.

Walker, J.B., Keirans, J.E. and Horak, I.G. (2000). The genus *Rhipicephalus* (Acari, Ixodidae): A guide to the brown ticks of the World, Cambridge University Press, Cambridge.

Wang, H-H, Corson, M.S, Grant, W.E. and Teel, P.D. (2017). Quantitative models of *Rhipicephalus* (*Boophilus*) ticks: historical review and synthesis. *Ecosphere.* 8: e01942.

Wang, H-H., Grant, W.E., Teel, P.D. Lohmeyer, K.H. and Pérez de León, A.A. (2020). Enhanced biosurveillance of high-consequence invasive pests: southern cattle fever ticks, *Rhipicephalus* (*Boophilus*) *microplus*, on livestock and wildlife. *Parasites Vectors* 13(1): 487.

Welc-Faleciak, R., Rodo, A., Siński, E. and Bajer, A. (2009). *Babesia canis* and other tick-borne infections in dogs in Central Poland. *Veterinary Parasitology.* 166: 191-198.

Wen, S., Feng, D., Chen, D., Yang, L., and Xu, Z. (2020). Molecular epidemiology and evolution of Haemophilus influenzae. *Infection Genetics and Evolution*. 80: 104205.

Wesonga, F.D., Kitala, P.M., Gathuma, J.M., Njenga, M.J. and Ngumi, P.N. (2010). An assessment of tick-borne diseases constraints to livestock production in a

smallholder livestock production system in Machakos District, Kenya. *Livestock Research for Rural Development*. 22(6).

Wildman, M.J., Smith, E.G., Groves, J., Beattie, J.M., Caul, E.O., Ayres, J.G. (2002). Chronic fatigue following infection by *Coxiella burnetii* (Q fever): ten-year follow-up of the 1989 UK outbreak cohort. *QJM: An International Journal of Medicine*. 95(8): 527-38.

Williams, P.D., Eichstadt, S.L., Kokjohn, T.A. and Martin, E.L. (2007). Effects of ultraviolet radiation on the gram-positive marine bacterium *Microbacterium maritypicum*. *Current Microbiology*. 55(1):1-7.

Wong, K.Y., Chan, Y.C. and Wong, C.Y. (2010). *Corynebacterium striatum* as an emerging pathogen. *Journal of Hospital Infection.* 76: 371-372

Wu-Chuang, A., Hodžić, A., Mateos-Hernández, L., Estrada-Peña, A., Obregon, D. and Cabezas-Cruz, A. (2021). Current debates and advances in tick microbiome research. *Current Research in Parasitology & Vector-Borne Diseases.* 1:100036.

Xiong, B., and Kocher, T. D. (1991). Comparison of mitochondrial DNA sequences of seven morphospecies of black flies (Diptera: Sim- uliidae). *Genome.* 34: 306-311.

Xiong, B., and Kocher, T. D. (1993). Phylogeny of sibling species of *Simulium uenustum* and *S. verecundum* (Diptera: Simulidael based on sequences of the mitochondrial 16S rRNA gene. *Molecular Phylogenetics and Evolution.* 2(4): 293-303.

Xu, S., Liu, Y., Gao, J., Zhou, M., Yang, J., He, F., Kastelic, J.P., Deng, Z. and Han, B. (2021). Comparative Genomic Analysis of *Streptococcus dysgalactiae subspecies dysgalactiae* Isolated from *Bovine Mastitis* in China. *Frontiers in Microbiology*. 12: 751863.

Yan, Y., Lu, C., Gong, P., Pei, Z, Peng, Y., Jian, F., Wang, R., Zhang, L., Qi, M. and Ning., C. (2022). Molecular detection and phylogeny of *Anaplasma* spp. closely related to *Anaplasma phagocytophilum* in small ruminants from China. *Ticks and Tick-borne Diseases.* 13: 101992.

Yawa, M., Nyangiwe, N., Kadzere, C.T., Muchenje, V., Mpendulo, T.C. and Marufu, M.C., (2019). In search of the *Rhipicephalus (Boophilus) microplus* in the western-central regions of the eastern Cape Province, South Africa. *Ticks and Tick-Borne Diseases.* 10: 564-567.

Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C. and Glöckner, F.O. (2014). The SILVA and "all-species living tree project (LTP)" taxonomic frameworks. *Nucleic Acids Research*.42(1): D643-d648.

Yin, H., Luo, J., Guan, G., Gao, Y., Lu, B., Zhang, Q., Ma, M., Lu, W., Lu, C., Yuan, Z., Guo, S., Wang, B., Du, H., Schnittger, L., Ahmed, J. and Jongejan, F. (2002). Transmission of an unidentified *Theileria* species to small ruminants by *Haemaphysalis qinghaiensis* ticks collected in the field. *Parasitology Research. 88*: s25-s27.

Yin, X., Guo, S., Ding, C., Cao, M., Kawabata, H., Sato, K., Ando, S., Fujita, H., Kawamori, F., Su, H., Shimada, M., Shimamura, Y., Masuda, S. and Ohashi, N. (2018). Spotted fever group rickettsiae in inner Mongolia, China, 2015-2016. *Emerging Infectious Diseases*. 24(11): 2105-2107.

Yokota, A. (2012). Cultivation of uncultured bacteria of the class Ktedonobacteria in the Phylum Chloroflexi. *Makara Journal of Science*: 16 (1): 1.

Yu, X.J., Liang, M.F., Zhang, S.Y., Liu, Y., Li, J.D., Sun, Y.L., Zhang, L., Zhang, Q.F., Popov, V.L., Li, C., Qu, J., Li, Q., Zhang, Y.P., Hai, R., Wu, W., Wang, Q., Zhan, F.X., Wang, X.J., Kan, B., Wang, S.W., Wan, K.L., Jing, H.Q., Lu, J.X., Yin, W.W.,

Zhou, H., Guan, X.H., Liu, J.F., Bi, Z.Q., Liu, G.H., Ren, J., Wang, H., Zhao, Z., Song, J.D., He, J.R., Wan, T., Zhang, J.S., Fu, X.P., Sun, L.N., Dong, X.P., Feng, Z.J., Yang, W.Z., Hong, T., Zhang, Y., Walker, D.H., Wang, Y. and Li, D.X. (2011) Fever with thrombocytopenia associated with a novel bunyavirus in China. *New England Journal of Medicine.* 364:1523-1532.

Yu, X.J., Liang, M.F., Zhang, S.Y., Liu, Y., Li, J.D., Sun, Y.L., Zhang, L., Zhang, Q.F., Popov, V.L., Li, C., Qu, J., Li, Q., Zhang, Y.P., Hai, R., Wu, W., Wang, Q., Zhan, F.X., Wang, X.J., Kan, B., Wang, S.W., Wan, K.L., Jing, H.Q., Lu, J.X., Yin, W.W., Zhou, H., Guan, X.H., Liu, J.F., Bi, Z.Q., Liu, G.H., Ren, J., Wang, H., Zhao, Z., Song, J.D., He, J.R., Wan, T., Zhang, J.S., Fu, X.P., Sun, L.N., Dong, X.P., Feng, Z.J., Yang, W.Z., Hong, T., Zhang, Y., Walker, D.H., Wang, Y. and Li, D.X. (2011) Fever with thrombocytopenia associated with a novel bunyavirus in China. *New England Journal of Medicine.* 364:1523–1532.

Zaragoza, N.E., Orellana, C.A., Moonen, G.A., Moutafis, G. and Marcellin, E. (2019). Vaccine production to protect animals against pathogenic Clostridia. *Toxins* (Basel).11(9): 525.

Zhang, R., Huang, Z., Yu, G. and Zhang, Z. (2019). Characterization of microbiota diversity of field-collected *Haemaphysalis longicornis* (Acari: Ixodidae) with regard to sex and blood meals. *Journal of Basic Microbiology*. 59(2): 215-223.

Zhang, W., Zhu, H.H., Yuan, M., Yao, Q., Tang, R, Lin, M., Yang, S.Z., Li, Z.K. and Chen M. (2010). Microbacterium *radiodurans* sp. nov., a UV radiation-resistant bacterium isolated from soil. *International Journal of Systematic and Evolutionary Microbiology*. 60(Pt 11): 2665-2670.

Zolnik, C.P., Falco, R.C., Daniels, T.J. and Kolokotronis, S-O. 2018. Transient influence of blood meal and natural environment on blacklegged tick bacterial

Zolnik, C.P., Prill, R.J., Falco, R.C., Daniels, T.J. and Kolokotronis, S. (2016). Microbiome changes through ontogeny of a tick pathogen vector. *Molecular Ecology*. 25: 4963–4977.

8. Appendix

8.1 BLASTn search results for tick identification samples targeting the*16S rRNA* gene

Horse_h6_16S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	658	658	98%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi evertsi isolate 130SA 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	658	658	98%	0.0	100.00%	401	KJ613642.1
Rhipicephalus evertsi clone 209/589_14740_159 mitochondrion, complete genome	Rhipicephalus e…	658	658	98%	0.0	100.00%	14740	KY457538.1
Rhipicephalus evertsi clone 1M_14379_26 mitochondrion, complete genome	Rhipicephalus e…	658	658	98%	0.0	100.00%	14739	KY457537.1
Rhipicephalus evertsi isolate F9P33 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	647	647	98%	0.0	99.44%	390	OP971116.1
Rhipicephalus evertsi isolate F9P32 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	647	647	98%	0.0	99.44%	390	OP971115.1

Horse_h7_16S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

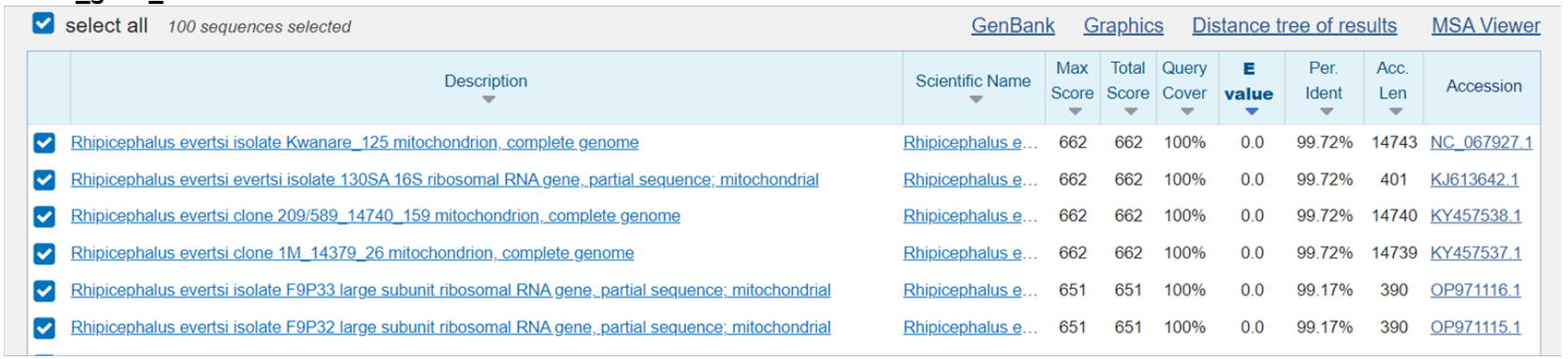
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	667	667	100%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi evertsi isolate 130SA 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	667	667	100%	0.0	100.00%	401	KJ613642.1
Rhipicephalus evertsi clone 209/589_14740_159 mitochondrion, complete genome	Rhipicephalus e…	667	667	100%	0.0	100.00%	14740	KY457538.1
Rhipicephalus evertsi clone 1M_14379_26 mitochondrion, complete genome	Rhipicephalus e…	667	667	100%	0.0	100.00%	14739	KY457537.1
Rhipicephalus evertsi isolate F9P33 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	656	656	100%	0.0	99.45%	390	OP971116.1
Rhipicephalus evertsi isolate F9P32 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	656	656	100%	0.0	99.45%	390	OP971115.1

Horse_h9_16S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus ev…	662	662	100%	0.0	99.72%	14743	NC_067927.1
Rhipicephalus evertsi evertsi isolate 130SA 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus ev…	662	662	100%	0.0	99.72%	401	KJ613642.1
Rhipicephalus evertsi clone 209/589_14740_159 mitochondrion, complete genome	Rhipicephalus ev…	662	662	100%	0.0	99.72%	14740	KY457538.1
Rhipicephalus evertsi clone 1M_14379_26 mitochondrion, complete genome	Rhipicephalus ev…	662	662	100%	0.0	99.72%	14739	KY457537.1
Rhipicephalus evertsi isolate F9P33 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus ev…	651	651	100%	0.0	99.17%	390	OP971116.1
Rhipicephalus evertsi isolate F9P32 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus ev…	651	651	100%	0.0	99.17%	390	OP971115.1

Goat_gmf1_16S



select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	662	662	100%	0.0	99.72%	14743	NC_067927.1
Rhipicephalus evertsi evertsi isolate 130SA 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	662	662	100%	0.0	99.72%	401	KJ613642.1
Rhipicephalus evertsi clone 209/589_14740_159 mitochondrion, complete genome	Rhipicephalus e…	662	662	100%	0.0	99.72%	14740	KY457538.1
Rhipicephalus evertsi clone 1M_14379_26 mitochondrion, complete genome	Rhipicephalus e…	662	662	100%	0.0	99.72%	14739	KY457537.1
Rhipicephalus evertsi isolate F9P33 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	651	651	100%	0.0	99.17%	390	OP971116.1
Rhipicephalus evertsi isolate F9P32 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	651	651	100%	0.0	99.17%	390	OP971115.1

Goat_gmf3_16S



select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	662	662	100%	0.0	99.72%	14743	NC_067927.1
Rhipicephalus evertsi evertsi isolate 130SA 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	662	662	100%	0.0	99.72%	401	KJ613642.1
Rhipicephalus evertsi clone 209/589_14740_159 mitochondrion, complete genome	Rhipicephalus e…	662	662	100%	0.0	99.72%	14740	KY457538.1
Rhipicephalus evertsi clone 1M_14379_26 mitochondrion, complete genome	Rhipicephalus e…	662	662	100%	0.0	99.72%	14739	KY457537.1
Rhipicephalus evertsi isolate F9P33 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	651	651	100%	0.0	99.17%	390	OP971116.1
Rhipicephalus evertsi isolate F9P32 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	651	651	100%	0.0	99.17%	390	OP971115.1

Goat_gmm3_16S



select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	667	667	100%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi evertsi isolate 130SA 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	667	667	100%	0.0	100.00%	401	KJ613642.1
Rhipicephalus evertsi clone 209/589_14740_159 mitochondrion, complete genome	Rhipicephalus e…	667	667	100%	0.0	100.00%	14740	KY457538.1
Rhipicephalus evertsi clone 1M_14379_26 mitochondrion, complete genome	Rhipicephalus e…	667	667	100%	0.0	100.00%	14739	KY457537.1
Rhipicephalus evertsi isolate F9P33 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	656	656	100%	0.0	99.45%	390	OP971116.1
Rhipicephalus evertsi isolate F9P32 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	656	656	100%	0.0	99.45%	390	OP971115.1

Dog_S3_16S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus sanguineus isolate 56 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus s…	675	675	100%	0.0	100.00%	441	MG651947.1
Rhipicephalus sanguineus M414 mitochondrial gene for 16S ribosomal RNA, partial sequence	Rhipicephalus s…	675	675	100%	0.0	100.00%	404	LC422405.1
Rhipicephalus sanguineus isolate 4981 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus s…	675	675	100%	0.0	100.00%	410	MH018842.1
Rhipicephalus sanguineus isolate 4983 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus s…	675	675	100%	0.0	100.00%	410	MH018841.1
Rhipicephalus sanguineus isolate 4976 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus s…	675	675	100%	0.0	100.00%	410	MH018840.1
Rhipicephalus sanguineus isolate 4977 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus s…	675	675	100%	0.0	100.00%	410	MH018839.1

Dog_SAN2_16S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus sanguineus isolate 56 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus sa…	671	671	100%	0.0	99.73%	441	MG651947.1
Rhipicephalus sanguineus isolate sanguineus0926 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus sa…	671	671	100%	0.0	99.73%	413	MH513312.1
Rhipicephalus sanguineus M414 mitochondrial gene for 16S ribosomal RNA, partial sequence	Rhipicephalus sa…	671	671	100%	0.0	99.73%	404	LC422405.1
Rhipicephalus sanguineus isolate 4981 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus sa…	671	671	100%	0.0	99.73%	410	MH018842.1
Rhipicephalus sanguineus isolate 4983 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus sa…	671	671	100%	0.0	99.73%	410	MH018841.1
Rhipicephalus sanguineus isolate 4976 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus sa…	671	671	100%	0.0	99.73%	410	MH018840.1

Dog_SAN3_16S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus sanguineus isolate 56 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus s…	675	675	100%	0.0	100.00%	441	MG651947.1
Rhipicephalus sanguineus M414 mitochondrial gene for 16S ribosomal RNA, partial sequence	Rhipicephalus s…	675	675	100%	0.0	100.00%	404	LC422405.1
Rhipicephalus sanguineus isolate 4981 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus s…	675	675	100%	0.0	100.00%	410	MH018842.1
Rhipicephalus sanguineus isolate 4983 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus s…	675	675	100%	0.0	100.00%	410	MH018841.1
Rhipicephalus sanguineus isolate 4976 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus s…	675	675	100%	0.0	100.00%	410	MH018840.1
Rhipicephalus sanguineus isolate 4977 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus s…	675	675	100%	0.0	100.00%	410	MH018839.1

RE7_Sheep_16S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	658	658	100%	0.0	99.72%	14743	NC_067927.1
Rhipicephalus evertsi evertsi isolate 130SA 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	658	658	100%	0.0	99.72%	401	KJ613642.1
Rhipicephalus evertsi clone 209/589_14740_159 mitochondrion, complete genome	Rhipicephalus e…	658	658	100%	0.0	99.72%	14740	KY457538.1
Rhipicephalus evertsi clone 1M_14379_26 mitochondrion, complete genome	Rhipicephalus e…	658	658	100%	0.0	99.72%	14739	KY457537.1
Rhipicephalus evertsi isolate F9P33 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	647	647	100%	0.0	99.17%	390	OP971116.1
Rhipicephalus evertsi isolate F9P32 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	647	647	100%	0.0	99.17%	390	OP971115.1

RE11_Sheep_16S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	662	662	99%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi evertsi isolate 130SA 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	662	662	99%	0.0	100.00%	401	KJ613642.1
Rhipicephalus evertsi clone 209/589_14740_159 mitochondrion, complete genome	Rhipicephalus e…	662	662	99%	0.0	100.00%	14740	KY457538.1
Rhipicephalus evertsi clone 1M_14379_26 mitochondrion, complete genome	Rhipicephalus e…	662	662	99%	0.0	100.00%	14739	KY457537.1
Rhipicephalus evertsi isolate F9P33 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	651	651	99%	0.0	99.44%	390	OP971116.1
Rhipicephalus evertsi isolate F9P32 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	651	651	99%	0.0	99.44%	390	OP971115.1

RE15_Sheep_16S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	664	664	99%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi evertsi isolate 130SA 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	664	664	99%	0.0	100.00%	401	KJ613642.1
Rhipicephalus evertsi clone 209/589_14740_159 mitochondrion, complete genome	Rhipicephalus e…	664	664	99%	0.0	100.00%	14740	KY457538.1
Rhipicephalus evertsi clone 1M_14379_26 mitochondrion, complete genome	Rhipicephalus e…	664	664	99%	0.0	100.00%	14739	KY457537.1
Rhipicephalus evertsi isolate F9P33 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	652	652	99%	0.0	99.44%	390	OP971116.1
Rhipicephalus evertsi isolate F9P32 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	652	652	99%	0.0	99.44%	390	OP971115.1

R1_Cattle_16S



select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	667	667	100%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi evertsi isolate 130SA 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	667	667	100%	0.0	100.00%	401	KJ613642.1
Rhipicephalus evertsi clone 209/589_14740_159 mitochondrion, complete genome	Rhipicephalus e…	667	667	100%	0.0	100.00%	14740	KY457538.1
Rhipicephalus evertsi clone 1M_14379_26 mitochondrion, complete genome	Rhipicephalus e…	667	667	100%	0.0	100.00%	14739	KY457537.1
Rhipicephalus evertsi isolate F9P33 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	656	656	100%	0.0	99.45%	390	OP971116.1
Rhipicephalus evertsi isolate F9P32 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	656	656	100%	0.0	99.45%	390	OP971115.1

R2_Cattle_16S



select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	665	665	100%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi evertsi isolate 130SA 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	665	665	100%	0.0	100.00%	401	KJ613642.1
Rhipicephalus evertsi clone 209/589_14740_159 mitochondrion, complete genome	Rhipicephalus e…	665	665	100%	0.0	100.00%	14740	KY457538.1
Rhipicephalus evertsi clone 1M_14379_26 mitochondrion, complete genome	Rhipicephalus e…	665	665	100%	0.0	100.00%	14739	KY457537.1
Rhipicephalus evertsi isolate F9P33 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	654	654	100%	0.0	99.44%	390	OP971116.1
Rhipicephalus evertsi isolate F9P32 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	654	654	100%	0.0	99.44%	390	OP971115.1

R3_Cattle_16S



select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	665	665	100%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi evertsi isolate 130SA 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	665	665	100%	0.0	100.00%	401	KJ613642.1
Rhipicephalus evertsi clone 209/589_14740_159 mitochondrion, complete genome	Rhipicephalus e…	665	665	100%	0.0	100.00%	14740	KY457538.1
Rhipicephalus evertsi clone 1M_14379_26 mitochondrion, complete genome	Rhipicephalus e…	665	665	100%	0.0	100.00%	14739	KY457537.1
Rhipicephalus evertsi isolate F9P33 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	654	654	100%	0.0	99.44%	390	OP971116.1
Rhipicephalus evertsi isolate F9P32 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus e…	654	654	100%	0.0	99.44%	390	OP971115.1

Clinvet_Rm1_16S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Ixodida sp. clone MK1 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Ixodida sp.	673	673	100%	0.0	100.00%	407	MN961110.1
Rhipicephalus microplus isolate CJ1-2021 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus m…	667	667	99%	0.0	100.00%	428	OP613368.1
Rhipicephalus microplus isolate CJ282-2020 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus m…	667	667	99%	0.0	100.00%	428	OP613367.1
Rhipicephalus microplus voucher WC2 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus m…	667	667	99%	0.0	100.00%	434	OQ725522.1
Rhipicephalus microplus voucher WC1 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus m…	667	667	99%	0.0	100.00%	432	OQ725521.1
Rhipicephalus microplus voucher LG6 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus m…	667	667	99%	0.0	100.00%	429	OQ725520.1

Clinvet_Rd1_16S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus microplus isolate CJ1-2021 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus m…	673	673	100%	0.0	100.00%	428	OP613368.1
Rhipicephalus microplus isolate CJ282-2020 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus m…	673	673	100%	0.0	100.00%	428	OP613367.1
Rhipicephalus microplus voucher WC2 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus m…	673	673	100%	0.0	100.00%	434	OQ725522.1
Rhipicephalus microplus voucher WC1 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus m…	673	673	100%	0.0	100.00%	432	OQ725521.1
Rhipicephalus microplus voucher LG6 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus m…	673	673	100%	0.0	100.00%	429	OQ725520.1
Rhipicephalus microplus voucher LG5 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus m…	673	673	100%	0.0	100.00%	433	OQ725519.1

Clinvet_Rp1_16S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus appendiculatus isolate Rhap mitochondrion, partial genome	Rhipicephalus a…	667	667	99%	0.0	100.00%	9569	KC503257.1
Rhipicephalus appendiculatus ZT17-397_Rap mitochondrial gene for 16S rRNA, partial sequence	Rhipicephalus a…	667	667	99%	0.0	100.00%	402	LC634572.1
Rhipicephalus appendiculatus clone 453/3458_14733_42 mitochondrion, complete genome	Rhipicephalus a…	667	667	99%	0.0	100.00%	14733	KY457536.1
Rhipicephalus appendiculatus clone 3001_14733_91 mitochondrion, complete genome	Rhipicephalus a…	667	667	99%	0.0	100.00%	14733	KY457535.1
Mitochondrion Rhipicephalus appendiculatus 16S ribosomal RNA (16S rRNA) gene	Rhipicephalus a…	662	662	99%	0.0	99.72%	455	L34301.1
Rhipicephalus appendiculatus clone T218 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus a…	656	656	99%	0.0	99.45%	405	MN266913.2

Re1_Clinvet_16S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus ev…	662	662	100%	0.0	99.72%	14743	NC_067927.1
Rhipicephalus evertsi evertsi isolate 130SA 16S ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus ev…	662	662	100%	0.0	99.72%	401	KJ613642.1
Rhipicephalus evertsi clone 209/589_14740_159 mitochondrion, complete genome	Rhipicephalus ev…	662	662	100%	0.0	99.72%	14740	KY457538.1
Rhipicephalus evertsi clone 1M_14379_26 mitochondrion, complete genome	Rhipicephalus ev…	662	662	100%	0.0	99.72%	14739	KY457537.1
Rhipicephalus evertsi isolate F9P33 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus ev…	651	651	100%	0.0	99.17%	390	OP971116.1
Rhipicephalus evertsi isolate F9P32 large subunit ribosomal RNA gene, partial sequence; mitochondrial	Rhipicephalus ev…	651	651	100%	0.0	99.17%	390	OP971115.1

8.2 BLASTn search results for tick identification samples targeting the *CO1* gene

Horse_h6_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus glabroscutatus isolate Rg1_LE_QAC_2016_Rg208 cytochrome oxidase subunit I (CO1) gene, pa…	Rhipicephalus gl…	996	996	100%	0.0	99.82%	548	MN453672.1
Rhipicephalus evertsi evertsi isolate Ree9_LE_QAC_2019_Ree436 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus ev…	996	996	100%	0.0	99.82%	548	MN453671.1
Rhipicephalus evertsi evertsi isolate Ree12_LE_LER_2019_Ree702 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus ev…	996	996	100%	0.0	99.82%	548	MN453669.1
Rhipicephalus evertsi evertsi isolate Ree1_LE_MAS_2017_Ree228 cytochrome c oxidase subunit I (CO1) gene…	Rhipicephalus ev…	996	996	100%	0.0	99.82%	544	MK551201.1
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus ev…	996	996	100%	0.0	99.82%	14743	NC_067927.1
Rhipicephalus evertsi cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus ev…	996	996	100%	0.0	99.82%	707	MZ351136.1

Horse_h7_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus glabroscutatus isolate Rg1_LE_QAC_2016_Rg208 cytochrome oxidase subunit I (CO1) gene, p…	Rhipicephalus gl…	1002	1002	100%	0.0	100.00%	548	MN453672.1
Rhipicephalus evertsi evertsi isolate Ree9_LE_QAC_2019_Ree436 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453671.1
Rhipicephalus evertsi evertsi isolate Ree12_LE_LER_2019_Ree702 cytochrome oxidase subunit I (CO1) gene…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453669.1
Rhipicephalus evertsi evertsi isolate Ree1_LE_MAS_2017_Ree228 cytochrome c oxidase subunit I (CO1) gen…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	544	MK551201.1
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	707	MZ351136.1

Horse_h9_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus glabroscutatus isolate Rg1_LE_QAC_2016_Rg208 cytochrome oxidase subunit I (CO1) gene, p…	Rhipicephalus gl…	1002	1002	100%	0.0	100.00%	548	MN453672.1
Rhipicephalus evertsi evertsi isolate Ree9_LE_QAC_2019_Ree436 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453671.1
Rhipicephalus evertsi evertsi isolate Ree12_LE_LER_2019_Ree702 cytochrome oxidase subunit I (CO1) gene…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453669.1
Rhipicephalus evertsi evertsi isolate Ree1_LE_MAS_2017_Ree228 cytochrome c oxidase subunit I (CO1) gen…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	544	MK551201.1
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	707	MZ351136.1

Goat_gmf1_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus glabroscutatus isolate Rg1_LE_QAC_2016_Rg208 cytochrome oxidase subunit I (CO1) gene, p…	Rhipicephalus gl…	1002	1002	100%	0.0	100.00%	548	MN453672.1
Rhipicephalus evertsi evertsi isolate Ree9_LE_QAC_2019_Ree436 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453671.1
Rhipicephalus evertsi evertsi isolate Ree12_LE_LER_2019_Ree702 cytochrome oxidase subunit I (CO1) gene…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453669.1
Rhipicephalus evertsi evertsi isolate Ree1_LE_MAS_2017_Ree228 cytochrome c oxidase subunit I (CO1) gen…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	544	MK551201.1
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	707	MZ351136.1

Goat_gmf3_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus glabroscutatus isolate Rg1_LE_QAC_2016_Rg208 cytochrome oxidase subunit I (CO1) gene, pa…	Rhipicephalus gl…	998	998	100%	0.0	99.82%	548	MN453672.1
Rhipicephalus evertsi evertsi isolate Ree9_LE_QAC_2019_Ree436 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus ev…	998	998	100%	0.0	99.82%	548	MN453671.1
Rhipicephalus evertsi evertsi isolate Ree12_LE_LER_2019_Ree702 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus ev…	998	998	100%	0.0	99.82%	548	MN453669.1
Rhipicephalus evertsi evertsi isolate Ree1_LE_MAS_2017_Ree228 cytochrome c oxidase subunit I (CO1) gene…	Rhipicephalus ev…	998	998	100%	0.0	99.82%	544	MK551201.1
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus ev…	998	998	100%	0.0	99.82%	14743	NC_067927.1
Rhipicephalus evertsi cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus ev…	998	998	100%	0.0	99.82%	707	MZ351136.1

Goat_gmm3_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus glabroscutatus isolate Rg1_LE_QAC_2016_Rg208 cytochrome oxidase subunit I (CO1) gene, p…	Rhipicephalus gl…	1002	1002	100%	0.0	100.00%	548	MN453672.1
Rhipicephalus evertsi evertsi isolate Ree9_LE_QAC_2019_Ree436 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453671.1
Rhipicephalus evertsi evertsi isolate Ree12_LE_LER_2019_Ree702 cytochrome oxidase subunit I (CO1) gene…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453669.1
Rhipicephalus evertsi evertsi isolate Ree1_LE_MAS_2017_Ree228 cytochrome c oxidase subunit I (CO1) gen…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	544	MK551201.1
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	707	MZ351136.1

Dog_SAN2_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus leporis isolate Bas-Sassandra-C cytochrome oxidase subunit I (COI) gene, partial cds; mitochond…	Rhipicephalus le…	996	996	100%	0.0	99.82%	630	KX757917.1
Rhipicephalus leporis isolate Bas-Sassandra-B cytochrome oxidase subunit I (COI) gene, partial cds; mitochond…	Rhipicephalus le…	990	990	100%	0.0	99.63%	630	KX757913.1
Rhipicephalus leporis isolate Bas-Sassandra-A cytochrome oxidase subunit I (COI) gene, partial cds; mitochond…	Rhipicephalus le…	979	979	100%	0.0	99.26%	630	KX757912.1
Rhipicephalus leporis voucher INHM:TC1338 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus le…	979	979	100%	0.0	99.26%	632	KM235720.1
Rhipicephalus leporis isolate Turkana-A cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus le…	968	968	100%	0.0	98.89%	633	KX757911.1
Rhipicephalus linnaei isolate P1/22:20-1 mitochondrion, complete genome	Rhipicephalus lin…	968	968	100%	0.0	98.89%	14716	OM994390.1
Rhipicephalus sanguineus sensu lato 'tropical lineage' isolate A3021 cytochrome c oxidase subunit I (COI) gene…	Rhipicephalus lin…	957	957	100%	0.0	98.52%	688	MF425995.1
Rhipicephalus sanguineus sensu lato 'tropical lineage' isolate A3015 cytochrome c oxidase subunit I (COI) gene…	Rhipicephalus lin…	957	957	100%	0.0	98.52%	703	MF425994.1
Rhipicephalus sanguineus sensu lato 'tropical lineage' isolate A3014 cytochrome c oxidase subunit I (COI) gene…	Rhipicephalus lin…	957	957	100%	0.0	98.52%	702	MF425993.1
Rhipicephalus sanguineus sensu lato 'tropical lineage' isolate A3013 cytochrome c oxidase subunit I (COI) gene…	Rhipicephalus lin…	957	957	100%	0.0	98.52%	693	MF425992.1
Rhipicephalus sanguineus voucher CM3 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus sa…	957	957	100%	0.0	98.52%	682	OQ704678.1
Rhipicephalus sanguineus voucher CM2 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus sa…	957	957	100%	0.0	98.52%	682	OQ704677.1
Rhipicephalus sanguineus voucher CM1 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus sa…	957	957	100%	0.0	98.52%	682	OQ704676.1

Dog_SAN3_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus leporis isolate Bas-Sassandra-C cytochrome oxidase subunit I (COI) gene, partial cds; mitochond…	Rhipicephalus le…	996	996	100%	0.0	99.82%	630	KX757917.1
Rhipicephalus leporis isolate Bas-Sassandra-B cytochrome oxidase subunit I (COI) gene, partial cds; mitochond…	Rhipicephalus le…	990	990	100%	0.0	99.63%	630	KX757913.1
Rhipicephalus leporis isolate Bas-Sassandra-A cytochrome oxidase subunit I (COI) gene, partial cds; mitochond…	Rhipicephalus le…	979	979	100%	0.0	99.26%	630	KX757912.1
Rhipicephalus leporis voucher INHM:TC1338 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus le…	979	979	100%	0.0	99.26%	632	KM235720.1
Rhipicephalus leporis isolate Turkana-A cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus le…	968	968	100%	0.0	98.89%	633	KX757911.1
Rhipicephalus linnaei isolate P1/22:20-1 mitochondrion, complete genome	Rhipicephalus lin…	968	968	100%	0.0	98.89%	14716	OM994390.1
Rhipicephalus sanguineus isolate WZ001 cytochrome oxidase subunit I gene, partial cds; mitochondrial	Rhipicephalus sa…	957	957	100%	0.0	98.52%	664	MG969504.1
Rhipicephalus sanguineus sensu lato 'tropical lineage' isolate A3021 cytochrome c oxidase subunit I (COI) gene…	Rhipicephalus lin…	957	957	100%	0.0	98.52%	688	MF425995.1
Rhipicephalus sanguineus sensu lato 'tropical lineage' isolate A3015 cytochrome c oxidase subunit I (COI) gene…	Rhipicephalus lin…	957	957	100%	0.0	98.52%	703	MF425994.1
Rhipicephalus sanguineus sensu lato 'tropical lineage' isolate A3014 cytochrome c oxidase subunit I (COI) gene…	Rhipicephalus lin…	957	957	100%	0.0	98.52%	702	MF425993.1
Rhipicephalus sanguineus sensu lato 'tropical lineage' isolate A3013 cytochrome c oxidase subunit I (COI) gene…	Rhipicephalus lin…	957	957	100%	0.0	98.52%	693	MF425992.1
Rhipicephalus sanguineus voucher CM3 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus sa…	957	957	100%	0.0	98.52%	682	OQ704678.1
Rhipicephalus sanguineus voucher CM2 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus sa…	957	957	100%	0.0	98.52%	682	OQ704677.1
Rhipicephalus sanguineus voucher CM1 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus sa…	957	957	100%	0.0	98.52%	682	OQ704676.1
Rhipicephalus sanguineus voucher QZ16 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus sa…	957	957	100%	0.0	98.52%	682	OQ704675.1

RE7_Sheep_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus glabroscutatus isolate Rg1_LE_QAC_2016_Rg208 cytochrome oxidase subunit I (CO1) gene, pa…	Rhipicephalus gl…	998	998	100%	0.0	99.82%	548	MN453672.1
Rhipicephalus evertsi evertsi isolate Ree9_LE_QAC_2019_Ree436 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus ev…	998	998	100%	0.0	99.82%	548	MN453671.1
Rhipicephalus evertsi evertsi isolate Ree12_LE_LER_2019_Ree702 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus ev…	998	998	100%	0.0	99.82%	548	MN453669.1
Rhipicephalus evertsi evertsi isolate Ree1_LE_MAS_2017_Ree228 cytochrome c oxidase subunit I (CO1) gene…	Rhipicephalus ev…	998	998	100%	0.0	99.82%	544	MK551201.1
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus ev…	998	998	100%	0.0	99.82%	14743	NC_067927.1
Rhipicephalus evertsi cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus ev…	998	998	100%	0.0	99.82%	707	MZ351136.1

RE11_Sheep_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus glabroscutatus isolate Rg1_LE_QAC_2016_Rg208 cytochrome oxidase subunit I (CO1) gene, p…	Rhipicephalus gl…	1002	1002	100%	0.0	100.00%	548	MN453672.1
Rhipicephalus evertsi evertsi isolate Ree9_LE_QAC_2019_Ree436 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453671.1
Rhipicephalus evertsi evertsi isolate Ree12_LE_LER_2019_Ree702 cytochrome oxidase subunit I (CO1) gene…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453669.1
Rhipicephalus evertsi evertsi isolate Ree1_LE_MAS_2017_Ree228 cytochrome c oxidase subunit I (CO1) gen…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	544	MK551201.1
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	707	MZ351136.1

RE15_Sheep_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus glabroscutatus isolate Rg1_LE_QAC_2016_Rg208 cytochrome oxidase subunit I (CO1) gene, p…	Rhipicephalus gl…	1002	1002	100%	0.0	100.00%	548	MN453672.1
Rhipicephalus evertsi evertsi isolate Ree9_LE_QAC_2019_Ree436 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453671.1
Rhipicephalus evertsi evertsi isolate Ree12_LE_LER_2019_Ree702 cytochrome oxidase subunit I (CO1) gene…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453669.1
Rhipicephalus evertsi evertsi isolate Ree1_LE_MAS_2017_Ree228 cytochrome c oxidase subunit I (CO1) gen…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	544	MK551201.1
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	707	MZ351136.1

R1_Cattle_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus glabroscutatus isolate Rg1_LE_QAC_2016_Rg208 cytochrome oxidase subunit I (CO1) gene, p…	Rhipicephalus gl…	1002	1002	100%	0.0	100.00%	548	MN453672.1
Rhipicephalus evertsi evertsi isolate Ree9_LE_QAC_2019_Ree436 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453671.1
Rhipicephalus evertsi evertsi isolate Ree12_LE_LER_2019_Ree702 cytochrome oxidase subunit I (CO1) gene…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453669.1
Rhipicephalus evertsi evertsi isolate Ree1_LE_MAS_2017_Ree228 cytochrome c oxidase subunit I (CO1) gen…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	544	MK551201.1
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	707	MZ351136.1

R2_Cattle_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus glabroscutatus isolate Rg1_LE_QAC_2016_Rg208 cytochrome oxidase subunit I (CO1) gene, p…	Rhipicephalus gl…	1002	1002	100%	0.0	100.00%	548	MN453672.1
Rhipicephalus evertsi evertsi isolate Ree9_LE_QAC_2019_Ree436 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453671.1
Rhipicephalus evertsi evertsi isolate Ree12_LE_LER_2019_Ree702 cytochrome oxidase subunit I (CO1) gene…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453669.1
Rhipicephalus evertsi evertsi isolate Ree1_LE_MAS_2017_Ree228 cytochrome c oxidase subunit I (CO1) gen…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	544	MK551201.1
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	707	MZ351136.1

R3_Cattle_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus glabroscutatus isolate Rg1_LE_QAC_2016_Rg208 cytochrome oxidase subunit I (CO1) gene, p…	Rhipicephalus gl…	1002	1002	100%	0.0	100.00%	548	MN453672.1
Rhipicephalus evertsi evertsi isolate Ree9_LE_QAC_2019_Ree436 cytochrome oxidase subunit I (CO1) gene,…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453671.1
Rhipicephalus evertsi evertsi isolate Ree12_LE_LER_2019_Ree702 cytochrome oxidase subunit I (CO1) gene…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	548	MN453669.1
Rhipicephalus evertsi evertsi isolate Ree1_LE_MAS_2017_Ree228 cytochrome c oxidase subunit I (CO1) gen…	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	544	MK551201.1
Rhipicephalus evertsi isolate Kwanare_125 mitochondrion, complete genome	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	14743	NC_067927.1
Rhipicephalus evertsi cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	Rhipicephalus e…	1002	1002	100%	0.0	100.00%	707	MZ351136.1

Clinvet_Rm1_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus microplus voucher TK-17 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus mi…	1002	1002	100%	0.0	100.00%	631	OQ750591.1
Rhipicephalus microplus voucher TK-41 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus mi…	1002	1002	100%	0.0	100.00%	628	OQ750590.1
Rhipicephalus microplus voucher TK-50 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus mi…	1002	1002	100%	0.0	100.00%	649	OQ750588.1
Rhipicephalus microplus voucher TK-19 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus mi…	1002	1002	100%	0.0	100.00%	626	OQ750587.1
Rhipicephalus microplus voucher TK-27 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus mi…	1002	1002	100%	0.0	100.00%	637	OQ750586.1
Rhipicephalus microplus voucher TK-60 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus mi…	1002	1002	100%	0.0	100.00%	628	OQ750585.1

Clinvet_Rd1_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus microplus voucher TK-17 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus mi…	1002	1002	100%	0.0	100.00%	631	OQ750591.1
Rhipicephalus microplus voucher TK-41 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus mi…	1002	1002	100%	0.0	100.00%	628	OQ750590.1
Rhipicephalus microplus voucher TK-50 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus mi…	1002	1002	100%	0.0	100.00%	649	OQ750588.1
Rhipicephalus microplus voucher TK-19 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus mi…	1002	1002	100%	0.0	100.00%	626	OQ750587.1
Rhipicephalus microplus voucher TK-27 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus mi…	1002	1002	100%	0.0	100.00%	637	OQ750586.1
Rhipicephalus microplus voucher TK-60 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Rhipicephalus mi…	1002	1002	100%	0.0	100.00%	628	OQ750585.1

Clinvet_Rp1_CO1

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus appendiculatus isolate Lake_Mburo_UG6 cytochrome c oxidase subunit I (COI) gene, partial cd…	Rhipicephalus a…	1002	1002	100%	0.0	100.00%	586	MN756038.1
Rhipicephalus appendiculatus isolate Lake_Mburo_UG2 cytochrome c oxidase subunit I (COI) gene, partial cd…	Rhipicephalus a…	1002	1002	100%	0.0	100.00%	586	MN756037.1
Rhipicephalus appendiculatus voucher KM3 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitocho…	Rhipicephalus a…	1002	1002	100%	0.0	100.00%	582	MT181227.1
Rhipicephalus appendiculatus isolate Ra1_SA_EC_2018_Ra365 cytochrome c oxidase subunit I (CO1) gene,…	Rhipicephalus a…	1002	1002	100%	0.0	100.00%	544	MK551199.1
Rhipicephalus appendiculatus isolate RNK03 haplotype CH7 cytochrome c oxidase subunit I (COI) gene, parti…	Rhipicephalus a…	1002	1002	100%	0.0	100.00%	586	MF458938.1
Rhipicephalus appendiculatus isolate W036B haplotype CH7 cytochrome c oxidase subunit I (COI) gene, parti…	Rhipicephalus a…	1002	1002	100%	0.0	100.00%	586	MF458925.1

8.3 BLASTn search results for tick identification samples targeting the *ITS2* gene

Horse_h6_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal …	Rhipicephalus ev…	2002	2239	100%	0.0	99.91%	9216	KY457503.1
Rhipicephalus evertsi clone 2_8998_58 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal R…	Rhipicephalus ev…	2002	2239	100%	0.0	99.91%	8998	KY457502.1
Rhipicephalus evertsi evertsi isolate 10T_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed …	Rhipicephalus ev…	1991	2234	100%	0.0	99.72%	1142	DQ849266.1
Rhipicephalus evertsi evertsi isolate 11K_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed s…	Rhipicephalus ev…	1991	2234	100%	0.0	99.72%	1142	DQ849265.1
Rhipicephalus evertsi internal transcribed spacer 2, partial sequence	Rhipicephalus ev…	1971	2206	100%	0.0	99.17%	1212	MZ351146.1
Rhipicephalus evertsi mimeticus internal transcribed spacer 2, complete sequence	Rhipicephalus ev…	1941	2184	100%	0.0	98.90%	1135	AF271279.1

Horse_h7_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal …	Rhipicephalus ev…	1997	2235	100%	0.0	99.82%	9216	KY457503.1
Rhipicephalus evertsi clone 2_8998_58 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal R…	Rhipicephalus ev…	1997	2235	100%	0.0	99.82%	8998	KY457502.1
Rhipicephalus evertsi evertsi isolate 10T_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed …	Rhipicephalus ev…	1986	2230	100%	0.0	99.63%	1142	DQ849266.1
Rhipicephalus evertsi evertsi isolate 11K_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed s…	Rhipicephalus ev…	1986	2230	100%	0.0	99.63%	1142	DQ849265.1
Rhipicephalus evertsi internal transcribed spacer 2, partial sequence	Rhipicephalus ev…	1965	2202	100%	0.0	99.08%	1212	MZ351146.1
Rhipicephalus evertsi mimeticus internal transcribed spacer 2, complete sequence	Rhipicephalus ev…	1936	2180	100%	0.0	98.81%	1135	AF271279.1

Horse_h9_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal …	Rhipicephalus ev…	1991	2226	100%	0.0	99.72%	9216	KY457503.1
Rhipicephalus evertsi clone 2_8998_58 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal R…	Rhipicephalus ev…	1991	2226	100%	0.0	99.72%	8998	KY457502.1
Rhipicephalus evertsi evertsi isolate 10T_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed …	Rhipicephalus ev…	1980	2221	100%	0.0	99.54%	1142	DQ849266.1
Rhipicephalus evertsi evertsi isolate 11K_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed s…	Rhipicephalus ev…	1980	2221	100%	0.0	99.54%	1142	DQ849265.1
Rhipicephalus evertsi internal transcribed spacer 2, partial sequence	Rhipicephalus ev…	1960	2079	100%	0.0	98.99%	1212	MZ351146.1
Rhipicephalus evertsi mimeticus internal transcribed spacer 2, complete sequence	Rhipicephalus ev…	1930	2171	100%	0.0	98.71%	1135	AF271279.1

Goat_gmf3_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal …	Rhipicephalus ev…	1949	2158	100%	0.0	99.53%	9216	KY457503.1
Rhipicephalus evertsi clone 2_8998_58 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal R…	Rhipicephalus ev…	1949	2158	100%	0.0	99.53%	8998	KY457502.1
Rhipicephalus evertsi evertsi isolate 10T_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed …	Rhipicephalus ev…	1949	2163	100%	0.0	99.53%	1142	DQ849266.1
Rhipicephalus evertsi evertsi isolate 11K_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed s…	Rhipicephalus ev…	1949	2163	100%	0.0	99.53%	1142	DQ849265.1
Rhipicephalus evertsi internal transcribed spacer 2, partial sequence	Rhipicephalus ev…	1938	2063	100%	0.0	99.25%	1212	MZ351146.1
Rhipicephalus evertsi mimeticus internal transcribed spacer 2, complete sequence	Rhipicephalus ev…	1905	2119	100%	0.0	98.78%	1135	AF271279.1

Dog_S3_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus turanicus internal transcribed spacer 2, complete sequence	Rhipicephalus tu…	1956	2191	100%	0.0	99.72%	1119	KM272204.1
Rhipicephalus sanguineus strain Egypt 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2…	Rhipicephalus sa…	1956	2191	100%	0.0	99.72%	1165	JQ412127.1
Rhipicephalus pumilio isolate dxstp-its2 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer …	Rhipicephalus pu…	1951	2186	100%	0.0	99.63%	1316	MZ536629.1
Rhipicephalus sanguineus isolate xjswp-1 5.8S ribosomal RNA gene, partial sequence; internal transcribed space…	Rhipicephalus sa…	1951	2186	100%	0.0	99.63%	1318	MZ408104.1
Rhipicephalus turanicus isolate 6D_Turan_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed sp…	Rhipicephalus tu…	1951	2186	100%	0.0	99.63%	1124	DQ849267.1
Rhipicephalus sanguineus strain Porto Alegre internal transcribed spacer 2, partial sequence	Rhipicephalus sa…	1945	2180	100%	0.0	99.53%	1135	MH616088.1

Dog_SAN2_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus turanicus internal transcribed spacer 2, complete sequence	Rhipicephalus tu…	1951	2182	100%	0.0	99.53%	1119	KM272204.1
Rhipicephalus sanguineus strain Egypt 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2…	Rhipicephalus sa…	1951	2182	100%	0.0	99.53%	1165	JQ412127.1
Rhipicephalus pumilio isolate dxstp-its2 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer …	Rhipicephalus pu…	1945	2176	100%	0.0	99.44%	1316	MZ536629.1
Rhipicephalus sanguineus isolate xjswp-1 5.8S ribosomal RNA gene, partial sequence; internal transcribed space…	Rhipicephalus sa…	1945	2176	100%	0.0	99.44%	1318	MZ408104.1
Rhipicephalus turanicus isolate 6D_Turan_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed sp…	Rhipicephalus tu…	1945	2176	100%	0.0	99.44%	1124	DQ849267.1
Rhipicephalus sanguineus strain Porto Alegre internal transcribed spacer 2, partial sequence	Rhipicephalus sa…	1940	2171	100%	0.0	99.34%	1135	MH616088.1

Sheep_RE7_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal …	Rhipicephalus ev…	1997	2235	100%	0.0	99.82%	9216	KY457503.1
Rhipicephalus evertsi clone 2_8998_58 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal R…	Rhipicephalus ev…	1997	2235	100%	0.0	99.82%	8998	KY457502.1
Rhipicephalus evertsi evertsi isolate 10T_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed …	Rhipicephalus ev…	1986	2230	100%	0.0	99.63%	1142	DQ849266.1
Rhipicephalus evertsi evertsi isolate 11K_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed s…	Rhipicephalus ev…	1986	2230	100%	0.0	99.63%	1142	DQ849265.1
Rhipicephalus evertsi internal transcribed spacer 2, partial sequence	Rhipicephalus ev…	1965	2202	100%	0.0	99.08%	1212	MZ351146.1
Rhipicephalus evertsi mimeticus internal transcribed spacer 2, complete sequence	Rhipicephalus ev…	1936	2180	100%	0.0	98.81%	1135	AF271279.1

Sheep_RE11_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal …	Rhipicephalus ev…	1991	2226	100%	0.0	99.72%	9216	KY457503.1
Rhipicephalus evertsi clone 2_8998_58 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal R…	Rhipicephalus ev…	1991	2226	100%	0.0	99.72%	8998	KY457502.1
Rhipicephalus evertsi evertsi isolate 10T_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed …	Rhipicephalus ev…	1980	2221	100%	0.0	99.54%	1142	DQ849266.1
Rhipicephalus evertsi evertsi isolate 11K_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed s…	Rhipicephalus ev…	1980	2221	100%	0.0	99.54%	1142	DQ849265.1
Rhipicephalus evertsi internal transcribed spacer 2, partial sequence	Rhipicephalus ev…	1960	2193	100%	0.0	98.99%	1212	MZ351146.1
Rhipicephalus evertsi mimeticus internal transcribed spacer 2, complete sequence	Rhipicephalus ev…	1930	2171	100%	0.0	98.71%	1135	AF271279.1

Sheep_RE15_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal …	Rhipicephalus ev…	1995	2230	100%	0.0	99.72%	9216	KY457503.1
Rhipicephalus evertsi clone 2_8998_58 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal R…	Rhipicephalus ev…	1995	2230	100%	0.0	99.72%	8998	KY457502.1
Rhipicephalus evertsi evertsi isolate 10T_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed …	Rhipicephalus ev…	1984	2224	100%	0.0	99.54%	1142	DQ849266.1
Rhipicephalus evertsi evertsi isolate 11K_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed s…	Rhipicephalus ev…	1984	2224	100%	0.0	99.54%	1142	DQ849265.1
Rhipicephalus evertsi internal transcribed spacer 2, partial sequence	Rhipicephalus ev…	1967	2200	100%	0.0	99.17%	1212	MZ351146.1
Rhipicephalus evertsi mimeticus internal transcribed spacer 2, complete sequence	Rhipicephalus ev…	1940	2180	100%	0.0	98.81%	1135	AF271279.1

R1_Cattle_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal …	Rhipicephalus ev…	1993	2224	100%	0.0	99.72%	9216	KY457503.1
Rhipicephalus evertsi clone 2_8998_58 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal R…	Rhipicephalus ev…	1993	2224	100%	0.0	99.72%	8998	KY457502.1
Rhipicephalus evertsi evertsi isolate 10T_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed …	Rhipicephalus ev…	1982	2219	100%	0.0	99.54%	1142	DQ849266.1
Rhipicephalus evertsi evertsi isolate 11K_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed s…	Rhipicephalus ev…	1982	2219	100%	0.0	99.54%	1142	DQ849265.1
Rhipicephalus evertsi internal transcribed spacer 2, partial sequence	Rhipicephalus ev…	1962	2081	100%	0.0	98.99%	1212	MZ351146.1
Rhipicephalus evertsi mimeticus internal transcribed spacer 2, complete sequence	Rhipicephalus ev…	1932	2169	100%	0.0	98.71%	1135	AF271279.1

R2_Cattle_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal …	Rhipicephalus ev…	1982	2217	100%	0.0	99.54%	9216	KY457503.1
Rhipicephalus evertsi clone 2_8998_58 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal R…	Rhipicephalus ev…	1982	2217	100%	0.0	99.54%	8998	KY457502.1
Rhipicephalus evertsi evertsi isolate 10T_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed …	Rhipicephalus ev…	1971	2211	100%	0.0	99.36%	1142	DQ849266.1
Rhipicephalus evertsi evertsi isolate 11K_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed s…	Rhipicephalus ev…	1971	2211	100%	0.0	99.36%	1142	DQ849265.1
Rhipicephalus evertsi internal transcribed spacer 2, partial sequence	Rhipicephalus ev…	1951	2184	100%	0.0	98.81%	1212	MZ351146.1
Rhipicephalus evertsi mimeticus internal transcribed spacer 2, complete sequence	Rhipicephalus ev…	1921	2162	100%	0.0	98.53%	1135	AF271279.1

R3_Cattle_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal …	Rhipicephalus ev…	1997	2235	100%	0.0	99.82%	9216	KY457503.1
Rhipicephalus evertsi clone 2_8998_58 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal R…	Rhipicephalus ev…	1997	2235	100%	0.0	99.82%	8998	KY457502.1
Rhipicephalus evertsi evertsi isolate 10T_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed …	Rhipicephalus ev…	1986	2230	100%	0.0	99.63%	1142	DQ849266.1
Rhipicephalus evertsi evertsi isolate 11K_Eve_S 5.8S ribosomal RNA gene, partial sequence; internal transcribed s…	Rhipicephalus ev…	1986	2230	100%	0.0	99.63%	1142	DQ849265.1
Rhipicephalus evertsi internal transcribed spacer 2, partial sequence	Rhipicephalus ev…	1965	2202	100%	0.0	99.08%	1212	MZ351146.1
Rhipicephalus evertsi mimeticus internal transcribed spacer 2, complete sequence	Rhipicephalus ev…	1936	2180	100%	0.0	98.81%	1135	AF271279.1

Clinvet_Rm1_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus microplus isolate JX-2 voucher NCCDCITS171 5.8S ribosomal RNA gene, partial sequence; intern…	Rhipicephalus mi…	2026	2136	100%	0.0	99.73%	1342	MG721035.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S riboso…	Rhipicephalus mi…	2026	2136	100%	0.0	99.73%	8908	KY457506.1
Rhipicephalus microplus isolate 11-2 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, c…	Rhipicephalus mi…	2026	2136	100%	0.0	99.73%	1521	KX450287.1
Rhipicephalus microplus internal transcribed spacer 2, partial sequence	Rhipicephalus mi…	2026	2136	100%	0.0	99.73%	1247	MZ351149.1
Rhipicephalus microplus isolate BomiB 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2…	Rhipicephalus mi…	2026	2136	100%	0.0	99.73%	1287	KC503273.1
Rhipicephalus microplus isolate 4Palu 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2,…	Rhipicephalus mi…	2026	2136	100%	0.0	99.73%	1287	KC503265.1

Clinvet_Rd1_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus microplus isolate JX-2 voucher NCCDCITS171 5.8S ribosomal RNA gene, partial sequence; intern…	Rhipicephalus mi…	2026	2254	100%	0.0	99.73%	1342	MG721035.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S riboso…	Rhipicephalus mi…	2026	2254	100%	0.0	99.73%	8908	KY457506.1
Rhipicephalus microplus isolate 11-2 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, c…	Rhipicephalus mi…	2026	2254	100%	0.0	99.73%	1521	KX450287.1
Rhipicephalus microplus internal transcribed spacer 2, partial sequence	Rhipicephalus mi…	2026	2254	100%	0.0	99.73%	1247	MZ351149.1
Rhipicephalus microplus isolate BomiB 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2…	Rhipicephalus mi…	2026	2254	100%	0.0	99.73%	1287	KC503273.1
Rhipicephalus microplus isolate 2Ken 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2,…	Rhipicephalus mi…	2026	2254	100%	0.0	99.73%	1285	KC503264.1

Clinvet_Rp1_ITS2

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus appendiculatus clone 14_8656_820 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8…	Rhipicephalus a…	1925	2143	100%	0.0	100.00%	8656	KY457500.1
Rhipicephalus appendiculatus haplotype 3 internal transcribed spacer 2, partial sequence	Rhipicephalus a…	1925	2143	100%	0.0	100.00%	1149	KX276952.1
Rhipicephalus appendiculatus isolate S23M 5.8S ribosomal RNA gene, partial sequence; internal transcribed s…	Rhipicephalus a…	1925	2143	100%	0.0	100.00%	1273	OQ557788.1
Rhipicephalus appendiculatus internal transcribed spacer 2, partial sequence	Rhipicephalus a…	1925	2143	100%	0.0	100.00%	1199	MZ351144.1
Rhipicephalus appendiculatus internal transcribed spacer 2, partial sequence	Rhipicephalus a…	1925	2143	100%	0.0	100.00%	1160	MZ351143.1
Rhipicephalus appendiculatus isolate A_SimoB_SP 5.8S ribosomal RNA gene, partial sequence; internal transc…	Rhipicephalus a…	1925	2143	100%	0.0	100.00%	1116	DQ849239.1

8.4 BLASTn search results for tick identification samples targeting the*18S rRNA* gene

Horse_h6_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus sanguineus isolate R2.9 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1448	1448	100%	0.0	99.62%	813	OP962707.1
Rhipicephalus sanguineus isolate R2.8 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1448	1448	100%	0.0	99.62%	815	OP962706.1
Rhipicephalus sanguineus isolate R2.4 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1448	1448	100%	0.0	99.62%	819	OP962703.1
Rhipicephalus sanguineus isolate R2.3 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1448	1448	100%	0.0	99.62%	816	OP962702.1
Rhipicephalus sanguineus isolate R2.2 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1448	1448	100%	0.0	99.62%	816	OP962701.1
Rhipicephalus sanguineus isolate R2.1 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1448	1448	100%	0.0	99.62%	815	OP962700.1

Horse_h7_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus sanguineus isolate R2.9 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1447	1447	99%	0.0	99.75%	813	OP962707.1
Rhipicephalus sanguineus isolate R2.8 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1447	1447	99%	0.0	99.75%	815	OP962706.1
Rhipicephalus sanguineus isolate R2.4 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1447	1447	99%	0.0	99.75%	819	OP962703.1
Rhipicephalus sanguineus isolate R2.3 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1447	1447	99%	0.0	99.75%	816	OP962702.1
Rhipicephalus sanguineus isolate R2.2 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1447	1447	99%	0.0	99.75%	816	OP962701.1
Rhipicephalus sanguineus isolate R2.1 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1447	1447	99%	0.0	99.75%	815	OP962700.1

Horse_h9_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus sanguineus isolate R2.8 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1454	1454	100%	0.0	99.75%	815	OP962706.1
Rhipicephalus sanguineus isolate R2.4 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1454	1454	100%	0.0	99.75%	819	OP962703.1
Rhipicephalus sanguineus isolate R2.9 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1452	1452	99%	0.0	99.87%	813	OP962707.1
Rhipicephalus sanguineus isolate R2.3 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1452	1452	99%	0.0	99.87%	816	OP962702.1
Rhipicephalus sanguineus isolate R2.2 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1452	1452	99%	0.0	99.87%	816	OP962701.1
Rhipicephalus sanguineus isolate R2.1 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1452	1452	99%	0.0	99.87%	815	OP962700.1

Goat_gmf1_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus zambeziensis clone 221_9750_843 18S ribosomal RNA gene, internal transcribed spacer 1, 5...	Rhipicephalus z...	1439	1439	100%	0.0	99.37%	9750	KY457509.1
Rhipicephalus simus clone 7_9296_958 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S riboso...	Rhipicephalus si...	1439	1439	100%	0.0	99.37%	9296	KY457508.1
Rhipicephalus zambeziensis clone 307_9750_71 18S ribosomal RNA gene, internal transcribed spacer 1, 5....	Rhipicephalus z...	1439	1439	100%	0.0	99.37%	9750	KY457507.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ri...	Rhipicephalus m...	1439	1439	100%	0.0	99.37%	8908	KY457506.1
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribos...	Rhipicephalus e...	1439	1439	100%	0.0	99.37%	9216	KY457503.1
Rhipicephalus appendiculatus clone 6_8584_532 18S ribosomal RNA gene, internal transcribed spacer 1, 5....	Rhipicephalus a...	1439	1439	100%	0.0	99.37%	8584	KY457501.1

Goat_gmf3_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus zambeziensis clone 221_9750_843 18S ribosomal RNA gene, internal transcribed spacer 1, 5...	Rhipicephalus z...	1454	1454	100%	0.0	99.75%	9750	KY457509.1
Rhipicephalus simus clone 7_9296_958 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S riboso...	Rhipicephalus si...	1454	1454	100%	0.0	99.75%	9296	KY457508.1
Rhipicephalus zambeziensis clone 307_9750_71 18S ribosomal RNA gene, internal transcribed spacer 1, 5....	Rhipicephalus z...	1454	1454	100%	0.0	99.75%	9750	KY457507.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ri...	Rhipicephalus m...	1454	1454	100%	0.0	99.75%	8908	KY457506.1
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribos...	Rhipicephalus e...	1454	1454	100%	0.0	99.75%	9216	KY457503.1
Rhipicephalus appendiculatus clone 6_8584_532 18S ribosomal RNA gene, internal transcribed spacer 1, 5....	Rhipicephalus a...	1454	1454	100%	0.0	99.75%	8584	KY457501.1

Goat_gmm3_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus zambeziensis clone 221_9750_843 18S ribosomal RNA gene, internal transcribed spacer 1, 5...	Rhipicephalus z...	1454	1454	100%	0.0	99.75%	9750	KY457509.1
Rhipicephalus simus clone 7_9296_958 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S riboso...	Rhipicephalus si...	1454	1454	100%	0.0	99.75%	9296	KY457508.1
Rhipicephalus zambeziensis clone 307_9750_71 18S ribosomal RNA gene, internal transcribed spacer 1, 5....	Rhipicephalus z...	1454	1454	100%	0.0	99.75%	9750	KY457507.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ri...	Rhipicephalus m...	1454	1454	100%	0.0	99.75%	8908	KY457506.1
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribos...	Rhipicephalus e...	1454	1454	100%	0.0	99.75%	9216	KY457503.1
Rhipicephalus appendiculatus clone 6_8584_532 18S ribosomal RNA gene, internal transcribed spacer 1, 5....	Rhipicephalus a...	1454	1454	100%	0.0	99.75%	8584	KY457501.1

Dog_SAN2_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus sanguineus isolate R2.5 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1463	1463	100%	0.0	100.00%	814	OP962704.1
Rhipicephalus zambeziensis clone 221_9750_843 18S ribosomal RNA gene, internal transcribed spacer 1,…	Rhipicephalus z…	1459	1459	99%	0.0	100.00%	9750	KY457509.1
Rhipicephalus simus clone 7_9296_958 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribos…	Rhipicephalus si…	1459	1459	99%	0.0	100.00%	9296	KY457508.1
Rhipicephalus zambeziensis clone 307_9750_71 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus z…	1459	1459	99%	0.0	100.00%	9750	KY457507.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S …	Rhipicephalus …	1459	1459	99%	0.0	100.00%	8908	KY457506.1
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribo…	Rhipicephalus e…	1459	1459	99%	0.0	100.00%	9216	KY457503.1

Dog_SAN3_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus sanguineus isolate R2.5 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s…	1463	1463	100%	0.0	100.00%	814	OP962704.1
Rhipicephalus zambeziensis clone 221_9750_843 18S ribosomal RNA gene, internal transcribed spacer 1,…	Rhipicephalus z…	1459	1459	99%	0.0	100.00%	9750	KY457509.1
Rhipicephalus simus clone 7_9296_958 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribos…	Rhipicephalus si…	1459	1459	99%	0.0	100.00%	9296	KY457508.1
Rhipicephalus zambeziensis clone 307_9750_71 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus z…	1459	1459	99%	0.0	100.00%	9750	KY457507.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S …	Rhipicephalus …	1459	1459	99%	0.0	100.00%	8908	KY457506.1
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribo…	Rhipicephalus e…	1459	1459	99%	0.0	100.00%	9216	KY457503.1

Dog_S3_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus zambeziensis clone 221_9750_843 18S ribosomal RNA gene, internal transcribed spacer 1,…	Rhipicephalus z…	1461	1461	99%	0.0	100.00%	9750	KY457509.1
Rhipicephalus simus clone 7_9296_958 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribos…	Rhipicephalus si…	1461	1461	99%	0.0	100.00%	9296	KY457508.1
Rhipicephalus zambeziensis clone 307_9750_71 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus z…	1461	1461	99%	0.0	100.00%	9750	KY457507.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S …	Rhipicephalus …	1461	1461	99%	0.0	100.00%	8908	KY457506.1
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribo…	Rhipicephalus e…	1461	1461	99%	0.0	100.00%	9216	KY457503.1
Rhipicephalus appendiculatus clone 6_8584_532 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus a…	1461	1461	99%	0.0	100.00%	8584	KY457501.1

RE7_Sheep_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus sanguineus isolate R2.9 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1454	1454	100%	0.0	99.75%	813	OP962707.1
Rhipicephalus sanguineus isolate R2.8 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1454	1454	100%	0.0	99.75%	815	OP962706.1
Rhipicephalus sanguineus isolate R2.4 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1454	1454	100%	0.0	99.75%	819	OP962703.1
Rhipicephalus sanguineus isolate R2.3 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1454	1454	100%	0.0	99.75%	816	OP962702.1
Rhipicephalus sanguineus isolate R2.2 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1454	1454	100%	0.0	99.75%	816	OP962701.1
Rhipicephalus sanguineus isolate R2.1 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1454	1454	100%	0.0	99.75%	815	OP962700.1

RE11_Sheep_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus sanguineus isolate R2.9 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1458	1458	100%	0.0	99.87%	813	OP962707.1
Rhipicephalus sanguineus isolate R2.8 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1458	1458	100%	0.0	99.87%	815	OP962706.1
Rhipicephalus sanguineus isolate R2.4 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1458	1458	100%	0.0	99.87%	819	OP962703.1
Rhipicephalus sanguineus isolate R2.3 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1458	1458	100%	0.0	99.87%	816	OP962702.1
Rhipicephalus sanguineus isolate R2.2 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1458	1458	100%	0.0	99.87%	816	OP962701.1
Rhipicephalus sanguineus isolate R2.1 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1458	1458	100%	0.0	99.87%	815	OP962700.1

RE15_Sheep_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus sanguineus isolate R2.9 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1454	1454	100%	0.0	99.75%	813	OP962707.1
Rhipicephalus sanguineus isolate R2.8 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1454	1454	100%	0.0	99.75%	815	OP962706.1
Rhipicephalus sanguineus isolate R2.4 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1454	1454	100%	0.0	99.75%	819	OP962703.1
Rhipicephalus sanguineus isolate R2.3 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1454	1454	100%	0.0	99.75%	816	OP962702.1
Rhipicephalus sanguineus isolate R2.2 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1454	1454	100%	0.0	99.75%	816	OP962701.1
Rhipicephalus sanguineus isolate R2.1 small subunit ribosomal RNA gene, partial sequence	Rhipicephalus s...	1454	1454	100%	0.0	99.75%	815	OP962700.1

R1_Cattle_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus zambeziensis clone 221_9750_843 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus z…	1450	1450	99%	0.0	99.75%	9750	KY457509.1
Rhipicephalus simus clone 7_9296_958 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S riboso…	Rhipicephalus si…	1450	1450	99%	0.0	99.75%	9296	KY457508.1
Rhipicephalus zambeziensis clone 307_9750_71 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus z…	1450	1450	99%	0.0	99.75%	9750	KY457507.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ri…	Rhipicephalus m…	1450	1450	99%	0.0	99.75%	8908	KY457506.1
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribos…	Rhipicephalus e…	1450	1450	99%	0.0	99.75%	9216	KY457503.1
Rhipicephalus appendiculatus clone 6_8584_532 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus a…	1450	1450	99%	0.0	99.75%	8584	KY457501.1

R2_Cattle_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus zambeziensis clone 221_9750_843 18S ribosomal RNA gene, internal transcribed spacer 1,…	Rhipicephalus z…	1461	1461	99%	0.0	100.00%	9750	KY457509.1
Rhipicephalus simus clone 7_9296_958 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribos…	Rhipicephalus si…	1461	1461	99%	0.0	100.00%	9296	KY457508.1
Rhipicephalus zambeziensis clone 307_9750_71 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus z…	1461	1461	99%	0.0	100.00%	9750	KY457507.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S …	Rhipicephalus …	1461	1461	99%	0.0	100.00%	8908	KY457506.1
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribo…	Rhipicephalus e…	1461	1461	99%	0.0	100.00%	9216	KY457503.1
Rhipicephalus appendiculatus clone 6_8584_532 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus a…	1461	1461	99%	0.0	100.00%	8584	KY457501.1

R3_Cattle18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus zambeziensis clone 221_9750_843 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus z…	1456	1456	99%	0.0	99.87%	9750	KY457509.1
Rhipicephalus simus clone 7_9296_958 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S riboso…	Rhipicephalus si…	1456	1456	99%	0.0	99.87%	9296	KY457508.1
Rhipicephalus zambeziensis clone 307_9750_71 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus z…	1456	1456	99%	0.0	99.87%	9750	KY457507.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ri…	Rhipicephalus m…	1456	1456	99%	0.0	99.87%	8908	KY457506.1
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribos…	Rhipicephalus e…	1456	1456	99%	0.0	99.87%	9216	KY457503.1
Rhipicephalus appendiculatus clone 6_8584_532 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus a…	1456	1456	99%	0.0	99.87%	8584	KY457501.1

Clinvet_Rm1_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus zambeziensis clone 221_9750_843 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus z…	1456	1456	99%	0.0	99.87%	9750	KY457509.1
Rhipicephalus simus clone 7_9296_958 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S riboso…	Rhipicephalus si…	1456	1456	99%	0.0	99.87%	9296	KY457508.1
Rhipicephalus zambeziensis clone 307_9750_71 18S ribosomal RNA gene, internal transcribed spacer 1, 5.…	Rhipicephalus z…	1456	1456	99%	0.0	99.87%	9750	KY457507.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ri…	Rhipicephalus m…	1456	1456	99%	0.0	99.87%	8908	KY457506.1
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribos…	Rhipicephalus e…	1456	1456	99%	0.0	99.87%	9216	KY457503.1
Rhipicephalus appendiculatus clone 6_8584_532 18S ribosomal RNA gene, internal transcribed spacer 1, 5,…	Rhipicephalus a…	1456	1456	99%	0.0	99.87%	8584	KY457501.1

Clinvet_Rd1_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus zambeziensis clone 221_9750_843 18S ribosomal RNA gene, internal transcribed spacer 1,…	Rhipicephalus z…	1461	1461	99%	0.0	100.00%	9750	KY457509.1
Rhipicephalus simus clone 7_9296_958 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribos…	Rhipicephalus si…	1461	1461	99%	0.0	100.00%	9296	KY457508.1
Rhipicephalus zambeziensis clone 307_9750_71 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus z…	1461	1461	99%	0.0	100.00%	9750	KY457507.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S …	Rhipicephalus …	1461	1461	99%	0.0	100.00%	8908	KY457506.1
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribo…	Rhipicephalus e…	1461	1461	99%	0.0	100.00%	9216	KY457503.1
Rhipicephalus appendiculatus clone 6_8584_532 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus a…	1461	1461	99%	0.0	100.00%	8584	KY457501.1

RP1_Clinvet_18S

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus zambeziensis clone 221_9750_843 18S ribosomal RNA gene, internal transcribed spacer 1,…	Rhipicephalus z…	1463	1463	100%	0.0	100.00%	9750	KY457509.1
Rhipicephalus simus clone 7_9296_958 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribos…	Rhipicephalus si…	1463	1463	100%	0.0	100.00%	9296	KY457508.1
Rhipicephalus zambeziensis clone 307_9750_71 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus z…	1463	1463	100%	0.0	100.00%	9750	KY457507.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S …	Rhipicephalus …	1463	1463	100%	0.0	100.00%	8908	KY457506.1
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribo…	Rhipicephalus e…	1463	1463	100%	0.0	100.00%	9216	KY457503.1
Rhipicephalus appendiculatus clone 6_8584_532 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus a…	1463	1463	100%	0.0	100.00%	8584	KY457501.1

Re1_Clinvet_CO1



select all *100 sequences selected*

GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhipicephalus zambeziensis clone 221_9750_843 18S ribosomal RNA gene, internal transcribed spacer 1, 5…	Rhipicephalus z…	1452	1452	99%	0.0	99.75%	9750	KY457509.1
Rhipicephalus simus clone 7_9296_958 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S riboso…	Rhipicephalus si…	1452	1452	99%	0.0	99.75%	9296	KY457508.1
Rhipicephalus zambeziensis clone 307_9750_71 18S ribosomal RNA gene, internal transcribed spacer 1, 5,…	Rhipicephalus z…	1452	1452	99%	0.0	99.75%	9750	KY457507.1
Rhipicephalus microplus clone 60_8908_763 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ri…	Rhipicephalus m…	1452	1452	99%	0.0	99.75%	8908	KY457506.1
Rhipicephalus evertsi clone 2_9216_449 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribos…	Rhipicephalus e…	1452	1452	99%	0.0	99.75%	9216	KY457503.1
Rhipicephalus appendiculatus clone 6_8584_532 18S ribosomal RNA gene, internal transcribed spacer 1, 5.…	Rhipicephalus a…	1452	1452	99%	0.0	99.75%	8584	KY457501.1

8.5 BLASTn search results for pathogen identification

DD4_Dog_*23S rRNA* — *Coxiella spp.*

select all *100 sequences selected* — GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Uncultured Coxiella sp. clone JA21C 23S ribosomal RNA gene, partial sequence	uncultured …	957	957	93%	0.0	99.81%	678	ON872213.1
Coxiella-like endosymbiont Zt17-1050_CLE gene for 23S rRNA, partial sequence	Coxiella-like…	953	953	97%	0.0	98.52%	540	LC635215.1
Coxiella endosymbiont of Amblyomma nuttalli isolate Craf2019 chromosome, comple…	Coxiella end…	795	795	99%	0.0	92.63%	1003026	CP064834.1
Coxiella-like endosymbiont HS64 gene for 23S rRNA, partial sequence	Coxiella-like…	791	791	97%	0.0	93.04%	543	LC602388.1
Coxiella-like endosymbiont HS62 gene for 23S rRNA, partial sequence	Coxiella-like…	791	791	97%	0.0	93.04%	543	LC602387.1
Coxiella-like endosymbiont HS59 gene for 23S rRNA, partial sequence	Coxiella-like…	791	791	97%	0.0	93.04%	543	LC602386.1

Re12_Sheep_*23S rRNA*

select all *100 sequences selected* — GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Coxiella-like endosymbiont Zt17-968_CLE gene for 23S rRNA, partial sequence	Coxiella-like end…	981	981	96%	0.0	99.63%	537	LC635196.1
Coxiella-like endosymbiont ZT17-497_CLE gene for 23S rRNA, partial sequence	Coxiella-like end…	950	950	97%	0.0	98.51%	538	LC635227.1
Coxiella-like endosymbiont ZT17-188_CLE gene for 23S rRNA, partial sequence	Coxiella-like end…	944	944	97%	0.0	98.33%	538	LC635226.1
Coxiella-like endosymbiont isolate CLE RmCVSA chromosome, complete genome	Coxiella-like end…	931	931	99%	0.0	97.27%	1565262	CP094229.1
Coxiella-like endosymbiont ZT17-356_CLE gene for 23S rRNA, partial sequence	Coxiella-like end…	920	920	96%	0.0	97.58%	538	LC635230.1
Uncultured Coxiella sp. clone JA22C 23S ribosomal RNA gene, partial sequence	uncultured Coxi…	893	893	94%	0.0	97.16%	630	ON872214.1

Smf3_Sheep_*23S rRNA*

select all *100 sequences selected* — GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Coxiella-like endosymbiont isolate CLE RaCVSA chromosome, complete genome	Coxiella-like end…	1016	1198	99%	0.0	99.82%	1449412	CP094378.1
Coxiella-like endosymbiont Zt17-967_CLE gene for 23S rRNA, partial sequence	Coxiella-like end…	994	1169	96%	0.0	100.00%	538	LC635204.1
Uncultured Coxiella sp. clone JA23C 23S ribosomal RNA gene, partial sequence	uncultured Coxi…	972	1156	96%	0.0	99.62%	623	ON872215.1
Coxiella endosymbiont of Rhipicephalus sanguineus isolate MAR16E 23S ribosomal RNA gene, partial sequ…	Coxiella endosy…	918	1117	99%	0.0	96.58%	588	ON045539.1
Coxiella endosymbiont of Rhipicephalus sanguineus isolate MAR11A 23S ribosomal RNA gene, partial sequ…	Coxiella endosy…	915	1110	98%	0.0	96.57%	566	ON045536.1
Coxiella endosymbiont of Rhipicephalus sanguineus isolate YUMA1A 23S ribosomal RNA gene, partial sequ…	Coxiella endosy…	913	1112	98%	0.0	96.56%	583	ON045551.1

h3_horse_gltA ***Rickettsia***

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rickettsia aeschlimannii isolate Tyumen-13 citrate synthase (gltA) gene, complete cds	Rickettsia aesch…	706	706	100%	0.0	100.00%	1308	OR687096.1
Rickettsia aeschlimannii isolate Tyumen-12 citrate synthase (gltA) gene, complete cds	Rickettsia aesch…	706	706	100%	0.0	100.00%	1308	OR687095.1
Rickettsia aeschlimannii isolate Tyumen-9 citrate synthase (gltA) gene, complete cds	Rickettsia aesch…	706	706	100%	0.0	100.00%	1308	OR687092.1
Rickettsia aeschlimannii isolate Tyumen-8 citrate synthase (gltA) gene, complete cds	Rickettsia aesch…	706	706	100%	0.0	100.00%	1308	OR687091.1
Rickettsia aeschlimannii isolate Tyumen-2 citrate synthase (gltA) gene, complete cds	Rickettsia aesch…	706	706	100%	0.0	100.00%	1308	OR687085.1
Rickettsia sp. isolate Rm11 citrate synthase (gltA) gene, partial cds	Rickettsia sp.	702	702	99%	0.0	100.00%	380	MW114885.1
Rickettsia sp. isolate Ag12 citrate synthase (gltA) gene, partial cds	Rickettsia sp.	702	702	99%	0.0	100.00%	380	MW114884.1
Uncultured Rickettsia sp. clone ARRL2016-149 citrate synthase (gltA) gene, partial cds	uncultured Rick…	701	701	100%	0.0	99.74%	476	MN431834.1

h14_horse_gltA

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rickettsia aeschlimannii isolate Tyumen-13 citrate synthase (gltA) gene, complete cds	Rickettsia aesch…	706	706	100%	0.0	100.00%	1308	OR687096.1
Rickettsia aeschlimannii isolate Tyumen-12 citrate synthase (gltA) gene, complete cds	Rickettsia aesch…	706	706	100%	0.0	100.00%	1308	OR687095.1
Rickettsia aeschlimannii isolate Tyumen-9 citrate synthase (gltA) gene, complete cds	Rickettsia aesch…	706	706	100%	0.0	100.00%	1308	OR687092.1
Rickettsia aeschlimannii isolate Tyumen-8 citrate synthase (gltA) gene, complete cds	Rickettsia aesch…	706	706	100%	0.0	100.00%	1308	OR687091.1
Rickettsia aeschlimannii isolate Tyumen-2 citrate synthase (gltA) gene, complete cds	Rickettsia aesch…	706	706	100%	0.0	100.00%	1308	OR687085.1
Rickettsia sp. isolate Rm11 citrate synthase (gltA) gene, partial cds	Rickettsia sp.	702	702	99%	0.0	100.00%	380	MW114885.1
Rickettsia sp. isolate Ag12 citrate synthase (gltA) gene, partial cds	Rickettsia sp.	702	702	99%	0.0	100.00%	380	MW114884.1
Uncultured Rickettsia sp. clone ARRL2016-149 citrate synthase (gltA) gene, partial cds	uncultured Rick…	701	701	100%	0.0	99.74%	476	MN431834.1
Rickettsia aeschlimannii isolate Raeschlimannii_Tick15 citrate synthase (gltA) gene, partial cds	Rickettsia aesch…	701	701	100%	0.0	99.74%	1184	MH267736.1

h3_horse_OmpA

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rickettsia aeschlimannii isolate Ank-RcL276 OmpA (ompA) gene, partial cds	Rickettsia aeschl…	1162	1162	100%	0.0	100.00%	632	MG920561.1
Rickettsia aeschlimannii isolate Ro-775 outer membrane protein A (ompA) gene, partial cds	Rickettsia aeschl…	1162	1162	100%	0.0	100.00%	631	MF379308.1
Rickettsia aeschlimannii isolate Ro-718 outer membrane protein A (ompA) gene, partial cds	Rickettsia aeschl…	1162	1162	100%	0.0	100.00%	632	MF379306.1
Rickettsia aeschlimannii strain EgyRickHimp-El-Arish-16 outer membrane protein A (ompA) gene, partial cds	Rickettsia aeschl…	1162	1162	100%	0.0	100.00%	633	HQ335157.1
Rickettsia aeschlimannii isolate Ank-RcL271 OmpA (ompA) gene, partial cds	Rickettsia aeschl…	1160	1160	99%	0.0	100.00%	629	MG920559.1
Rickettsia aeschlimannii isolate Bolu-RcL855 OmpA (ompA) gene, partial cds	Rickettsia aeschl…	1158	1158	99%	0.0	100.00%	630	MG920564.1
Rickettsia aeschlimannii isolate Ro-210 outer membrane protein A (ompA) gene, partial cds	Rickettsia aeschl…	1157	1157	99%	0.0	100.00%	630	MF379299.1
Uncultured Rickettsia sp. clone 4F outer membrane protein A (ompA) gene, partial cds	uncultured Ricke…	1151	1151	100%	0.0	99.68%	710	KU596571.1

h14_horse_OmpA

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rickettsia aeschlimannii isolate Ank-RcL276 OmpA (ompA) gene, partial cds	Rickettsia aeschl…	1151	1151	100%	0.0	100.00%	632	MG920561.1
Rickettsia aeschlimannii isolate Ro-775 outer membrane protein A (ompA) gene, partial cds	Rickettsia aeschl…	1151	1151	100%	0.0	100.00%	631	MF379308.1
Rickettsia aeschlimannii isolate Ro-718 outer membrane protein A (ompA) gene, partial cds	Rickettsia aeschl…	1151	1151	100%	0.0	100.00%	632	MF379306.1
Rickettsia aeschlimannii strain EgyRickHimp-El-Arish-16 outer membrane protein A (ompA) gene, partial cds	Rickettsia aeschl…	1151	1151	100%	0.0	100.00%	633	HQ335157.1
Rickettsia aeschlimannii isolate Ank-RcL271 OmpA (ompA) gene, partial cds	Rickettsia aeschl…	1149	1149	99%	0.0	100.00%	629	MG920559.1
Rickettsia aeschlimannii isolate Bolu-RcL855 OmpA (ompA) gene, partial cds	Rickettsia aeschl…	1147	1147	99%	0.0	100.00%	630	MG920564.1
Rickettsia aeschlimannii isolate Ro-210 outer membrane protein A (ompA) gene, partial cds	Rickettsia aeschl…	1146	1146	99%	0.0	100.00%	630	MF379299.1

h1_horse_*uidA*

Escherichia Shigella

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Shigella sonnei strain SE6-1 chromosome, complete genome	Shigella sonnei	265	265	100%	3e-66	100.00%	4762774	CP055292.1
Escherichia coli strain EcPF15 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	4796742	CP054227.1
Escherichia coli strain EcPF16 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	4721932	CP054224.1
Escherichia coli strain AH62 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	4799791	CP055259.1
Escherichia coli strain AH25 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	5146159	CP055256.1
Escherichia coli strain AH01 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	4886157	CP055251.1

Smf3 _Sheep_ *uidA*

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Shigella sonnei strain SE6-1 chromosome, complete genome	Shigella sonnei	265	265	100%	3e-66	100.00%	4762774	CP055292.1
Escherichia coli strain EcPF15 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	4796742	CP054227.1
Escherichia coli strain EcPF16 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	4721932	CP054224.1
Escherichia coli strain AH62 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	4799791	CP055259.1
Escherichia coli strain AH25 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	5146159	CP055256.1
Escherichia coli strain AH01 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	4886157	CP055251.1

gkm1_goat_uidA

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Shigella sonnei strain SE6-1 chromosome, complete genome	Shigella sonnei	265	265	100%	3e-66	100.00%	4762774	CP055292.1
Escherichia coli strain EcPF15 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	4796742	CP054227.1
Escherichia coli strain EcPF16 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	4721932	CP054224.1
Escherichia coli strain AH62 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	4799791	CP055259.1
Escherichia coli strain AH25 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	5146159	CP055256.1
Escherichia coli strain AH01 chromosome, complete genome	Escherichia coli	265	265	100%	3e-66	100.00%	4886157	CP055251.1

gmm5_goat_*Ehr-F/Ehr-R biotin* ***Ehrlichia***

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Ehrlichia canis strain YZ-1 chromosome, complete genome	Ehrlichia canis	845	845	96%	0.0	98.94%	1314789	CP025749.1
Uncultured Ehrlichia sp. clone Dog_Hong Kong_04 16S ribosomal RNA gene, partial sequence	uncultured Ehrlichia sp.	845	845	96%	0.0	98.74%	480	OP236554.1
Ehrlichia canis strain 21-00950-0007 chromosome, complete genome	Ehrlichia canis	845	845	96%	0.0	98.94%	1312158	CP085275.1
Ehrlichia canis strain 20-02677-0001 chromosome, complete genome	Ehrlichia canis	845	845	96%	0.0	98.94%	1312048	CP085276.1
Ehrlichia canis strain 20-01904-0002 chromosome, complete genome	Ehrlichia canis	845	845	96%	0.0	98.94%	1312022	CP085277.1
Ehrlichia canis isolate TrKysEcan3 16S ribosomal RNA gene, partial sequence	Ehrlichia canis	845	845	96%	0.0	98.74%	1507	KJ513197.1

gkm1_goat_*Ehr-F/Ehr-R biotin*

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Ehrlichia canis strain YZ-1 chromosome, complete genome	Ehrlichia canis	845	845	96%	0.0	98.94%	1314789	CP025749.1
Uncultured Ehrlichia sp. clone Dog_Hong Kong_04 16S ribosomal RNA gene, partial sequence	uncultured Ehrlichia sp.	845	845	96%	0.0	98.74%	480	OP236554.1
Ehrlichia canis strain 21-00950-0007 chromosome, complete genome	Ehrlichia canis	845	845	96%	0.0	98.94%	1312158	CP085275.1
Ehrlichia canis strain 20-02677-0001 chromosome, complete genome	Ehrlichia canis	845	845	96%	0.0	98.94%	1312048	CP085276.1
Ehrlichia canis strain 20-01904-0002 chromosome, complete genome	Ehrlichia canis	845	845	96%	0.0	98.94%	1312022	CP085277.1
Ehrlichia canis isolate TrKysEcan3 16S ribosomal RNA gene, partial sequence	Ehrlichia canis	845	845	96%	0.0	98.74%	1507	KJ513197.1

gmf7_goat_*Ehr-F/Ehr-R biotin*

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Ehrlichia canis strain YZ-1 chromosome, complete genome	Ehrlichia canis	850	850	96%	0.0	99.15%	1314789	CP025749.1
Uncultured Ehrlichia sp. clone Dog_Hong Kong_04 16S ribosomal RNA gene, partial sequence	uncultured Ehrlichia sp.	850	850	96%	0.0	98.95%	480	OP236554.1
Ehrlichia canis strain 21-00950-0007 chromosome, complete genome	Ehrlichia canis	850	850	96%	0.0	99.15%	1312158	CP085275.1
Ehrlichia canis strain 20-02677-0001 chromosome, complete genome	Ehrlichia canis	850	850	96%	0.0	99.15%	1312048	CP085276.1
Ehrlichia canis strain 20-01904-0002 chromosome, complete genome	Ehrlichia canis	850	850	96%	0.0	99.15%	1312022	CP085277.1
Ehrlichia canis isolate TrKysEcan3 16S ribosomal RNA gene, partial sequence	Ehrlichia canis	850	850	96%	0.0	98.95%	1507	KJ513197.1

gmm5_goat_*E.c*

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Uncultured Ehrlichia sp. clone 120GR19 16S ribosomal RNA gene, partial sequence	uncultured Ehrli…	230	230	98%	8e-56	100.00%	390	MT229111.1
Uncultured Ehrlichia sp. clone 71GR19 16S ribosomal RNA gene, partial sequence	uncultured Ehrli…	230	230	98%	8e-56	100.00%	342	MT229110.1
Uncultured Ehrlichia sp. clone 70GR19 16S ribosomal RNA gene, partial sequence	uncultured Ehrli…	230	230	98%	8e-56	100.00%	342	MT229109.1
Ehrlichia canis isolate YucTiz4 16S ribosomal RNA gene, partial sequence	Ehrlichia canis	230	230	98%	8e-56	100.00%	315	MH374122.1
Ehrlichia canis isolate YucTiz3 16S ribosomal RNA gene, partial sequence	Ehrlichia canis	230	230	98%	8e-56	100.00%	315	MH374121.1
Ehrlichia canis isolate YucTiz2 16S ribosomal RNA gene, partial sequence	Ehrlichia canis	230	230	98%	8e-56	100.00%	315	MH374120.1

gmf7_goat_*E.c*

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Ehrlichia canis isolate NGR_ABK_23 16S ribosomal RNA gene, partial sequence	Ehrlichia canis	230	230	98%	8e-56	100.00%	359	KY434113.1
Ehrlichia canis isolate NGR_ABK_30 16S ribosomal RNA gene, partial sequence	Ehrlichia canis	230	230	98%	8e-56	100.00%	359	KY434112.1
Ehrlichia canis isolate NGR_ABK_39 16S ribosomal RNA gene, partial sequence	Ehrlichia canis	230	230	98%	8e-56	100.00%	358	KY434110.1
Ehrlichia canis isolate SLTX-7 16S ribosomal RNA gene, partial sequence	Ehrlichia canis	230	230	98%	8e-56	100.00%	306	MF059355.1
Ehrlichia canis isolate ROTX-1 16S ribosomal RNA gene, partial sequence	Ehrlichia canis	230	230	98%	8e-56	100.00%	303	MF059354.1
Ehrlichia canis isolate NTX17 16S ribosomal RNA gene, partial sequence	Ehrlichia canis	230	230	98%	8e-56	100.00%	304	MF059352.1

h14_horse_*Fr153F01/Fr1281R01* ***Francisella***

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Francisella-like endosymbiont of Hyalomma marginatum clone P35b 16S ribosomal RNA gene, partial sequence	Francisella-like e…	1962	1962	100%	0.0	99.72%	1448	MH645200.1
Francisella-like endosymbiont of Hyalomma marginatum clone P37b 16S ribosomal RNA gene, partial sequence	Francisella-like e…	1962	1962	100%	0.0	99.72%	1453	MH645199.1
Francisella-like endosymbiont of Hyalomma marginatum clone P38 16S ribosomal RNA gene, partial sequence	Francisella-like e…	1962	1962	100%	0.0	99.72%	1464	MH645198.1
Francisella-like endosymbiont of Dermacentor marginatus clone P39 16S ribosomal RNA gene, partial sequence	Francisella-like e…	1962	1962	100%	0.0	99.72%	1454	MH645197.1
Francisella-like endosymbiont of Hyalomma marginatum isolate Hyamar1 16S ribosomal RNA gene, partial seque…	Francisella-like e…	1962	1962	100%	0.0	99.72%	1252	MW287951.1
Francisella-like endosymbiont strain FLE02 16S ribosomal RNA gene, partial sequence	Francisella-like e…	1962	1962	100%	0.0	99.72%	1094	HQ705171.1

h40_horse_*Fr153F01/Fr1281R01*

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Uncultured Francisella sp. clone CRETAV-HUVDH 16S ribosomal RNA gene, partial sequence	uncultured Fran…	1964	1964	99%	0.0	99.81%	1358	OK481098.1
Francisella-like endosymbiont of Hyalomma excavatum isolate Hyaexc1 16S ribosomal RNA gene, partial se…	Francisella-like …	1956	1956	99%	0.0	99.72%	1252	MW287952.1
Francisella-like endosymbiont of Hyalomma excavatum isolate Hyaexc2 16S ribosomal RNA gene, partial se…	Francisella-like …	1951	1951	99%	0.0	99.63%	1252	MW287953.1
Francisella-like endosymbiont of Hyalomma lusitanicum isolate Hyalus2 16S ribosomal RNA gene, partial se…	Francisella-like …	1951	1951	99%	0.0	99.63%	1252	MW287950.1
Uncultured Francisella sp. clone FraApaulo 16S ribosomal RNA gene, partial sequence	uncultured Fran…	1945	1945	99%	0.0	99.53%	1247	MN998649.1
Francisella endosymbiont of Dermacentor auratus clone SSPG3 16S ribosomal RNA gene, partial sequence	Francisella endo…	1945	1945	99%	0.0	99.53%	1101	JQ764629.1

8.6 ASVs produced from South African *Rhipicephalus* Ticks

Taxonomy	Kingdom	Phylum	Class	Order	Family	Genus
seq1	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae (Subgroup 1)	*Granulicella*
seq2	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae (Subgroup 1)	*Terriglobus*
seq3	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	uncultured bacterium	*uncultured bacterium*
seq4	Bacteria	Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae (Subgroup 3)	*Bryobacter*
seq5	Bacteria	Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae (Subgroup 3)	*Candidatus Solibacter*
seq6	Bacteria	Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae (Subgroup 3)	
seq7	Bacteria	Acidobacteria	Acidobacteriia	Subgroup 2	uncultured Acidobacteria bacterium	*uncultured Acidobacteria bacterium*
seq8	Bacteria	Acidobacteria	Blastocatellia (Subgroup 4)		uncultured bacterium	*uncultured bacterium*
seq9	Bacteria	Acidobacteria	Blastocatellia (Subgroup 4)	Blastocatellales	Blastocatellaceae	*Aridibacter*
seq10	Bacteria	Acidobacteria	Blastocatellia (Subgroup 4)	Blastocatellales	Blastocatellaceae	*Blastocatella*
seq11	Bacteria	Acidobacteria	Blastocatellia (Subgroup 4)	Blastocatellales	Blastocatellaceae	*Stenotrophobacter*
seq12	Bacteria	Acidobacteria	Blastocatellia (Subgroup 4)	Blastocatellales	Blastocatellaceae	*uncultured*
seq13	Bacteria	Acidobacteria	Blastocatellia (Subgroup 4)	Blastocatellales	Blastocatellaceae	
seq14	Bacteria	Acidobacteria	Blastocatellia (Subgroup 4)	DS-100		
seq15	Bacteria	Acidobacteria	Blastocatellia (Subgroup 4)	Pyrinomonadales	Pyrinomonadaceae	*RB41*
seq16	Bacteria	Acidobacteria	Holophagae	Subgroup 7		
seq17	Bacteria	Acidobacteria	Subgroup 5			
seq18	Bacteria	Acidobacteria	Subgroup 6	Acidobacteria bacterium WX27	Acidobacteria bacterium WX27	*Acidobacteria bacterium WX27*
seq19	Bacteria	Acidobacteria	Subgroup 6	Unknown Order	Unknown Family	*Luteitalea*
seq20	Bacteria	Acidobacteria	Subgroup 6	Unknown Order	Unknown Family	*Vicinamibacter*

seq21	Bacteria	Acidobacteria	Subgroup 6	uncultured Acidobacteriales bacterium	uncultured Acidobacteriales bacterium	*uncultured Acidobacteriales bacterium*
seq22	Bacteria	Acidobacteria	Subgroup 6	uncultured microorganism	uncultured microorganism	*uncultured microorganism*
seq23	Bacteria	Acidobacteria	Subgroup 6	wastewater metagenome	wastewater metagenome	*wastewater metagenome*
seq24	Bacteria	Acidobacteria	Subgroup 6			
seq25	Bacteria	Actinobacteria	Acidimicrobiia	Microtrichales	Iamiaceae	*Iamia*
seq26	Bacteria	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	*Ilumatobacter*
seq27	Bacteria	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	*uncultured*
seq28	Bacteria	Actinobacteria	Acidimicrobiia	Microtrichales	Microtrichaceae	*uncultured*
seq29	Bacteria	Actinobacteria	Acidimicrobiia	Microtrichales	uncultured	*uncultured Acidimicrobiales bacterium*
seq30	Bacteria	Actinobacteria	Acidimicrobiia	Microtrichales	uncultured	*uncultured Actinomycetales bacterium*
seq31	Bacteria	Actinobacteria	Acidimicrobiia	uncultured	uncultured Acidimicrobidae bacterium	*uncultured Acidimicrobidae bacterium*
seq32	Bacteria	Actinobacteria	Acidimicrobiia	uncultured	uncultured bacterium	*uncultured bacterium*
seq33	Bacteria	Actinobacteria	Acidimicrobiia	uncultured		
seq34	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	*Arcanobacterium*
seq35	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	*Flaviflexus*
seq36	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	*Trueperella*
seq37	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	*Gardnerella*
seq38	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Corynebacterium*
seq39	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Corynebacterium 1*
seq40	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Lawsonella*
seq41	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	
seq42	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Dietziaceae	*Dietzia*
seq43	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	*Mycobacterium*
seq44	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	*Gordonia*
seq45	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	*Rhodococcus*
seq46	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	*Williamsia*
seq47	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Tsukamurellaceae	*Tsukamurella*

seq48	Bacteria	Actinobacteria	Actinobacteria	Frankiales	Acidothermaceae	*Acidothermus*
seq49	Bacteria	Actinobacteria	Actinobacteria	Frankiales	Cryptosporangiaceae	*Fodinicola*
seq50	Bacteria	Actinobacteria	Actinobacteria	Frankiales	Frankiaceae	*Jatrophihabitans*
seq51	Bacteria	Actinobacteria	Actinobacteria	Frankiales	Geodermatophilaceae	*Blastococcus*
seq52	Bacteria	Actinobacteria	Actinobacteria	Frankiales	Geodermatophilaceae	*Geodermatophilus*
seq53	Bacteria	Actinobacteria	Actinobacteria	Frankiales	Geodermatophilaceae	*Modestobacter*
seq54	Bacteria	Actinobacteria	Actinobacteria	Frankiales	Geodermatophilaceae	
seq55	Bacteria	Actinobacteria	Actinobacteria	Frankiales	Nakamurellaceae	*Nakamurella*
seq56	Bacteria	Actinobacteria	Actinobacteria	Kineosporiales	Kineosporiaceae	*Kineococcus*
seq57	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Bogoriellaceae	*Georgenia*
seq58	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Bogoriellaceae	
seq59	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Brevibacteriaceae	*Brevibacterium*
seq60	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	*Cellulomonas*
seq61	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Dermabacteraceae	*Brachybacterium*
seq62	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Dermabacteraceae	*Dermabacter*
seq63	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Dermabacteraceae	
seq64	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	*Ornithinicoccus*
seq65	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	*Ornithinimicrobium*
seq66	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	
seq67	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	*Agromyces*
seq68	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	*Curtobacterium*
seq69	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	*Leucobacter*
seq70	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	*Microbacterium*
seq71	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	*Schumannella*
seq72	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	
seq73	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	*Arthrobacter*
seq74	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	*Citricoccus*
seq75	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	*Enteractinococcus*
seq76	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	*Garicola*
seq77	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	*Glutamicibacter*

seq78	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	*Kocuria*
seq79	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	*Micrococcus*
seq80	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	*Nesterenkonia*
seq81	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	*Rothia*
seq82	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	*Yaniella*
seq83	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	
seq84	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Promicromonosporaceae	*Cellulosimicrobium*
seq85	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Sanguibacteraceae	*Sanguibacter*
seq86	Bacteria	Actinobacteria	Actinobacteria	Micrococcales		
seq87	Bacteria	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	*Actinoplanes*
seq88	Bacteria	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	*Luedemannella*
seq89	Bacteria	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	*Micromonospora*
seq90	Bacteria	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	
seq91	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	*Aeromicrobium*
seq92	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	*Marmoricola*
seq93	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	*Nocardioides*
seq94	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	
seq95	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Cutibacterium*
seq96	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Friedmanniella*
seq97	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Luteococcus*
seq98	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Propionicicella*
seq99	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Tessaracoccus*
seq100	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	*uncultured*
seq101	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	
seq102	Bacteria	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Amycolatopsis*
seq103	Bacteria	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Crossiella*
seq104	Bacteria	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Pseudonocardia*
seq105	Bacteria	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	*Streptomyces*
seq106	Bacteria	Actinobacteria	Actinobacteria	Streptosporangiales	Nocardiopsaceae	*Nocardiopsis*
seq107	Bacteria	Actinobacteria	Actinobacteria	Streptosporangiales	Thermomonosporaceae	*Actinomadura*

seq108	Bacteria	Actinobacteria	Actinobacteria	Streptosporangiales	Thermomonosporaceae	
seq109	Bacteria	Actinobacteria	Actinobacteria			
seq110	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Atopobiaceae	*Atopobium*
seq111	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Atopobiaceae	*Olsenella*
seq112	Bacteria	Actinobacteria	MB-A2-108	uncultured Actinomycetales bacterium	uncultured Actinomycetales bacterium	*uncultured Actinomycetales bacterium*
seq113	Bacteria	Actinobacteria	MB-A2-108			
seq114	Bacteria	Actinobacteria	Nitriliruptoria	Nitriliruptorales	Nitriliruptoraceae	*Egicoccus*
seq115	Bacteria	Actinobacteria	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	*Rubrobacter*
seq116	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	*Gaiella*
seq117	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	uncultured	*uncultured bacterium 5G12*
seq118	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	uncultured	*uncultured microorganism*
seq119	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	*Solirubrobacterales bacterium 67-14*
seq120	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	*metagenome*
seq121	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	*uncultured Solirubrobacter sp.*
seq122	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	*uncultured actinobacterium*
seq123	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	
seq124	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	*Conexibacter*
seq125	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	*Parviterribacter*
seq126	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	*Solirubrobacter*
seq127	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	*uncultured*
seq128	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales		
seq129	Bacteria	Armatimonadetes	Fimbriimonadia	Fimbriimonadales	Fimbriimonadaceae	*metagenome*
seq130	Bacteria	Armatimonadetes	Fimbriimonadia	Fimbriimonadales	Fimbriimonadaceae	*uncultured Fimbriimonas sp.*
seq131	Bacteria	Armatimonadetes	Fimbriimonadia	Fimbriimonadales	Fimbriimonadaceae	*uncultured bacterium*
seq132	Bacteria	Armatimonadetes	uncultured			
seq133	Bacteria	BRC1	uncultured bacterium	uncultured bacterium	uncultured bacterium	*uncultured bacterium*
seq134	Bacteria	BRC1				
seq135	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides*

seq136	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales RF16 group	*uncultured bacterium*
seq137	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes vadinHA17	*uncultured Bacteroidales bacterium*
seq138	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Dysgonomonadaceae	*Fermentimonas*
seq139	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Dysgonomonadaceae	*Petrimonas*
seq140	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Dysgonomonadaceae	*uncultured*
seq141	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Dysgonomonadaceae	
seq142	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	HAW-RM37-2	*uncultured bacterium*
seq143	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	M2PB4-65 termite group	*uncultured bacterium*
seq144	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Paludibacteraceae	*uncultured*
seq145	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	*Porphyromonas*
seq146	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Alloprevotella*
seq147	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotella*
seq148	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotella 1*
seq149	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotella 6*
seq150	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotella 7*
seq151	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae UCG-003*
seq152	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae UCG-004*
seq153	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*uncultured*
seq154	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	
seq155	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	*WCHB1-32*
seq156	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	*uncultured*
seq157	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Alistipes*
seq158	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*DMER64*
seq159	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae RC9 gut group*
seq160	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*SP3-e08*
seq161	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*dgA-11 gut group*
seq162	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*hoa5-07d05 gut group*
seq163	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	
seq164	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Tannerellaceae	*Macellibacteroides*

seq165	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Tannerellaceae	*Parabacteroides*
seq166	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	p-251-o5	*metagenome*
seq167	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	p-251-o5	*uncultured bacterium*
seq168	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	p-2534-18B5 gut group	*uncultured bacterium*
seq169	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	uncultured	*uncultured Bacteroidales bacterium*
seq170	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	*Cnuella*
seq171	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	*Ferruginibacter*
seq172	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	*Flaviaesturariibacter*
seq173	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	*Flavisolibacter*
seq174	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	*Heliimonas*
seq175	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	*Lacibacter*
seq176	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	*Sediminibacterium*
seq177	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	*Segetibacter*
seq178	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	*uncultured*
seq179	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	
seq180	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	*Algoriphagus*
seq181	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	*Imperialibacter*
seq182	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	*uncultured*
seq183	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	
seq184	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Cytophagaceae	*Rhodocytophaga*
seq185	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	*Adhaeribacter*
seq186	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	*Hymenobacter*
seq187	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	*Nibribacter*
seq188	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	*Pontibacter*
seq189	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	
seq190	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	*Ohtaekwangia*
seq191	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	*Siphonobacter*
seq192	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	*uncultured*
seq193	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	*Dyadobacter*

seq194	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	*Fibrella*
seq195	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	*Flectobacillus*
seq196	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	*Nibrella*
seq197	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	*Rhabdobacter*
seq198	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	*Spirosoma*
seq199	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*
seq200	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	*Myroides*
seq201	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	*Salinimicrobium*
seq202	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	NS9 marine group	
seq203	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	*Bergeyella*
seq204	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	*Chishuiella*
seq205	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	*Chryseobacterium*
seq206	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	*Cloacibacterium*
seq207	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	*Empedobacter*
seq208	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	*Moheibacter*
seq209	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	*Ornithobacterium*
seq210	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	*uncultured*
seq211	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	
seq212	Bacteria	Bacteroidetes	Bacteroidia	SM1A07	uncultured bacterium	*uncultured bacterium*
seq213	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	*Lentimicrobium*
seq214	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	*iron-reducing bacterium enrichment culture clone HN105*
seq215	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	*Arcticibacter*
seq216	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	*Mucilaginibacter*
seq217	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	*Pedobacter*
seq218	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	*Sphingobacterium*
seq219	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	*uncultured*
seq220	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	
seq221	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	env.OPS 17	

seq222	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales		
seq223	Bacteria	Bacteroidetes	Bacteroidia			
seq224	Bacteria	Bacteroidetes	Ignavibacteria	Ignavibacteriales	BSV40	
seq225	Bacteria	Bacteroidetes	Ignavibacteria	OPB56	uncultured bacterium KF-JG30-B11	*uncultured bacterium KF-JG30-B11*
seq226	Bacteria	Bacteroidetes	Ignavibacteria	OPB56		
seq227	Bacteria	Bacteroidetes	Rhodothermia	Rhodothermales	Rhodothermaceae	*Rubrivirga*
seq228	Bacteria	Bacteroidetes	Rhodothermia	Rhodothermales	Rhodothermaceae	*metagenome*
seq229	Bacteria	Bacteroidetes	Rhodothermia	Rhodothermales	Rhodothermaceae	
seq230	Bacteria	Chlamydiae	Chlamydiae	Chlamydiales	Parachlamydiaceae	
seq231	Bacteria	Chlamydiae	Chlamydiae	Chlamydiales	Simkaniaceae	*Candidatus Rhabdochlamydia*
seq232	Bacteria	Chloroflexi	AD3	uncultured Clostridium sp.	uncultured Clostridium sp.	*uncultured Clostridium sp.*
seq233	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	*Anaerolinea*
seq234	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	*Leptolinea*
seq235	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	*UTCFX1*
seq236	Bacteria	Chloroflexi	Anaerolineae	Ardenticatenales	Ardenticatenaceae	*uncultured*
seq237	Bacteria	Chloroflexi	Anaerolineae	Caldilineales	Caldilineaceae	*uncultured*
seq238	Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	*OLB13*
seq239	Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	*metagenome*
seq240	Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	*uncultured Chloroflexi bacterium*
seq241	Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	*uncultured gamma proteobacterium*
seq242	Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	
seq243	Bacteria	Chloroflexi	Anaerolineae	SBR1031	uncultured Caldilineaceae bacterium	*uncultured Caldilineaceae bacterium*
seq244	Bacteria	Chloroflexi	Anaerolineae	SJA-15		
seq245	Bacteria	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	*uncultured*
seq246	Bacteria	Chloroflexi	Chloroflexia	Kallotenuales	AKIW781	
seq247	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	*Paraburkholderia tropica*

seq248	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	*uncultured Chloroflexi bacterium*
seq249	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	*uncultured bacterium*
seq250	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	*uncultured soil bacterium*
seq251	Bacteria	Chloroflexi	Dehalococcoidia	S085	uncultured soil bacterium	*uncultured soil bacterium*
seq252	Bacteria	Chloroflexi	Dehalococcoidia	S085		
seq253	Bacteria	Chloroflexi	Gitt-GS-136	uncultured Caldilinea sp.	uncultured Caldilinea sp.	*uncultured Caldilinea sp.*
seq254	Bacteria	Chloroflexi	Gitt-GS-136			
seq255	Bacteria	Chloroflexi	JG30-KF-CM66			
seq256	Bacteria	Chloroflexi	KD4-96	uncultured bacterium	uncultured bacterium	*uncultured bacterium*
seq257	Bacteria	Chloroflexi	KD4-96			
seq258	Bacteria	Chloroflexi	Ktedonobacteria	C0119	uncultured bacterium	*uncultured bacterium*
seq259	Bacteria	Chloroflexi	Ktedonobacteria	C0119	uncultured soil bacterium	*uncultured soil bacterium*
seq260	Bacteria	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	*21-Mar*
seq261	Bacteria	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	*JG30a-KF-32*
seq262	Bacteria	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	
seq263	Bacteria	Chloroflexi	TK10			
seq264	Bacteria	Chloroflexi				
seq265	Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured bacterium	*uncultured bacterium*
seq266	Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales		
seq267	Bacteria	Cyanobacteria	Melainabacteria	Obscuribacterales	uncultured bacterium	*uncultured bacterium*
seq268	Bacteria	Cyanobacteria	Melainabacteria	Obscuribacterales	uncultured cyanobacterium	*uncultured cyanobacterium*
seq269	Bacteria	Cyanobacteria	Oxyphotobacteria	Nostocales	Nostocaceae	*Nostoc PCC-73102*
seq270	Bacteria	Cyanobacteria	Oxyphotobacteria	Oxyphotobacteria Incertae Sedis	Unknown Family	
seq271	Bacteria	Cyanobacteria	Oxyphotobacteria	Phormidesmiales	Nodosilineaceae	*Nodosilinea PCC-7104*
seq272	Bacteria	Cyanobacteria	Sericytochromatia	uncultured bacterium	uncultured bacterium	*uncultured bacterium*
seq273	Bacteria	Cyanobacteria	Sericytochromatia	uncultured soil bacterium	uncultured soil bacterium	*uncultured soil bacterium*
seq274	Bacteria	Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae	*Deinococcus*

seq275	Bacteria	Deinococcus-Thermus	Deinococci	Deinococcales	Trueperaceae	*Truepera*
seq276	Bacteria	Dependentiae	Babeliae	Babeliales	Vermiphilaceae	
seq277	Bacteria	Elusimicrobia	Elusimicrobia	Lineage IV	uncultured Termite group 1 bacterium	*uncultured Termite group 1 bacterium*
seq278	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Arcobacteraceae	*Arcobacter*
seq279	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Campylobacteraceae	*Campylobacter*
seq280	Bacteria	FBP	metagenome	metagenome	metagenome	*metagenome*
seq281	Bacteria	FBP	uncultured actinobacterium	uncultured actinobacterium	uncultured actinobacterium	*uncultured actinobacterium*
seq282	Bacteria	FBP	uncultured bacterium	uncultured bacterium	uncultured bacterium	*uncultured bacterium*
seq283	Bacteria	FBP	uncultured organism	uncultured organism	uncultured organism	*uncultured organism*
seq284	Bacteria	FBP	uncultured soil bacterium	uncultured soil bacterium	uncultured soil bacterium	*uncultured soil bacterium*
seq285	Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	*uncultured*
seq286	Bacteria	Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae	*Tumebacillus*
seq287	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Amphibacillus*
seq288	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Anoxybacillus*
seq289	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*
seq290	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Oceanobacillus*
seq291	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Pseudogracilibacillus*
seq292	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Sinobaca*
seq293	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	
seq294	Bacteria	Firmicutes	Bacilli	Bacillales	Family X	*Thermicanus*
seq295	Bacteria	Firmicutes	Bacilli	Bacillales	Family XI	*Gemella*
seq296	Bacteria	Firmicutes	Bacilli	Bacillales	Family XII	*Exiguobacterium*
seq297	Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	*Paenibacillus*
seq298	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	*Lysinibacillus*
seq299	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	*Psychrobacillus*
seq300	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	*Solibacillus*
seq301	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	*Sporosarcina*

seq302	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	
seq303	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Jeotgalicoccus*
seq304	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Macrococcus*
seq305	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*S31*
seq306	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Salinicoccus*
seq307	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*
seq308	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	
seq309	Bacteria	Firmicutes	Bacilli	Bacillales		
seq310	Bacteria	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	*Aerococcus*
seq311	Bacteria	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	*Facklamia*
seq312	Bacteria	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	*Globicatella*
seq313	Bacteria	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	
seq314	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	*Alkalibacterium*
seq315	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	*Alloiococcus*
seq316	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	*Atopostipes*
seq317	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	*Carnobacterium*
seq318	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	*Desemzia*
seq319	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	*Jeotgalibaca*
seq320	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	*Trichococcus*
seq321	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	*uncultured*
seq322	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	
seq323	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus*
seq324	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*Vagococcus*
seq325	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	
seq326	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*
seq327	Bacteria	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	*Weissella*
seq328	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	*Streptococcus*
seq329	Bacteria	Firmicutes	Bacilli	Lactobacillales		
seq330	Bacteria	Firmicutes	Bacilli			
seq331	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	*Christensenellaceae R-7 group*

seq332	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	*uncultured*
seq333	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	*Clostridium sensu stricto 1*
seq334	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	*Clostridium sensu stricto 13*
seq335	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	*Clostridium sensu stricto 18*
seq336	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	*Clostridium sensu stricto 8*
seq337	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	*Proteiniclasticum*
seq338	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	
seq339	Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	*Anaerofustis*
seq340	Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	*Eubacterium*
seq341	Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	*Garciella*
seq342	Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	*Pseudoramibacter*
seq343	Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	
seq344	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	*Anaerococcus*
seq345	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	*Ezakiella*
seq346	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	*Finegoldia*
seq347	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	*Gallicola*
seq348	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	*Helcococcus*
seq349	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	*Murdochiella*
seq350	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	*Parvimonas*
seq351	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	*Peptoniphilus*
seq352	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	*Tissierella*
seq353	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	*W5053*
seq354	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	*Anaerovorax*
seq355	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	*Family XIII AD3011 group*
seq356	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	*Mogibacterium*
seq357	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	*S5-A14a*
seq358	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	*[Eubacterium] nodatum group*
seq359	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	
seq360	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Acetitomaculum*
seq361	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Agathobacter*

seq362	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Anaerocolumna*
seq363	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Cellulosilyticum*
seq364	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Coprococcus 1*
seq365	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Coprococcus 3*
seq366	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Dorea*
seq367	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Lachnospiraceae NK3A20 group*
seq368	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Lachnospiraceae UCG-001*
seq369	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Lachnospiraceae UCG-002*
seq370	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Lachnospiraceae UCG-006*
seq371	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Lachnospiraceae UCG-010*
seq372	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Moryella*
seq373	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Oribacterium*
seq374	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Pseudobutyrivibrio*
seq375	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*[Eubacterium] hallii group*
seq376	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*[Ruminococcus] torques group*
seq377	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*uncultured*
seq378	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	
seq379	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptococcaceae	*Desulfotomaculum*
seq380	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptococcaceae	*Peptococcus*
seq381	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	*Clostridioides*
seq382	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	*Paeniclostridium*
seq383	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	*Peptostreptococcus*
seq384	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	*Romboutsia*
seq385	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Caproiciproducens*
seq386	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Faecalibacterium*
seq387	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Fastidiosipila*
seq388	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Hydrogenoanaerobacterium*
seq389	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Ruminiclostridium*
seq390	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Ruminiclostridium 1*

seq391	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Ruminococcaceae NK4A214 group*
seq392	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Ruminococcaceae UCG-002*
seq393	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Ruminococcaceae UCG-004*
seq394	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Ruminococcaceae UCG-005*
seq395	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Ruminococcaceae UCG-009*
seq396	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Ruminococcaceae UCG-010*
seq397	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Ruminococcaceae UCG-013*
seq398	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Ruminococcaceae UCG-014*
seq399	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Ruminococcus 1*
seq400	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Ruminococcus 2*
seq401	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Saccharofermentans*
seq402	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*[Eubacterium] coprostanoligenes group*
seq403	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*uncultured*
seq404	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	
seq405	Bacteria	Firmicutes	Clostridia	Clostridiales		
seq406	Bacteria	Firmicutes	Clostridia	M55-D21	uncultured Firmicutes bacterium	*uncultured Firmicutes bacterium*
seq407	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	*Turicibacter*
seq408	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	*Phascolarctobacterium*
seq409	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	*Veillonella*
seq410	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	*uncultured*
seq411	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	
seq412	Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	*Fusobacterium*
seq413	Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	*Oceanivirga*
seq414	Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	*Sneathia*
seq415	Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	
seq416	Bacteria	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	*Gemmatimonas*

seq417	Bacteria	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	*Gemmatirosa*
seq418	Bacteria	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	*uncultured*
seq419	Bacteria	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	
seq420	Bacteria	Gemmatimonadetes	Longimicrobia	Longimicrobiales	Longimicrobiaceae	*bacterium YC-ZSS-LKJ147*
seq421	Bacteria	Gemmatimonadetes	Longimicrobia	Longimicrobiales	Longimicrobiaceae	*metagenome*
seq422	Bacteria	Gemmatimonadetes	Longimicrobia	Longimicrobiales	Longimicrobiaceae	*uncultured bacterium*
seq423	Bacteria	Gemmatimonadetes	Longimicrobia	Longimicrobiales	Longimicrobiaceae	
seq424	Bacteria	Gemmatimonadetes	S0134 terrestrial group	uncultured bacterium	uncultured bacterium	*uncultured bacterium*
seq425	Bacteria	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	uncultured bacterium	*uncultured bacterium*
seq426	Bacteria	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41		
seq427	Bacteria	Latescibacteria				
seq428	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	*Nitrospira*
seq429	Bacteria	Patescibacteria	Gracilibacteria	Absconditabacteriales (SR1)	uncultured bacterium	*uncultured bacterium*
seq430	Bacteria	Patescibacteria	Gracilibacteria	JGI 0000069-P22		
seq431	Bacteria	Patescibacteria	Microgenomatia	Candidatus Pacebacteria	uncultured Microgenomates group bacterium	*uncultured Microgenomates group bacterium*
seq432	Bacteria	Patescibacteria	Microgenomatia	Candidatus Woesebacteria	uncultured bacterium	*uncultured bacterium*
seq433	Bacteria	Patescibacteria	Parcubacteria	Candidatus Nomurabacteria	uncultured bacterium	*uncultured bacterium*
seq434	Bacteria	Patescibacteria	Parcubacteria	GWA2-38-13b	uncultured deep-sea bacterium	*uncultured deep-sea bacterium*
seq435	Bacteria	Patescibacteria	Parcubacteria	uncultured Parcubacteria group bacterium	uncultured Parcubacteria group bacterium	*uncultured Parcubacteria group bacterium*
seq436	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	Candidatus Saccharibacteria bacterium UB2523	*Candidatus Saccharibacteria bacterium UB2523*
seq437	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadaceae	*Candidatus Saccharimonas*
seq438	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadaceae	*uncultured bacterium*

seq439	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadaceae	
seq440	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	uncultured bacterium SBR1071	*uncultured bacterium SBR1071*
seq441	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	uncultured bacterium	*uncultured bacterium*
seq442	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales		
seq443	Bacteria	Patescibacteria	WS6 (Dojkabacteria)	uncultured bacterium	uncultured bacterium	*uncultured bacterium*
seq444	Bacteria	Planctomycetes	Phycisphaerae	Phycisphaerales	Phycisphaeraceae	*SM1A02*
seq445	Bacteria	Planctomycetes	Phycisphaerae	Tepidisphaerales	Tepidisphaeraceae	*uncultured bacterium*
seq446	Bacteria	Planctomycetes	Phycisphaerae	Tepidisphaerales	WD2101 soil group	*uncultured bacterium*
seq447	Bacteria	Planctomycetes	Phycisphaerae	Tepidisphaerales	WD2101 soil group	*uncultured eubacterium WD283*
seq448	Bacteria	Planctomycetes	Phycisphaerae	Tepidisphaerales	WD2101 soil group	*uncultured planctomycete*
seq449	Bacteria	Planctomycetes	Phycisphaerae	Tepidisphaerales	WD2101 soil group	
seq450	Bacteria	Planctomycetes	Phycisphaerae	Tepidisphaerales	uncultured Planctomycetales bacterium	*uncultured Planctomycetales bacterium*
seq451	Bacteria	Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	*Fimbriiglobus*
seq452	Bacteria	Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	*Gemmata*
seq453	Bacteria	Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	*uncultured*
seq454	Bacteria	Planctomycetes	Planctomycetacia	Isosphaerales	Isosphaeraceae	*Singulisphaera*
seq455	Bacteria	Planctomycetes	Planctomycetacia	Isosphaerales	Isosphaeraceae	*uncultured*
seq456	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	*Blastopirellula*
seq457	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	*Pir4 lineage*
seq458	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	*Pirellula*
seq459	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	*Rhodopirellula*
seq460	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	*Roseimaritima*
seq461	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	*p-1088-a5 gut group*
seq462	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	*uncultured*
seq463	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	*Planctomicrobium*
seq464	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	*SH-PL14*
seq465	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	uncultured	*uncultured planctomycete*
seq466	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	uncultured	

seq467	Bacteria	Planctomycetes				
seq468	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acetobacter*
seq469	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acidiphilium*
seq470	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Belnapia*
seq471	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Craurococcus*
seq472	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Roseomonas*
seq473	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Rubritepida*
seq474	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*uncultured*
seq475	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	
seq476	Bacteria	Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	*Azospirillum*
seq477	Bacteria	Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	*Skermanella*
seq478	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Brevundimonas*
seq479	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Caulobacter*
seq480	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*
seq481	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*uncultured*
seq482	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	
seq483	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	*Hirschia*
seq484	Bacteria	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured	*uncultured alpha proteobacterium*
seq485	Bacteria	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured	*uncultured bacterium*
seq486	Bacteria	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured	
seq487	Bacteria	Proteobacteria	Alphaproteobacteria	Holosporales	Holosporaceae	*uncultured*
seq488	Bacteria	Proteobacteria	Alphaproteobacteria	Micavibrionales	Micavibrionaceae	*uncultured*
seq489	Bacteria	Proteobacteria	Alphaproteobacteria	Paracaedibacterales	Paracaedibacteraceae	*Candidatus Paracaedibacter*
seq490	Bacteria	Proteobacteria	Alphaproteobacteria	Puniceispirillales	Puniceispirillales Incertae Sedis	*Constrictibacter*
seq491	Bacteria	Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae	*Reyranella*
seq492	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*1174-901-12*
seq493	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*Bosea*
seq494	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*Methylobacterium*
seq495	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*Methylocella*

seq496	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*Microvirga*
seq497	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*Psychroglaciecola*
seq498	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*alphal cluster*
seq499	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*uncultured*
seq500	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	
seq501	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	D05-2	*uncultured bacterium*
seq502	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	*Devosia*
seq503	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	*uncultured bacterium*
seq504	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	
seq505	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Filomicrobium*
seq506	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	KF-JG30-B3	
seq507	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Labraceae	*Labrys*
seq508	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloligellaceae	*uncultured*
seq509	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Pleomorphomonadaceae	*Pleomorphomonas*
seq510	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Aliihoeflea*
seq511	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*
seq512	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Aminobacter*
seq513	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Aurantimonas*
seq514	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Aureimonas*
seq515	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Mesorhizobium*
seq516	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Ochrobactrum*
seq517	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Paenochrobactrum*
seq518	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Pseudochrobactrum*
seq519	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Shinella*
seq520	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*uncultured*
seq521	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	
seq522	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales Incertae Sedis	*Alsobacter*
seq523	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales Incertae Sedis	*Bauldia*
seq524	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales Incertae Sedis	*Nordella*

seq525	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	*Afipia*
seq526	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	*Bradyrhizobium (endoymbiont)*
seq527	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	*Rhodopseudomonas*
seq528	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	*uncultured*
seq529	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	
seq530	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales		
seq531	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Amaricoccus*
seq532	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Gemmobacter*
seq533	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Paracoccus*
seq534	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Rhodobacter*
seq535	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Roseovarius*
seq536	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Rubellimicrobium*
seq537	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	
seq538	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodopirillaceae	*Defluviicoccus*
seq539	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	uncultured	*gut metagenome*
seq540	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	uncultured	*uncultured soil bacterium*
seq541	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	uncultured	
seq542	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Fodinicurvataceae	*uncultured*
seq543	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Anaplasma*
seq544	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Ehrlichia*
seq545	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Wolbachia*
seq546	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Midichloriaceae	*Candidatus Midichloria*
seq547	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	*Rickettsia*
seq548	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	bac2nit3	*metagenome*
seq549	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Altererythrobacter*
seq550	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*DSSF69*
seq551	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Ellin6055*
seq552	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Novosphingobium*
seq553	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sandarakinorhabdus*
seq554	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingoaurantiacus*

seq555	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingobium*
seq556	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*
seq557	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*uncultured*
seq558	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	
seq559	Bacteria	Proteobacteria	Alphaproteobacteria	Tistrellales	Geminicoccaceae	*Candidatus Alysiosphaera*
seq560	Bacteria	Proteobacteria	Alphaproteobacteria	uncultured	uncultured forest soil bacterium	*uncultured forest soil bacterium*
seq561	Bacteria	Proteobacteria	Alphaproteobacteria			
seq562	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoracaceae	*Peredibacter*
seq563	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	*Bdellovibrio*
seq564	Bacteria	Proteobacteria	Deltaproteobacteria	Bradymonadales	Bradymonadaceae	*uncultured proteobacterium*
seq565	Bacteria	Proteobacteria	Deltaproteobacteria	Bradymonadales	uncultured marine bacterium	*uncultured marine bacterium*
seq566	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	*uncultured*
seq567	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	*Bilophila*
seq568	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Archangiaceae	*Anaeromyxobacter*
seq569	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	BIrii41	*Sorangiineae bacterium NIC37A_2*
seq570	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	BIrii41	
seq571	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	*Haliangium*
seq572	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	*Labilithrix*
seq573	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	*Pajaroellobacter*
seq574	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	*uncultured*
seq575	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Sandaracinaceae	*Sandaracinus*
seq576	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Sandaracinaceae	*uncultured*
seq577	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	bacteriap25	*metagenome*
seq578	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	bacteriap25	*uncultured Myxococcales bacterium*
seq579	Bacteria	Proteobacteria	Deltaproteobacteria	Oligoflexales	0319-6G20	*uncultured Roseobacter sp.*
seq580	Bacteria	Proteobacteria	Deltaproteobacteria	Oligoflexales	0319-6G20	*uncultured bacterium*
seq581	Bacteria	Proteobacteria	Deltaproteobacteria	Oligoflexales	0319-6G20	
seq582	Bacteria	Proteobacteria	Deltaproteobacteria	Oligoflexales	Oligoflexaceae	*Oligoflexus*

seq583	Bacteria	Proteobacteria	Deltaproteobacteria	Oligoflexales	Oligoflexaceae	*Silvanigrella*
seq584	Bacteria	Proteobacteria	Deltaproteobacteria	Oligoflexales	Oligoflexaceae	*uncultured*
seq585	Bacteria	Proteobacteria	Deltaproteobacteria	SAR324 clade(Marine group B)	uncultured bacterium	*uncultured bacterium*
seq586	Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	*Desulfovirga*
seq587	Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	*Aeromonas*
seq588	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Alishewanella*
seq589	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Rheinheimera*
seq590	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Idiomarinaceae	*Aliidiomarina*
seq591	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales		
seq592	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	A21b	*uncultured Rhodocyclaceae bacterium*
seq593	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*AAP99*
seq594	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Achromobacter*
seq595	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Acidovorax*
seq596	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Advenella*
seq597	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Alcaligenes*
seq598	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Aquabacterium*
seq599	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Burkholderia-Caballeronia-Paraburkholderia*
seq600	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Comamonas*
seq601	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Cupriavidus*

seq602	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Curvibacter*
seq603	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Delftia*
seq604	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Herbaspirillum*
seq605	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Lautropia*
seq606	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Limnobacter*
seq607	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Massilia*
seq608	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Noviherbaspirillum*
seq609	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Oligella*
seq610	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Paenalcaligenes*
seq611	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Parapusillimonas*
seq612	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Pseudorhodoferax*
seq613	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Pusillimonas*
seq614	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Ralstonia*
seq615	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Ramlibacter*
seq616	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Variovorax*
seq617	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Verticia*
seq618	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*uncultured*
seq619	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	

seq620	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Hydrogenophilaceae	*Thiobacillus*
seq621	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Neisseriaceae	*Alysiella*
seq622	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Neisseriaceae	*Neisseria*
seq623	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	*DSSD61*
seq624	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	*MND1*
seq625	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	*Nitrosospira*
seq626	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	*mle1-7*
seq627	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	*Denitratisoma*
seq628	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	*Methyloversatilis*
seq629	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	
seq630	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	TRA3-20	
seq631	Bacteria	Proteobacteria	Gammaproteobacteria	Cardiobacteriales	Wohlfahrtiimonadaceae	*Ignatzschineria*
seq632	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Cellvibrionaceae	*Cellvibrio*
seq633	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Cellvibrionaceae	*uncultured*
seq634	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Cellvibrionaceae	
seq635	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Porticoccaceae	*C1-B045*
seq636	Bacteria	Proteobacteria	Gammaproteobacteria	Coxiellales	Coxiellaceae	*Coxiella*
seq637	Bacteria	Proteobacteria	Gammaproteobacteria	Diplorickettsiales	Diplorickettsiaceae	*Aquicella*

seq638	Bacteria	Proteobacteria	Gammaproteobacteria	Diplorickettsiales	Diplorickettsiaceae	*Rickettsiella*
seq639	Bacteria	Proteobacteria	Gammaproteobacteria	EPR3968-O8a-Bc78	uncultured bacterium	*uncultured bacterium*
seq640	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Escherichia-Shigella*
seq641	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Hafnia-Obesumbacterium*
seq642	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Kosakonia*
seq643	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Morganella*
seq644	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Pantoea*
seq645	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Proteus*
seq646	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Providencia*
seq647	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Serratia*
seq648	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	
seq649	Bacteria	Proteobacteria	Gammaproteobacteria	Francisellales	Francisellaceae	*Francisella*
seq650	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis	Unknown Family	*Acidibacter*
seq651	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis	Unknown Family	*Candidatus Berkiella*
seq652	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	*Legionella*
seq653	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	*Halomonas*
seq654	Bacteria	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	*Actinobacillus*
seq655	Bacteria	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	*Haemophilus*

seq656	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*
seq657	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Alkanindiges*
seq658	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Enhydrobacter*
seq659	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Moraxella*
seq660	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Psychrobacter*
seq661	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	
seq662	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
seq663	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales		
seq664	Bacteria	Proteobacteria	Gammaproteobacteria	R7C24		
seq665	Bacteria	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	*Nevskia*
seq666	Bacteria	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	*uncultured*
seq667	Bacteria	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	*Steroidobacter*
seq668	Bacteria	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	*uncultured*
seq669	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	*Pseudofulvimonas*
seq670	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	*uncultured*
seq671	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Luteimonas*
seq672	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Lysobacter*
seq673	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Pseudoxanthomonas*

seq674	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*
seq675	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Xanthomonas*
seq676	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*uncultured*
seq677	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	
seq678	Bacteria	Proteobacteria	Gammaproteobacteria			
seq679	Bacteria	Proteobacteria				
seq680	Bacteria	Spirochaetes	Leptospirae	Leptospirales	Leptospiraceae	*Leptospira*
seq681	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	*Treponema 2*
seq682	Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	*uncultured*
seq683	Bacteria	Tenericutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae	*Mycoplasma*
seq684	Bacteria	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	*Candidatus Udaeobacter*
seq685	Bacteria	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	*Chthoniobacter*
seq686	Bacteria	Verrucomicrobia	Verrucomicrobiae	Opitutales	Opitutaceae	
seq687	Bacteria	Verrucomicrobia	Verrucomicrobiae	Opitutales	Puniceicoccaceae	*uncultured*
seq688	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Akkermansiaceae	*Akkermansia*
seq689	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	*Luteolibacter*

8.7 Tick identification: *Cytochrome oxidase subunit 1* (*CO1*) sequences

>Seq1 [organism=*Rhipicephalus evertsi evertsi*] [gmf1] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial

TTAAGTATAAGAATATTAATTCGACTTGAATTAGGCCAACCTGGAAGATTAATTGGTAAT
GACCAAATTTACAACGTAATTGTAACAGCCCATGCATTTATTATAATTTTTTTTTATGGTTA
TGCCAATTATAATTGGGGGATTTGGTAATTGACTTGTACCTATTATATTAGGTGCTCCTG
ATATAGCCTTCCCACGAATAAATAATATAAGATTCTGACTTTTACCCCCTTCCTTATTTTT
ACTAATTAATTCTTCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTTTATCCCCC
TTTATCATCGAATTTATCTCATTATGGTCCTTCTGTAGATTTAGCTATTTTCTCTTTACATC
TTGCTGGTGCATCTTCAATTTTAGGTGCAATTAATTTTATTACAACCATTTTAAATATACG
GTCAATTGGCATAAGAATAGAACGCATACCTTTATTTGTATGATCTGTATTAATCACTGC
TATTCTTTTACTTTTGTCTCTACCTGTCTTAGCAGGGGCCATTACAATATTATTAAC

>Seq2 [organism=*Rhipicephalus evertsi evertsi*] [gmf3] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial
TTAAGTATAAGAATATTAATTCGACTTGAATTAGGCCAACCTGGAAGATTAATTGGTAAT
GACCAAATTTACAACGTAATTGTAACAGCCCATGCATTTATTATAATTTTTTTTTATGGTTA
TGCCAATTATAATTGGGGGATTTGGTAATTGACTTGTACCTATTATATTAGGTGCTCCTG
ATATAGCCTTCCCACGAATAAATAATATAAGATTCTGACTTTTACCCCCTTCCTTATTTTT
ACTAATTAATTCTTCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTTTATCCCCC
TTTATCATCGAATTTATCTCATTATGGTCCTTCTGTAGATTTAGCTATTTTCTCTTTACATC
TTGCTGGTGCATCTTCAATTTTAGGTGCAATTAATTTTATTACAACCATTTTAAATATACG
GTCAATTGGCATAAGAATAGAACGCATACCTTTATTTGTATGATCTGTATTAATCACTGC
TATTCTTTTACTTTTGTCTCTACCTGTCTTAGCAGGGGCCATTACAATATTATTAAC

>Seq3 [organism=*Rhipicephalus evertsi evertsi*] [gmm3] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial

TTAAGTATAAGAATATTAATTCGACTTGAATTAGGCCAACCTGGAAGATTAATTGGTAAT
GACCAAATTTACAACGTAATTGTAACAGCCCATGCATTTATTATAATTTTTTTTTATGGTTA
TGCCAATTATAATTGGGGGATTTGGTAATTGACTTGTACCTATTATATTAGGTGCTCCTG
ATATAGCCTTCCCACGAATAAATAATATAAGATTCTGACTTTTACCCCCTTCCTTATTTTT
ACTAATTAATTCTTCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTTTATCCCCC
TTTATCATCGAATTTATCTCATTATGGTCCTTCTGTAGATTTAGCTATTTTCTCTTTACATC
TTGCTGGTGCATCTTCAATTTTAGGTGCAATTAATTTTATTACAACCATTTTAAATATACG

GTCAATTGGCATAAGAATAGAACGCATACCTTTATTTGTATGATCTGTATTAATCACTGC
TATTCTTTTACTTTTGTCTCTACCTGTCTTAGCAGGGGCCATTACAATATTATTAAC

>Seq4 [organism=*Rhipicephalus evertsi evertsi*] [h6] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial

TTAAGTATAAGAATATTAATTCGACTTGAATTAGGCCAACCTGGAAGATTAATTGGTAAT
GACCAAATTTACAACGTAATTGTAACAGCCCATGCATTTATTATAATTTTTTTTATGGTTA
TGCCAATTATAATTGGGGGATTTGGTAATTGACTTGTACCTATTATATTAGGTGCTCCTG
ATATAGCCTTCCCACGAATAAATAATATAAGATTCTGACTTTTACCCCCTTCCTTATTTTT
ACTAATTAATTCTTCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTTTATCCCCC
TTTATCATCGAATTTATCTCACTATGGTCCTTCTGTAGATTTAGCTATTTTCTCTTTACAT
CTTGCTGGTGCATCTTCAATTTTAGGTGCAATTAATTTTATTACAACCATTTTAAATATAC
GGTCAATTGGCATAAGAATAGAACGCATACCTTTATTTGTATGATCTGTATTAATCACTG
CTATTCTTTTACTTTTGTCTCTACCTGTCTTAGCAGGGGCCATTACAATATTATTAAC

>Seq5 [organism=*Rhipicephalus evertsi evertsi*] [h7] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial

TTAAGTATAAGAATATTAATTCGACTTGAATTAGGCCAACCTGGAAGATTAATTGGTAAT
GACCAAATTTACAACGTAATTGTAACAGCCCATGCATTTATTATAATTTTTTTTATGGTTA
TGCCAATTATAATTGGGGGATTTGGTAATTGACTTGTACCTATTATATTAGGTGCTCCTG
ATATAGCCTTCCCACGAATAAATAATATAAGATTCTGACTTTTACCCCCTTCCTTATTTTT
ACTAATTAATTCTTCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTTTATCCCCC
TTTATCATCGAATTTATCTCATTATGGTCCTTCTGTAGATTTAGCTATTTTCTCTTTACATC
TTGCTGGTGCATCTTCAATTTTAGGTGCAATTAATTTTATTACAACCATTTTAAATATACG
GTCAATTGGCATAAGAATAGAACGCATACCTTTATTTGTATGATCTGTATTAATCACTGC
TATTCTTTTACTTTTGTCTCTACCTGTCTTAGCAGGGGCCATTACAATATTATTAAC

>Seq6 [organism=*Rhipicephalus evertsi evertsi*] [h9] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial
TTAAGTATAAGAATATTAATTCGACTTGAATTAGGCCAACCTGGAAGATTAATTGGTAAT
GACCAAATTTACAACGTAATTGTAACAGCCCATGCATTTATTATAATTTTTTTTATGGTTA
TGCCAATTATAATTGGGGGATTTGGTAATTGACTTGTACCTATTATATTAGGTGCTCCTG
ATATAGCCTTCCCACGAATAAATAATATAAGATTCTGACTTTTACCCCCTTCCTTATTTTT
ACTAATTAATTCTTCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTTTATCCCCC
TTTATCATCGAATTTATCTCATTATGGTCCTTCTGTAGATTTAGCTATTTTCTCTTTACATC

TTGCTGGTGCATCTTCAATTTTAGGTGCAATTAATTTTATTACAACCATTTTAAATATACG
GTCAATTGGCATAAGAATAGAACGCATACCTTTATTTGTATGATCTGTATTAATCACTGC
TATTCTTTTACTTTTGTCTCTACCTGTCTTAGCAGGGGCCATTACAATATTATTAAC

>Seq7 [organism=*Rhipicephalus evertsi evertsi*] [R1] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial

TTAAGTATAAGAATATTAATTCGACTTGAATTAGGCCAACCTGGAAGATTAATTGGTAAT
GACCAAATTTACAACGTAATTGTAACAGCCCATGCATTTATTATAATTTTTTTTATGGTTA
TGCCAATTATAATTGGGGGATTTGGTAATTGACTTGTACCTATTATATTAGGTGCTCCTG
ATATAGCCTTCCCACGAATAAATAATATAAGATTCTGACTTTTACCCCCTTCCTTATTTTT
ACTAATTAATTCTTCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTTTATCCCCC
TTTATCATCGAATTTATCTCATTATGGTCCTTCTGTAGATTTAGCTATTTTCTCTTTACATC
TTGCTGGTGCATCTTCAATTTTAGGTGCAATTAATTTTATTACAACCATTTTAAATATACG
GTCAATTGGCATAAGAATAGAACGCATACCTTTATTTGTATGATCTGTATTAATCACTGC
TATTCTTTTACTTTTGTCTCTACCTGTCTTAGCAGGGGCCATTACAATATTATTAAC

>Seq8 [organism=*Rhipicephalus evertsi evertsi*] [R2] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial

TTAAGTATAAGAATATTAATTCGACTTGAATTAGGCCAACCTGGAAGATTAATTGGTAAT
GACCAAATTTACAACGTAATTGTAACAGCCCATGCATTTATTATAATTTTTTTTATGGTTA
TGCCAATTATAATTGGGGGATTTGGTAATTGACTTGTACCTATTATATTAGGTGCTCCTG
ATATAGCCTTCCCACGAATAAATAATATAAGATTCTGACTTTTACCCCCTTCCTTATTTTT
ACTAATTAATTCTTCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTTTATCCCCC
TTTATCATCGAATTTATCTCATTATGGTCCTTCTGTAGATTTAGCTATTTTCTCTTTACATC
TTGCTGGTGCATCTTCAATTTTAGGTGCAATTAATTTTATTACAACCATTTTAAATATACG
GTCAATTGGCATAAGAATAGAACGCATACCTTTATTTGTATGATCTGTATTAATCACTGC
TATTCTTTTACTTTTGTCTCTACCTGTCTTAGCAGGGGCCATTACAATATTATTAAC

>Seq9 [organism=*Rhipicephalus evertsi evertsi*] [R3] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial

TTAAGTATAAGAATATTAATTCGACTTGAATTAGGCCAACCTGGAAGATTAATTGGTAAT
GACCAAATTTACAACGTAATTGTAACAGCCCATGCATTTATTATAATTTTTTTTATGGTTA
TGCCAATTATAATTGGGGGATTTGGTAATTGACTTGTACCTATTATATTAGGTGCTCCTG
ATATAGCCTTCCCACGAATAAATAATATAAGATTCTGACTTTTACCCCCTTCCTTATTTTT
ACTAATTAATTCTTCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTTTATCCCCC

TTTATCATCGAATTTATCTCATTATGGTCCTTCTGTAGATTTAGCTATTTTCTCTTTACATC
TTGCTGGTGCATCTTCAATTTTAGGTGCAATTAATTTTATTACAACCATTTTAAATATACG
GTCAATTGGCATAAGAATAGAACGCATACCTTTATTTGTATGATCTGTATTAATCACTGC
TATTCTTTTACTTTTGTCTCTACCTGTCTTAGCAGGGGCCATTACAATATTATTAAC

>Seq10 [organism=*Rhipicephalus microplus*] [Rm2] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial
TTAAGTATAAGAATATTAATTCGACTTGAGCTAGGTCAACCAGGAAGATTAATTGGTAAT
GATCAAATTTATAATGTAATCGTAACTGCCCATGCATTTATTATGATTTTCTTTATAGTAA
TACCTATTATAATTGGGGGATTTGGAAATTGGCTTGTCCCTATTATATTAGGGGCCCCTG
ATATAGCTTTTCCACGTATAAATAATATAAGATTTTGGCTTTTACCCCCTTCATTATTTTTA
TTAATTAATTCATCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTGTATCCCCCA
TTATCATCAAATTTATCTCATTACGGCCCTTCTGTTGATTTAGCCATTTTTTCTCTTCACT
TAGCTGGGGCATCATCAATTTTAGGGGCAATTAATTTTATTACAACTATTTTAAATATACG
TTCTATTGGCATATCTATAGAACGTATACCTTTATTTGTCTGATCTGTTTTAATTACAGCC
ATTCTTTTATTATTATCTTTACCTGTTTTAGCAGGGGCTATTACTATATTGTTAAC

>Seq11 [organism=*Rhipicephalus evertsi evertsi*] [Re1] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial

TTAAGTATAAGAATATTAATTCGACTTGAATTAGGCCAACCTGGAAGATTAATTGGTAAT
GACCAAATTTACAACGTAATTGTAACAGCCCATGCATTTATTATAATTTTTTTTATGGTTA
TGCCAATTATAATTGGGGGATTTGGTAATTGACTTGTACCTATTATATTAGGTGCTCCTG
ATATAGCCTTCCCACGAATAAATAATATAAGATTCTGACTTTTACCCCCTTCCTTATTTTT
ACTAATTAATTCTTCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTTTATCCCCC
TTTATCATCGAATTTATCTCATTATGGTCCTTCTGTAGATTTAGCTATTTTCTCTTTACATC
TTGCTGGTGCATCTTCAATTTTAGGTGCAATTAATTTTATTACAACCATTTTAAATATACG
GTCAATTGGCATAAGAATAGAACGCATACCTTTATTTGTATGATCTGTATTAATCACTGC
TATTCTTTTACTTTTGTCTCTACCTGTCTTAGCAGGGGCCATTACAATATTATTAAC

>Seq12 [organism=*Rhipicephalus evertsi evertsi*] [Re11] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial

TTAAGTATAAGAATATTAATTCGACTTGAATTAGGCCAACCTGGAAGATTAATTGGTAAT
GACCAAATTTACAACGTAATTGTAACAGCCCATGCATTTATTATAATTTTTTTTATGGTTA
TGCCAATTATAATTGGGGGATTTGGTAATTGACTTGTACCTATTATATTAGGTGCTCCTG
ATATAGCCTTCCCACGAATAAATAATATAAGATTCTGACTTTTACCCCCTTCCTTATTTTT

ACTAATTAATTCTTCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTTTATCCCCC
TTTATCATCGAATTTATCTCATTATGGTCCTTCTGTAGATTTAGCTATTTTCTCTTTACATC
TTGCTGGTGCATCTTCAATTTTAGGTGCAATTAATTTTATTACAACCATTTTAAATATACG
GTCAATTGGCATAAGAATAGAACGCATACCTTTATTTGTATGATCTGTATTAATCACTGC
TATTCTTTTACTTTTGTCTCTACCTGTCTTAGCAGGGGCCATTACAATATTATTAAC

>Seq13 [organism=*Rhipicephalus evertsi evertsi*] [Re15] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial

TTAAGTATAAGAATATTAATTCGACTTGAATTAGGCCAACCTGGAAGATTAATTGGTAAT
GACCAAATTTACAACGTAATTGTAACAGCCCATGCATTTATTATAATTTTTTTTATGGTTA
TGCCAATTATAATTGGGGGATTTGGTAATTGACTTGTACCTATTATATTAGGTGCTCCTG
ATATAGCCTTCCCACGAATAAATAATATAAGATTCTGACTTTTACCCCCTTCCTTATTTTT
ACTAATTAATTCTTCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTTTATCCCCC
TTTATCATCGAATTTATCTCATTATGGTCCTTCTGTAGATTTAGCTATTTTCTCTTTACATC
TTGCTGGTGCATCTTCAATTTTAGGTGCAATTAATTTTATTACAACCATTTTAAATATACG
GTCAATTGGCATAAGAATAGAACGCATACCTTTATTTGTATGATCTGTATTAATCACTGC
TATTCTTTTACTTTTGTCTCTACCTGTCTTAGCAGGGGCCATTACAATATTATTAAC

>Seq14 [organism=*Rhipicephalus evertsi evertsi]* [Re7] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial

TTAAGTATAAGAATATTAATTCGACTTGAATTAGGCCAACCTGGAAGATTAATTGGTAAT
GACCAAATTTACAACGTAATTGTAACAGCCCATGCATTTATTATAATTTTTTTTATGGTTA
TGCCAATTATAATTGGGGGATTTGGTAATTGACTTGTACCTATTATATTAGGTGCTCCTG
ATATAGCCTTCCCACGAATAAATAATATAAGATTCTGACTTTTACCCCCTTCCTTATTTTT
AYTAATTAATTCTTCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTTTATCCCCC
TTTATCATCGAATTTATCTCATTATGGTCCTTCTGTAGATTTAGCTATTTTCTCTTTACATC
TTGCTGGTGCATCTTCAATTTTAGGTGCAATTAATTTTATTACAACCATTTTAAATATACG
GTCAATTGGCATAAGAATAGAACGCATACCTTTATTTGTATGATCTGTATTAATCACTGC
TATTCTTTTACTTTTGTCTCTACCTGTCTTAGCAGGGGCCATTACAATATTATTAAC

>Seq15 [organism=*Rhipicephalus microplus*] [Rm1] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial
TTAAGTATAAGAATATTAATTCGACTTGAGCTAGGTCAACCAGGAAGATTAATTGGTAAT
GATCAAATTTATAATGTAATCGTAACTGCCCATGCATTTATTATGATTTTCTTTATAGTAA
TACCTATTATAATTGGGGGATTTGGAAATTGGCTTGTCCCTATTATATTAGGGGCCCCTG

ATATAGCTTTTCCACGTATAAATAATATAAGATTTTGGCTTTTACCCCCTTCATTATTTTTA
TTAATTAATTCATCTTTAGTTGAATCAGGAGCAGGGACTGGATGAACTGTGTATCCCCCA
TTATCATCAAATTTATCTCATTACGGCCCTTCTGTTGATTTAGCCATTTTTTCTCTTCACT
TAGCTGGGGCATCATCAATTTTAGGGGCAATTAATTTTATTACAACTATTTTAAATATACG
TTCTATTGGCATATCTATAGAACGTATACCTTTATTTGTCTGATCTGTTTTAATTACAGCC
ATTCTTTTATTATTATCTTTACCTGTTTTAGCAGGGGCTATTACTATATTGTTAAC

>Seq16 [organism=*Rhipicephalus appendiculatus*] [Ra1] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial
TTAAGAATAAGAATATTAATTCGAATAGAATTAGGTCAACCAGGAACTTTAATTGGTAAT
GATCAAATTTATAATGTAATTGTAACTGCTCATGCATTTATTATAATTTTTTTTATAGTAAT
ACCTATTATAATTGGAGGATTTGGAAACTGATTAGTTCCTATTATAATTGGGGCTCCAGA
TATAGCATTTCCTCGAATAAATAATATAAGATTTTGACTTCTTCCTCCTTCTTTATTTTTAC
TAATTAATTCTTCTTTAGTAGAATCTGGAGCTGGAACAGGATGAACAGTTTATCCTCCTT
TATCCTCAAATCTTTCTCATTATGGGCCTTCAGTAGATCTTGCAATTTTTTCTTTACATCT
TGCAGGAGCATCTTCAATTTTAGGGGCAATCAATTTTATTACAACTATTATCAACATACG
TTCTATTGGAATAACTATAGAACGAATACCTTTATTTGTTTGATCAGTTTTAATTACAGCT
ATTTTACTGTTATTATCATTACCAGTTTTAGCTGGTGCTATTACAATATTATTAAC

>Seq17 [organism=*Rhipicephalus sanguineus*] [SAN2] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial
TTAAGAATAAGAATATTAATCCGCATAGAATTAGGTCAACCAGGAACTTTAATTGGAAAC
GATCAAATTTATAATGTAATTGTAACAGCACATGCATTTATTATAATTTTTTTTATAGTTAT
ACCAATTATAATCGGAGGATTTGGAAATTGATTAGTTCCTATTATGTTAGGAGCTCCAGA
TATAGCATTTCCACGAATAAATAATATAAGATTTTGGTTACTTCCTCCTTCTCTATTTCTA
CTAATTAATTCTTCATTAATCGAGTCAGGAGCAGGTACAGGATGAACAGTTTACCCCCC
CTTATCATCAAATTTATCACACTACGGACCATCAGTAGATTTAGCTATCTTTTCTCTGCAT
CTTGCCGGTGCTTCTTCAATTTTAGGTGCAATTAATTTCATTACAACTATTGTAAATATAC
GATCCATTGGAATAACAATAGAACGAATACCATTATTTGTATGATCTGTTTTAATTACTGC
AATTTTATTATTATTATCTTTACCAGTCTTAGCTGGTGCAATTACAATATTATTAAC

>Seq18 [organism=*Rhipicephalus sanguineus*] [SAN2] *cytochrome oxidase subunit I* (*CO1*) gene, partial sequence, mitochondrial
TTAAGAATAAGAATATTAATCCGCATAGAATTAGGTCAACCAGGAACTTTAATTGGAAAC
GATCAAATTTATAATGTAATTGTAACAGCACATGCATTTATTATAATTTTTTTTATAGTTAT
ACCAATTATAATCGGAGGATTTGGAAATTGATTAGTTCCTATTATGTTAGGAGCTCCAGA
TATAGCATTTCCACGAATAAATAATATAAGATTTTGGTTACTTCCTCCTTCTCTATTTCTA

CTAATTAATTCTTCATTAATCGAGTCAGGAGCAGGTACAGGATGAACAGTTTACCCCCC
CTTATCATCAAATTTATCACACTACGGACCATCAGTAGATTTAGCTATCTTTTCTCTGCAT
CTTGCCGGTGCTTCTTCAATTTTAGGTGCAATTAATTTCATTACAACTATTGTAAATATAC
GATCCATTGGAATAACAATAGAACGAATACCATTATTTGTATGATCTGTTTTAATTACTGC
AATTTTATTATTATTATCTTTACCAGTCTTAGCTGGTGCAATTACAATATTATTAAC

8.8 Tick identification: *16S ribosomal RNA* gene sequences

>Seq1 [organism=*Rhipicephalus evertsi evertsi*] [h6] *16S ribosomal RNA gene* cds; partial sequence; mitochondrial

AAGGATTTTTAATTGAATGCTAAGAGAATGGAATTTCAGGGAAAAACTTTTTTAAAATTAA
AAATTGAACTTTTTTTAATTTGTGCAGAAACAATTATTTTAATTAAGGACAAGAAGACCCT
AAGAATTTATGAATTTAAATAATATATTTTCATATTAATTAAATTTTGGCTGGGGCGGCTG
AGAAATATTAAAAACTTTTTGTTTTAACATGACCCATTATTAATGATTAAATGAAAAAATAC
TCTAGGGATAACAGCGTTATATTTTTTGATAGACCATATTGACAAAAAAGTTTGCGACCT
CGATGTTGGATTAGGATACTATTTTAATGAAGATATTAAAATAAGAAGTTTGTTCAA

>Seq2 [organism=*Rhipicephalus evertsi evertsi*] [gmf1] *16S ribosomal RNA* gene cds; partial sequence; mitochondrial

TAAGATTTTAATTGAATGCTAAGAGAATGGAATTTCAGGGAAAAACTTTTTTAAAATTAAA
AATTGAACTTTTTTTAATTTGTGCAGAAACAATTATTTTAATTAAGGACAAGAAGACCCTA
AGAATTTATGAATTTAAATAATATATTTTCATATTAATTAAATTTTGGCTGGGGCGGCTGA
GAAATATTAAAAACTTTTTGTTTTAACATGACCCATTATTAATGATTAAATGAAAAAATACT
CTAGGGATAACAGCGTTATATTTTTTGATAGACCATATTGACAAAAAAGTTTGCGACCTC
GATGTTGGATTAGGATACTATTTTAATGAAGATATTAAAATAAGAAGGTTTGTTCAA

>Seq3 [organism=*Rhipicephalus evertsi evertsi*] [gmf3] *16S ribosomal RNA* gene cds; partial sequence; mitochondrial

TAAGATTTTAATTGAATGCTAAGAGAATGGAATTTCAGGGAAAAACTTTTTTAAAATTAAA
AATTGAACTTTTTTTAATTTGTGCAGAAACAATTATTTTAATTAAGGACAAGAAGACCCTA
AGAATTTATGAATTTAAATAATATATTTTCATATTAATTAAATTTTGGCTGGGGCGGCTGA
GAAATATTAAAAACTTTTTGTTTTAACATGACCCATTATTAATGATTAAATGAAAAAATACT
CTAGGGATAACAGCGTTATATTTTTTGATAGACCATATTGACAAAAAAGTTTGCGACCTC
GATGTTGGATTAGGATACTATTTTAATGAAGATATTAAAATAAGAAAGTTTGTTCAA

>Seq4 [organism=*Rhipicephalus evertsi evertsi*] [gmm3] *16S ribosomal RNA* gene cds; partial sequence; mitochondrial

TAAGATTTTAATTGAATGCTAAGAGAATGGAATTTCAGGGAAAAACTTTTTTAAAATTAAA
AATTGAACTTTTTTTAATTTGTGCAGAAACAATTATTTTAATTAAGGACAAGAAGACCCTA
AGAATTTATGAATTTAAATAATATATTTTCATATTAATTAAATTTTGGCTGGGGCGGCTGA
GAAATATTAAAAACTTTTTGTTTTAACATGACCCATTATTAATGATTAAATGAAAAAATACT
CTAGGGATAACAGCGTTATATTTTTTGATAGACCATATTGACAAAAAAGTTTGCGACCTC
GATGTTGGATTAGGATACTATTTTAATGAAGATATTAAAATAAGAAGTTTGTTCAA

>Seq5 [organism=*Rhipicephalus evertsi evertsi*] [h7] *16S ribosomal RNA* gene cds; partial sequence; mitochondrial

TAAGATTTTAATTGAATGCTAAGAGAATGGAATTTCAGGGAAAAACTTTTTTAAAATTAAA
AATTGAACTTTTTTTAATTTGTGCAGAAACAATTATTTTAATTAAGGACAAGAAGACCCTA
AGAATTTATGAATTTAAATAATATATTTTCATATTAATTAAATTTTGGCTGGGGCGGCTGA
GAAATATTAAAAACTTTTTGTTTTAACATGACCCATTATTAATGATTAAATGAAAAAATACT
CTAGGGATAACAGCGTTATATTTTTTGATAGACCATATTGACAAAAAAGTTTGCGACCTC
GATGTTGGATTAGGATACTATTTTAATGAAGATATTAAAATAAGAAGTTTGTTCAA

>Seq6 [organism=*Rhipicephalus evertsi evertsi*] [h9] *16S ribosomal RNA* gene cds; partial sequence; mitochondrial

TAAGATTTTAATTGAATGCTAAGAGAATGGAATTTCAGGGGAAAACTTTTTTAAAATTAAA
AATTGAACTTTTTTTAATTTGTGCAGAAACAATTATTTTAATTAAGGACAAGAAGACCCTA
AGAATTTATGAATTTAAATAATATATTTTCATATTAATTAAATTTTGGCTGGGGCGGCTGA
GAAATATTAAAAACTTTTTGTTTTAACATGACCCATTATTAATGATTAAATGAAAAAATACT
CTAGGGATAACAGCGTTATATTTTTTGATAGACCATATTGACAAAAAAGTTTGCGACCTC
GATGTTGGATTAGGATACTATTTTAATGAAGATATTAAAATAAGAAGTTTGTTCAA

>Seq7 [organism=*Rhipicephalus microplus*] [Rm1] *16S ribosomal RNA* gene cds; partial sequence; mitochondrial

AAGGATTTTAATTGAATGCTAAGAGAATGGAATATCAAAAAACAACTTTCTTAAAATTAAA
AATTGAAATTTTTTTAATTTGTGTAAAAACAATTATAAAAATTAAAGACAAGAAGACCCTA
AGAATTTTTAAAATTTAAATAATACATTTTTTGTTTTAATTAAATTTTAACTGGGGCGGTTA
AAAAATATTAAAAACTTTTTAATTTAAAAATGACCCATTATTAATGAAAATATGATTAAATA

CTCTAGGGATAACAGCGTTATATTTTTTGATAGATCATATTGACAAAAAAGTTTGCGACC
TCGATGTTGGATTAGGATACTTTTTTAATGAAGATATTAAAATAAGAAGTTTGTTCAA

>Seq8 [organism=*Rhipicephalus microplus*] [Rm2] *16S ribosomal RNA gene* cds; partial sequence; mitochondrial

TAAGATTTTAATTGAATGCTAAGAGAATGGAATATCAAAAAACAACTTTCTTAAAATTAAA
AATTGAAATTTTTTTAATTTGTGTAAAAACAATTATAAAAATTAAAGACAAGAAGACCCTA
AGAATTTTTAAAATTTAAATAATACATTTTTTGTTTTAATTAAATTTTAACTGGGGCGGTTA
AAAAATATTAAAAACTTTTTAATTTAAAAATGACCCATTATTAATGAAAATATGATTAAATA
CTCTAGGGATAACAGCGTTATATTTTTTGATAGATCATATTGACAAAAAAGTTTGCGACC
TCGATGTTGGATTAGGATACTTTTTTAATGAAGATATTAAAATAAGAAGTTTGTTCAA

>Seq9 [organism=*Rhipicephalus appendiculatus]* [Ra1] *16S ribosomal RNA* gene cds; partial sequence; mitochondrial

TAAGATTTTAATTGAATGCTAAGAGAATGGAATTTCAGGGAAAAACTTTATTTAAACTAAT
AATTAAAGTTTTTTTAATTTGTGCAGAAACAATTATATAAATTAAGGACAAGAAGACCCTA
TGAATTTATAAAACTTATTTTTTTTATTAATATAACTAATAAGTTTTAGCTGGGGCGGCAA
GAAAATATAAAAAACTTTTCATTATTAAAATGATCCATTATTAATGATTAAATGAAAAAATA
CTCTAGGGATAACAGCGTTATATTTTTTGATAGATCATATTGACAAAAAAGTTTGCGACC
TCGATGTTGGATTAGGATACTTTTTTAATGCAGATATTAAAAAAAGAAGTTTGTTCCA

>Seq10 [organism=*Rhipicephalus sanguineus*] [S3D] *16S ribosomal RNA* gene cds; partial sequence; mitochondrial

TAAGATTTTAATTGAATGCTAAGAGAATGGAAGTCCAGAAAAAAACTTTTTTTAAATTAAA
AATTGAAGTTTTTTTAATTTGTGCAGAAACAATTATTTATATTAAAGACAAGAAGACCCTA
TGAATTTATTAAATTTTTATTTAATATGTAATTACTATTAGAAAAATTTTGGCTGGGGCGG
CTAGAAAATATTATGAACTTTTTAAAAATAAAATGATCCATTATTAATGATTTTATGTTAAA
TACTCTAGGGATAACAGCGTTATATTTTTTGATAGATCATATTGACAAAAAAGTTTGCGA
CCTCGATGTTGGATTAGGATACTTTTTTAATGAAGATATTAAAAAAAGAAGTTTGTTCAA

>Seq11 [organism=*Rhipicephalus evertsi evertsi*] [RE7S] *16S ribosomal RNA* gene cds; partial sequence; mitochondrial
TAAGATTTTAATTGAATGCTAAGAGAATGGAATTTCAGGGAAAAACTTTTTTAAAATTAAA
AATTGAACTTTTTTTAATTTGTGCAGAAACAATTATTTTAATTAAGGACAAGAAGACCCTA
AGAATTTATGAATTTAAATAATATATTTTCATATTAATTAAATTTTGGCTGGGGCGGCTGA

GAAATATTAAAAACTTTTTGTTTTAACATGACCCATTATTAATGATTAAATGAAAAAATACT
CTAGGGATAACAGCGTTATATTTTTTGATAGACCATATTGACAAAAAAGTTTGCGACCTC
GATGTTGGATTAGGATACTATTTTAATGAAGATATTAAAATAAGAAAGTTTGTTC

>Seq12 [organism=*Rhipicephalus evertsi evertsi*] [RE11S] *16S ribosomal RNA gene* cds; partial sequence; mitochondrial

AAGGATTTTAATTGAATGCTAAGAGAATGGAATTTCAGGGAAAAACTTTTTTAAAATTAAA
AATTGAACTTTTTTTAATTTGTGCAGAAACAATTATTTTAATTAAGGACAAGAAGACCCTA
AGAATTTATGAATTTAAATAATATATTTTCATATTAATTAAATTTTGGCTGGGGCGGCTGA
GAAATATTAAAAACTTTTTGTTTTAACATGACCCATTATTAATGATTAAATGAAAAAATACT
CTAGGGATAACAGCGTTATATTTTTTGATAGACCATATTGACAAAAAAGTTTGCGACCTC
GATGTTGGATTAGGATACTATTTTAATGAAGATATTAAAATAAGAAGTTTGTTCAA

>Seq13 [organism=*Rhipicephalus evertsi evertsi*] [RE15S] *16S ribosomal RNA gene* cds; partial sequence; mitochondrial

TAAGATTTTAATTGAATGCTAAGAGAATGGAATTTCAGGGAAAAACTTTTTTAAAATTAAA
AATTGAACTTTTTTTAATTTGTGCAGAAACAATTATTTTAATTAAGGACAAGAAGACCCTA
AGAATTTATGAATTTAAATAATATATTTTCATATTAATTAAATTTTGGCTGGGGCGGCTGA
GAAATATTAAAAACTTTTTGTTTTAACATGACCCATTATTAATGATTAAATGAAAAAATACT
CTAGGGATAACAGCGTTATATTTTTTGATAGACCATATTGACAAAAAAGTTTGCGACCTC
GATGTTGGATTAGGATACTATTTTAATGAAGATATTAAAATAAGAAGTTTGTTCCA

>Seq14 [organism=*Rhipicephalus evertsi evertsi*] [R1C] *16S ribosomal RNA* gene cds; partial sequence; mitochondrial

TAAGATTTTAATTGAATGCTAAGAGAATGGAATTTCAGGGAAAAACTTTTTTAAAATTAAA
AATTGAACTTTTTTTAATTTGTGCAGAAACAATTATTTTAATTAAGGACAAGAAGACCCTA
AGAATTTATGAATTTAAATAATATATTTTCATATTAATTAAATTTTGGCTGGGGCGGCTGA
GAAATATTAAAAACTTTTTGTTTTAACATGACCCATTATTAATGATTAAATGAAAAAATACT
CTAGGGATAACAGCGTTATATTTTTTGATAGACCATATTGACAAAAAAGTTTGCGACCTC
GATGTTGGATTAGGATACTATTTTAATGAAGATATTAAAATAAGAAGTTTGTTCAA

>Seq15 [organism=*Rhipicephalus evertsi evertsi*] [R2C] *16S ribosomal RNA* gene cds; partial sequence; mitochondrial
TAAGATTTTAATTGAATGCTAAGAGAATGGAATTTCAGGGAAAAACTTTTTTAAAATTAAA
AATTGAACTTTTTTTAATTTGTGCAGAAACAATTATTTTAATTAAGGACAAGAAGACCCTA

AGAATTTATGAATTTAAATAATATATTTTCATATTAATTAAATTTTGGCTGGGGCGGCTGA
GAAATATTAAAAACTTTTTGTTTTAACATGACCCATTATTAATGATTAAATGAAAAAATACT
CTAGGGATAACAGCGTTATATTTTTTGATAGACCATATTGACAAAAAAGTTTGCGACCTC
GATGTTGGATTAGGATACTATTTTAATGAAGATATTAAAATAAGAAGTTTGTTCA

>Seq16 [organism=*Rhipicephalus evertsi evertsi*] [R3C] *16S ribosomal RNA* gene cds; partial sequence; mitochondrial

TAAGATTTTAATTGAATGCTAAGAGAATGGAATTTCAGGGAAAAACTTTTTTAAAATTAAA
AATTGAACTTTTTTTAATTTGTGCAGAAACAATTATTTTAATTAAGGACAAGAAGACCCTA
AGAATTTATGAATTTAAATAATATATTTTCATATTAATTAAATTTTGGCTGGGGCGGCTGA
GAAATATTAAAAACTTTTTGTTTTAACATGACCCATTATTAATGATTAAATGAAAAAATACT
CTAGGGATAACAGCGTTATATTTTTTGATAGACCATATTGACAAAAAAGTTTGCGACCTC
GATGTTGGATTAGGATACTATTTTAATGAAGATATTAAAATAAGAAGTTTGTTCA

>Seq17 [organism=*Rhipicephalus evertsi evertsi*] [Re1] *16S ribosomal RNA gene* cds; partial sequence; mitochondrial

TAAGATTTTAATTGAATGCTAAGAGAATGGAATTTCAGGGGAAAACTTTTTTAAAATTAAA
AATTGAACTTTTTTTAATTTGTGCAGAAACAATTATTTTAATTAAGGACAAGAAGACCCTA
AGAATTTATGAATTTAAATAATATATTTTCATATTAATTAAATTTTGGCTGGGGCGGCTGA
GAAATATTAAAAACTTTTTGTTTTAACATGACCCATTATTAATGATTAAATGAAAAAATACT
CTAGGGATAACAGCGTTATATTTTTTGATAGACCATATTGACAAAAAAGTTTGCGACCTC
GATGTTGGATTAGGATACTATTTTAATGAAGATATTAAAATAAGAAGTTTGTTCAA

>Seq18 [organism=*Rhipicephalus sanguineus*] [SAN2D] *16S ribosomal RNA gene* cds; partial sequence; mitochondrial

TAAGATTTTAATTGAATGCTAAGAGAATGGAAGTCCAGAAAAAAACTTTTTTTAAATTAAA
AATTGAAGTTTTTTTAATTTGTGCAGAAACAATTATTTATATTAAAGACAAGAAGACCCTA
TGAATTTATTAAATTTTTATTTAATATGTAATTACTATTAGAAAAATTTTGGCTGGGGCGG
CTAGAAAATATTATRAACTTTTTAAAAATAAAATGATCCATTATTAATGATTTTATGTTAAA
TACTCTAGGGATAACAGCGTTATATTTTTTGATAGATCATATTGACAAAAAAGTTTGCGA
CCTCGATGTTGGATTAGGATACTTTTTTAATGAAGATATTAAAAAAAGAAGTTTGTTCAA

>Seq19 [organism=*Rhipicephalus sanguineus*] [SAN3D] *16S ribosomal RNA* gene cds; partial sequence; mitochondrial

TAAGATTTTAATTGAATGCTAAGAGAATGGAAGTCCAGAAAAAAACTTTTTTTAAATTAAA
AATTGAAGTTTTTTTAATTTGTGCAGAAACAATTATTTATATTAAAGACAAGAAGACCCTA
TGAATTTATTAAATTTTTATTTAATATGTAATTACTATTAGAAAAATTTTGGCTGGGGCGG
CTAGAAAATATTATGAACTTTTTAAAAATAAAATGATCCATTATTAATGATTTTATGTTAAA
TACTCTAGGGATAACAGCGTTATATTTTTTGATAGATCATATTGACAAAAAAGTTTGCGA
CCTCGATGTTGGATTAGGATACTTTTTTAATGAAGATATTAAAAAAAGAAGTTTGTTCAA

8.9 Tick identification: *Internal transcribed spacer 2 (ITS2*)

>Seq1 [organism=*Rhipicephalus sanguineus*] [S3D] *ITS2 internal transcribed spacer 2 ribosomal RNA gene* cds; partial sequence

TCACATATCAAGAGAGACTTCGGCGCACAAGGGAACGTGAGCCGTCGACTCGTTTTGA
CCGTGTCGGCATCACGGACAGCACGTTGAACGCTACGGCAAAGCGACAACGGCCGCT
CGAAAGGCGAGAGACTGTGGCGAACGTCGTGCCGGAGGCCCGACCGAAACGGGGCA
ACCGACTGCACCGAGGTGATGGCACCAACAAAGACCGCCGCAGGACTCGAATCGGAA
TGAGCCTGCAGGGAAAGCGCGGTCGAGGTAGCGTACTCCTCTCTGCGACCGAGGGCG
CAAATCTGCGAGAGACTCGGACGCGCAACTGGAACGCACGGTGCATACGGGGAGCGA
AAGCCGGCCAGCGAGCTCCCCCAGCCGTGTGCGAAAGTGCGCGAGATCGCATTTTTG
CGCTTGTTGCCTTCGGAGTAAGCCGGGTCGCCCGCAGACCGGGCGCTCGAACACGCG
AGACGGTGACGGCGCCCCTCCGGGCTCTGCCGCGCGAACAGGGAACGTTCGTGCGC
GGAGGTGAGGGGTGCGCCCGCGAGCGGTTCGGTGCGTACGTGGCCTGCGGGCAGAA
GGTGCGGCGACTCGTCGCGAAGCGGAAAAATGTCTCCCGCTCGAGTGCGCCACGTTG
CGAAGCCTGAAGCGTTCCGTCGTAGTCCGCCGTCGGTCCAAGTGCTTCGCAGTTCCCG
TCCCGTGCAAAACTGGGCCACTCCAGTTGGGGCGGGGGCGACGCTACACGAGACGAT
GCCTCTCGCCAGGCTGCGAGTCGCCCTTGCGGCGACCGGCCTCGGCGGTGTTTGGGC
TTTCGAACACGGTCGTTTATCACGCAACTGCTCGGACGACGCACGCGTGCAGCGGGAT
ACCGCTTGCCAGCCTTGTCAAGATGCGGCCCTGTACAGGGTTGCGGGCGGACTTGGT
AGGGCGTCGTACTCGGTTCGCGATGGATTTACGAACGTGTTCCGTCACTTCCAAGTCA
CACCGGTTGTGCGCCGCACGCGCCGCAGCGGGGAAGCCGATTGCCAGCCTCGTCAAG
ATGCGGCCCTGTACAGGGTTGCGGGCGGACTTGGTAGGGCGC

>Seq2 [organism=*Rhipicephalus microplus*] [Rm2] *ITS2 internal transcribed spacer 2 ribosomal RNA* gene cds; partial sequence

TCACATATCAAGAGAGCCTTCGGCGCACAAGGGAACGTGAGCCGTCGACTCGTTTTGA
CCGCGTCGGCAACACGGACAGCACGCTGAACACCTCACAGCGAGCGCCAACAGCGGC
CACTCAAGGGCGAGACGGTGGCGACCGTCGTGCCAGAGCCCAACCGAAACGGGGGC

GACCGACTGCATTGAGGATGTGGCACCTCGTTGAGACCGCCGCAGGACTTCGAGTCG
GAAGGAAGCCTGCAGGGAAAGTGCGGTCGAGGTTGCGTACTCCTCTCTGCGACCGRG
CGCGCAAGAGCTGCGAGAGCCACGGACGCGCAACTTTAACGCACGGTAAACACGAGG
AGCGAAAGCCGGCCAGCAAAGCTTCTCCAGCCGTGCGCAAAGTGCGCGAGATCGCAG
CCTTGCGTTGCGCTTGTTGCCCTCGAAGTAAGCAGGGTGTCCCGTAGACCGGGCGCTC
GAACACGCTGCGGGGCCGTGCCTCCTCCAGGCTTTGCCGCGCGAACAGGGAACGTTC
GCGCGCAAAGCGCAGGGAGGTGAGGAGGCTGCGCCCGACGTTTGCGGTTCGCTGCG
TACGCGGTTGATGCGGGAGAGCACGGCGCGACGACTTGCCGCGAAGCGGAAAAAGTC
TCCCGCACGAGTTGGCGAAACGTTGGCGAAGCTTAAGGCGTTCTCGTCGTAGTCCGCC
GTCGGTCTAAGTGCTTCGCAGTTCCCGTCCCGTTCAAAAAACTGGGCCACTCCAGTTG
GGGCGGGGGCGACGCTACACGAGACGATGCCTCTCGCCAGGCTGCGTGGCTGCCCTT
GCGGCGGCGGCGACTGGGCCTCGGCGGTGTTTGGGCTTTCGACACGGTCGTTTATCA
CGCAACTGCTCGGACGACGCACGCGCGCAGCGGAATGCCGCTTGCCAGCCTTGTGAA
GATGTGACCCTGTACAGGGTTGCGGGCGCACTTGGTAGGGCGTCGTACTCGGTTCGC
GATGGGTTTACGAACGTGTCCCGTCACTTCCACGTCACACCGGTTGTGCGCCGCACGC
GTGCAGCGGGGAAGCCGATTGCCAGCCTTGTGAAGAAGTGGCCCTGTACAGGGTTGC
GGGCGCACTTGGTAGGGCGA

>Seq3 [organism=*Rhipicephalus microplus*] [Rm1] *ITS2 internal transcribed spacer 2 ribosomal RNA* gene cds; partial sequence

TCACATATCAAGAGAGCCTTCGGCGCACAAGGGAACGTGAGCCGTCGACTCGTTTTGA
CCGCGTCGGCAACACGGACAGCACGCTGAACACCTCACAGCGAGCGCCAACAGCGGC
CACTCAAGGGCGAGACGGTGGCGACCGTCGTGCCAGAGCCCAACCGAAACGGGGGC
GACCGACTGCATTGAGGATGTGGCACCTCGTTGAGACCGCCGCAGGACTTCGAGTCG
GAAGGAAGCCTGCAGGGAAAGTGCGGTCGAGGTTGCGTACTCCTCTCTGCGACCGGG
CGCGCAAGAGCTGCGAGAGCCACGGACGCGCAACTTTAACGCACGGTAAACACGAGG
AGCGAAAGCCGGCCAGCAAAGCTTCTCCAGCCGTGCGCAAAGTGCGCGAGATCGCAG
CCTTGCGTTGCGCTTGTTGCCCTCGAAGTAAGCAGGGTGTCCCGTAGACCGGGCGCTC
GAACACGCTGCGGGGCCGTGCCTCCTCCAGGCTTTGCCGCGCGAACAGGGAACGTTC
GCGCGCAAAGCGCAGGGAGGTGAGGAGGCTGCGCCCGACGTTTGCGGTTCGCTGCG
TACGCGGTTGATGCGGAGAGCACGGCGCGACGACTTGCCGCGAARCGGAAAAAGTCT
CCCGCACGAGTTGGCGAAACGTTGGCGAAGCTTAAGGCGTTCTCGTCGTAGTCCGCC
GTCGGTCTAAGTGCTTCGCAGTTCCCGTCCCGTTCAAAAAACTGGGCCACTCCAGTTG
GGGCGGGGGCGACGCTACACGAGACGATGCCTCTCGCCAGGCTGCGTGGCTGCCCTT
GCGGCGGCGGCGACTGGCCTCGGCGGTGTTTGGGCTTTCGACACGGTCGTTTATCAC
GCAACTGCTCGGACGACGCACGCGCGCAGCGGAATGGCCGCTTGCCAGCCTTGTGAA

GATGTGACCCTGTACAGGGTTGCGGGCGCACTTGGTAGGGCGTCGTACTCGGTTCGC
GATGGGTTTACGAACGTGTCCCGTCACTTCCACGTCACACCGGTTGTGCGCCGCACGC
GTGCAGCGGGGAAGCCGATTGCCAGCCTTGTGAAGAAGTGGCCCTGTACAGGGTTGC
GGGCGCRCTTGGTAGGGCGA

>Seq4 [organism=*Rhipicephalus appendiculatus*] [Ra1] *ITS2 internal transcribed spacer 2 ribosomal RNA* gene cds; partial sequence

TCACATATCAAGAGAGACTTCGGCGCACAAGGGAACGTGGGCCGTCGACTCGTTTTGA
CCGCGTCGGCATCATGGACAGCACGTGGAACGCAATAGCACGCGCCGACTGCCGCTC
GAAGGCGAGACGGCGGCGACCGCCGTGTCGGAGCTCGACCGAAACGGGGCGACCGA
CAGCACTGAGGTGACGGCACCATCAAAGACCGCCGCAGGACTCGAATCGGAAAGAGC
CTGCAGGGAAAGCGCGGTCGAGGTGTCCGACTCCTCTCTGCGACCGAGAGCGCGAGA
TATGCGAGAGACTCGGAAGCGCCACTTGAACGCACGGAGAGCGAAAGCCGGCCAGCT
AGCTCGAAGTGCGAAGTGCGCGTGATCGCATTTTTCTCGTGCCTGTTGCCCTCGGAGT
AAGCCGGGTTGCCCGCGTACCGGGCGCTCGAACACGCGACGGTGCTGGTGCCCCTCC
GGGCTCTGCCGCACGAACAGGGAACGTTCGCGCGCTGAGCGGGGAGGTGAGGGGCG
CGCCCGATTAAGCGGTTCGGTGCGTACGTGGTATGCGGGGAGAACTGCACGGCGACT
CGTCGCGAAGCGGAAAATGTCTCCCGCACGAGTTGGCGCCACGTTGGCGAAGCTTCA
AGCGTTCCCGTCGTAGTCCGCCGTCGGTCCAAGTGCTTCGCAGTTCCCGTCCCGTACA
AAACTGGGCCACTCCAGTTGGGGCGGGGGCGACGCTACACGAGACGATGCCTCTCGC
CAGGCCGCGAGCCGCCTTGGGGCGGCCGGCCTCGGCGGTGTTTGGGCTTATCGACA
CGGTCGTTTATCACGCAACTGCTCGGACGACGCACGCGTGCAGCGGGATACCGCTTG
CCAGCCTTGTGAAGATGTGGCCCTGTACAGGGTTGCGGGCGCACTTGGTAGGGCGTC
GTACTCGGTTCGCGATGGGTTTACGAACGTGTCCCGTCACTTCCAAGTCACACCGGTT
GTGCGCCGCACGCGCGCAGCGGGGAAGCCGATTGCCAGCCTTGTGAAGATGTGGCCC
TGTACAGGGTTGCGG

>Seq5 [organism=*Rhipicephalus sanguineus*] [SAN2] *ITS2 internal transcribed spacer 2 ribosomal RNA* gene cds; partial sequence

TCACATATCAAGAGAGACTTCGGCGCACAAGGGAACGTGAGCCGTCGACTCGTTTTGA
CCGTGTCGGCATCACGGACAGCACGTTGAACGCTACGGCAAAGCGACAACGGCCGCT
CGAAAGGCGAGAGACTGTGGCGAACGTCGTGCCGGAGGMCCGACCGAAACGGGGCA
ACCGACTGCACCGAGGTGATGGCACCAACAAAGACCGCCGCAGGACTCGAATCGGAA
TGAGCCTGCAGGGAAAGCGCGGTCGAGGTAGCGTACTCCTCTCTGCGACCGAGGGCG
CAAATCTGCGAGAGACTCGGACGCGCAACTGGAACGCACGGTGCATACGGGGAGCGA

AAGCCGGCCAGCGAGCTCCCCCAGCCGTGTGCGAAAGTGCGCGAGATCGCATTTTTG
CGCTTGTTGCCTTCGGAGTAAGCCGGGTCGCCCGCAGACCGGGCGCTCGAACACGCG
AGACGGTGACGGCGCCCCTCCGGGCTCTGCCGCGCGAACAGGGAACGTTCGTGCGC
GGAGGTGAGGGGTGCGCCCGCGAGCGGTTCGGTGCGTACGTGGCCTGCGGGCAGAA
GGTGCGGCGACTCGTCGCGAAGCGGAAAAATGTCTCCCGCTCGAGTGCGCCACGTTG
CGAAGCCTGAAGCGTTCCGTCGTAGTCCGCCGTCGGTCCAAGTGCTTCGCAGTTCCCG
TCCCGTGCAAAACTGGGCCACTCCAGTTGGGGCGGGGGCGACGCTACACGAGACGAT
GCCTCTCGCCAGGCTGCGAGTCGCCCTTGCGGCGACCGGCCTCGGCGGTGTTTGGGC
TTTCGAACACGGTCGTTTATCACGCAACTGCTCGGACGACGCACGCGTGCAGCGGGAT
ACCGCTTGCCAGCCTTGTCAAGATGCGGCCCTGTACAGGGTTGCGGGCGGACTTGGT
AGGGCGTCGTACTCGGTTCGCGATGGATTTACGAACGTGTTCCGTCACTTCCAAGTCA
CACCGGTTGTGCGCCGCACGCGCGCARCGGGGAAGCCGATTGCCARCCTCGTCAAGA
TGCGGCCCTGTACAGGGTTGCGGGCGGACTTGGTAGGGCGC

>Seq6 [organism=*Rhipicephalus evertsi evertsi*] [RE7] *ITS2 internal transcribed spacer 2 ribosomal RNA* gene cds; partial sequence

TCACATATCAAGAGAGACTTCGGGCGCACCAAGGGAACGTGGGCCGTCGACTCGTTTT
GACCGCGTCGGCATCACGGACAGCACGTTGAACGCTGCAGCAAGCGCCAACGGCCGC
TCGAAGGCGAGACGGCGGCGACCGTCGTGCCGGAGCCCGACCGAAACGGGGGCGAC
CGACTGCATTGAGGTTATGGCACCAACAGAGACCGCCGCAGGACTTTGAATCGGAAGA
AAGCCTGCAGGGAAAGTGCGGCCGAGGTTGCGGACTCCTCTCTGCGACCGGGAGCGC
GAAATCTTGCGAGAGACACGGACGCGCAACTTTTAACGCACGGTGAACACGGGGAGC
GAAAGCCGGCCAGCGAAGCTCCTCCAGCCGTGCGCGAAGTGCGCGGGATCGCATCTT
TTCTTGCGCCTGTTGCCCTCCGAGTAAGCCGGGCTGCCCGCAGACCGGGCGCTCGAA
CACGCGATGGTGCCGTGCCCCTCCGGGCTCCGCCGCACGAACAGGGAACGTTCGCGC
GCGGAGCGGGGAGGTGAGGGGCGCGCCCGATATTGCGGTTCGCTGCGTACGCGGCC
TTCGGGGAGCACTGCACGGCGACTCGCCGCGAAGCGGAGAGTGTCTCCCGCTCGAGT
TGGCGAAACGTTGGCGAAGCTTCAGGCGTTCTCGTCGTAGTCCGCCGTCGGTCCAAGT
GCTTCGCAGTTCCCGTCCCGTTCAAAAACTGGGCCACTCCAGTTGGGGCGGGGGCGA
CGCTACACGAGACGATGCCTCTCGCCAGGCTGCGGGCCGCCCTCGCGGCGGCGACC
GGCCTCGGCGGTGTTTGGGCTTTCGACACTGTCGTTTATCACGCAACTGCTCGGACGA
CGCACGCGCGCAGCGGGATACCGCTTGCCAGCCTCGTGAAGATGTGGCCCTGTACAG
GGTTGCGGGCGCACTTGGTAGGGCGTCGTACTCGGTTCGCGATGGGTTTACGAACGT
GTCCCGTCACTTCCACGTCACACCGGTTGTGCGCCGCACGCGTGCAGCGGGGAAGCC
GATTGCCAGCCTTGTGAAGATGTGGCCCTGTACAGGGTTGCGGGCGCACTTGGTAGG
GCGC

>Seq7 [organism=*Rhipicephalus evertsi evertsi*] [RE11] *ITS2 internal transcribed spacer 2 ribosomal RNA* gene cds; partial sequence

TCACATATCAAGAGAGACTTCGGCGCACAAGGGAACGTGGGCCCGTCGACTCGTTTTG
ACCGCGTCGGCATCACGGACAGCACGTTGAACGCTGCAGCAAGCGCCAACGGCCGCT
CGAAGGCGAGACGGCGGCGACCGTCGTGCCGGAGCCCGACCGAAACGGGGGGCGAC
CGACTGCATTGAGGTTATGGCACCAACAGAGACCGCCGCAGGACTTTGAATCGGAAGA
AAGCCTGCAGGGAAAGTGCGGCCGAGGTTGCGGACTCCTCTCTGCGACCGGGAGCGC
GAAATCTTGCGAGAGACACGGACGCGCAACTTTTAACGCACGGTGAACACGGGGAGC
GAAAGCCGGCCAGCGAAGCTCCTCCAGCCGTGCGCGAAGTGCGCGGGATCGCATCTT
TTCTTGCGCCTGTTGCCCTCCGAGTAAGCCGGGCTGCCCGCAGACCGGGCGCTCGAA
CACGCGATGGTGCCGTGCCCCTCCGGGCTCCGCCGCACGAACAGGGAACGTTCGCGC
GCGGAGCGGGGAGGTGAGGGGCGCGCCCGATATTGCGGTTCGCTGCGTACGCGGCC
TTCGGGGAGCACTGCACGGCGACTCGCCGCGAAGCGGAGAGTGTCTCCCGCTCGAGT
TGGCGAAACGTTGGCGAAGCTTCAGGCGTTCTCGTCGTAGTCCGCCGTCGGTCCAAGT
GCTTCGCAGTTCCCGTCCCGTTCAAAAACTGGGCCACTCCAGTTGGGGCGGGGGCGA
CGCTACACGAGACGATGCCTCTCGCCAGGCTGCGGGCCGCCCTCGCGGCGGCGACC
GGCCTCGGCGGTGTTTGGGCTTTCGACACTGTCGTTTATCACGCAACTGCTCGGACGA
CGCACGCGCGCAGCGGGATACCGCTTGCCAGCCTCGTGAAGATGTGGCCCTGTACAG
GGTTGCGGGCGCACTTGGTAGGGCGTCGTACTCGGTTCGCGATGGGTTTACGAACGT
GTCCCGTCACTTCCACGTCACACCGGTTGTGCGCCGCACGCGTGCAGCGGGGAAGCC
GATTGCCAGCCTTGGTGAAGATGTGGCCCTGTACAGGGTTGCGGGCGCACTTGGTAG
GGCGC

>Seq8 [organism=*Rhipicephalus evertsi evertsi*] [RE2] *ITS2 internal transcribed spacer 2 ribosomal RNA* gene cds; partial sequence

TCACATATCAAGAGAGACTTCGGCGCACAAGGGAACGTGGGCCGTCGACTCGTTTTGA
CCGCGTCGGCATCACGGACAGCACGTTGAACGCTGCAGCAAGCGCCAACGGCCGCTC
GAAGGCGAGACGGCGGCGACCGTCGTGCCGGAGCCCGACCGAAACGGGGGGCGACC
GACTGCATTGAGGTTATGGCACCAACAGAGACCGCCGCAGGACTTTGAATCGGAAGAA
AGCCTGCAGGGAAAGTGCGGCCGAGGTTGCGGACTCCTCTCTGCGACCGGGAGCGC
GAAATCTTGCGAGAGACACGGACGCGCAACTTTTAACGCACGGTGAACACGGGGAGC
GAAAGCCGGCCAGCGAAGCTCCTCCAGCCGTGCGCGAAGTGCGCGGGATCGCATCTT
TTCTTGCGCCTGTTGCCCTCCGAGTAAGCCGGGCTGCCCGCAGACCGGGCGCTCGAA
CACGCGATGGTGCCGTGCCCCTCCGGGCTCCGCCGCACGAACAGGGAACGTTCGCGC

GCGGAGCGGGGAGGTGAGGGGCGCGCCCGATATTGCGGTTCGCTGCGTACGCGGCC
TTCGGGGAGCACTGCACGGCGACTCGCCGCGAAGCGGAGARTGTCTCCCGCTCGAGT
TGGCGAAACGTTGGCGAAGCTTCAGGCGTTCTCGTCGTAGTCCGCCGTCGGTCCAAGT
GCTTCGCAGTTCCCGTCCCGTTCAAAAACTGGGCCACTCCAGTTGGGGCGGGGGCGA
CGCTACACGAGACGATGCCTCTCGCCAGGCTGCGGGCCGCCCTCGCGGCGGCGACC
GGCCTCGGCGGTGTTTGGGCTTTCGACACTGTCGTTTATCACGCAACTGCTCGGACGA
CGCACGCGCGCAGCGGGATACCGCTTGCCAGCCTCGTGAAGATGTGGCCCTGTACAG
GGTTGCGGGCGCACTTGGTAGGGCGTCGTACTCGGTTCGCGATGGGTTTACGAACGT
GTCCCGTCACTTCCACGTCACACCGGTTGTGCGCCGCACGCGTGCAGCGGGGAAGCC
GATTGCCAGCCTTGTGAAGATGTGGCCCTGTACAGGGTTGCGGGCGCACTTGGTAGG
GGCGC

>Seq9 [organism=*Rhipicephalus evertsi evertsi*] [h6] *ITS2 internal transcribed spacer 2 ribosomal RNA gene* cds; partial sequence

TCACATATCAAGAGAGACTTCGGCGCACAAGGGAACGTGGGCCGTCGACTCGTTTTGA
CCGCGTCGGCATCACGGACAGCACGTTGAACGCTGCAGCAAGCGCCAACGGCCGCTC
GAAGGCGAGACGGCGGCGACCGTCGTGCCGGAGCCCGACCGAAACGGGGGCGACCG
ACTGCATTGAGGTTATGGCACCAACAGAGACCGCCGCAGGACTTTGAATCGGAAGAAA
GCCTGCAGGGAAAGTGCGGCCGAGGTTGCGGACTCCTCTCTGCGACCGGGAGCGCG
AAATCTTGCGAGAGACACGGACGCGCAACTTTTAACGCACGGTGAACACGGGGAGCGA
AAGCCGGCCAGCGAAGCTCCTCCAGCCGTGCGCGAAGTGCGCGGGATCGCATCTTTT
CTTGCGCCTGTTGCCCTCCGAGTAAGCCGGGCTGCCCGCAGACCGGGCGCTCGAACA
CGCGATGGTGCCGTGCCCCTCCGGGCTCCGCCGCACGAACAGGGAACGTTCGCGCG
CGGAGCGGGGAGGTGAGGGGCGCGCCCGATATTGCGGTTCGCTGCGTACGCGGCCT
TCGGGGAGCACTGCACGGCGACTCGCCGCGAAGCGGAGAGTGTCTCCCGCTCGAGTT
GGCGAAACGTTGGCGAAGCTTCAGGCGTTCTCGTCGTAGTCCGCCGTCGGTCCAAGT
GCTTCGCAGTTCCCGTCCCGTTCAAAAACTGGGCCACTCCAGTTGGGGCGGGGGCGA
CGCTACACGAGACGATGCCTCTCGCCAGGCTGCGGGCCGCCCTCGCGGCGGCGACC
GGCCTCGGCGGTGTTTGGGCTTTCGACACTGTCGTTTATCACGCAACTGCTCGGACGA
CGCACGCGCGCAGCGGGATACCGCTTGCCAGCCTCGTGAAGATGTGGCCCTGTACAG
GGTTGCGGGCGCACTTGGTAGGGCGTCGTACTCGGTTCGCGATGGGTTTACGAACGT
GTCCCGTCACTTCCACGTCACACCGGTTGTGCGCCGCACGCGTGCAGCGGGGAAGCC
GATTGCCAGCCTTGTGAAGATGTGGCCCTGTACAGGGTTGCGGGCGCACTTGGTAGG
GCCGC

>Seq10 [organism=*Rhipicephalus evertsi evertsi*] [h7] *ITS2 internal transcribed spacer 2 ribosomal RNA* gene cds; partial sequence

TCACATATCAAGAGAGACTTCGGCGCACCAAGGGAACGTGGGCCGTCGACTCGTTTTG
ACCGCGTCGGCATCACGGACAGCACGTTGAACGCTGCAGCAAGCGCCAACGGCCGCT
CGAAGGCGAGACGGCGGCGACCGTCGTGCCGGAGCCCGACCGAAACGGGGGCGACC
GACTGCATTGAGGTTATGGCACCAACAGAGACCGCCGCAGGACTTTGAATCGGAAGAA
AGCCTGCAGGGAAAGTGCGGCCGAGGTTGCGGACTCCTCTCTGCGACCGGGAGCGC
GAAATCTTGCGAGAGACACGGACGCGCAACTTTTAACGCACGGTGAACACGGGGAGC
GAAAGCCGGCCCAGCGAAGCTCCTCCAGCCGTGCGCGAAGTGCGCGGGATCGCATCT
TTTCTTGCGCCTGTTGCCCTCCGAGTAAGCCGGGCTGCCCGCAGACCGGGCGCTCGA
ACACGCGATGGTGCCGTGCCCCTCCGGGCTCCGCCGCACGAACAGGGAACGTTCGCG
CGCGGAGCGGGGAGGTGAGGGGCGCGCCCGATATTGCGGTTCGCTGCGTACGCGGC
CTTCGGGGAGCACTGCACGGCGACTCGCCGCGAAGCGGAGAGTGTCTCCCGCTCGAG
TTGGCGAAACGTTGGCGAAGCTTCAGGCGTTCTCGTCGTAGTCCGCCGTCGGTCCAAG
TGCTTCGCAGTTCCCGTCCCGTTCAAAAACTGGGCCACTCCAGTTGGGGCGGGGGCG
ACGCTACACGAGACGATGCCTCTCGCCAGGCTGCGGGCCGCCCTCGCGGCGGCGAC
CGGCCTCGGCGGTGTTTGGGCTTTCGACACTGTCGTTTATCACGCAACTGCTCGGACG
ACGCACGCGCGCAGCGGGATACCGCTTGCCAGCCTCGTGAAGATGTGGCCCTGTACA
GGGTTGCGGGCGCACTTGGTAGGGCGTCGTACTCGGTTCGCGATGGGTTTACGAACG
TGTCCCGTCACTTCCACGTCACACCGGTTGTGCGCCGCACGCGTGCAGCGGGGAAGC
CGATTGCCAGCCTTGTGAAGATGTGGCCCTGTACAGGGTTGCGGGCGCACTTGGTAG
GGCGC

>Seq11 [organism=*Rhipicephalus evertsi evertsi*] [h9] *ITS2 internal transcribed spacer 2 ribosomal RNA* gene cds; partial sequence

TCACATATCAAGAGAGACTTCGGCGCAACAAGGGAACGTGGGCCGTCGACTCGTTTTG
ACCGCGTCGGCATCACGGACAGCACGTTGAACGCTGCAGCAAGCGCCAACGGCCGCT
CGAAGGCGAGACGGCGGCGACCGTCGTGCCGGAGCCCGACCGAAACGGGGGCGACC
GACTGCATTGAGGTTATGGCACCAACAGAGACCGCCGCAGGACTTTGAATCGGAAGAA
AGCCTGCAGGGAAAGTGCGGCCGAGGTTGCGGACTCCTCTCTGCGAACCGGGAGCGC
GAAATCTTGCGAGAGACACGGACGCGCAACTTTTAACGCACGGTGAACACGGGGAGC
GAAAGCCGGCCAGCGAAGCTCCTCCAGCCGTGCGCGAAGTGCGCGGGATCGCATCTT
TTCTTGCGCCTGTTGCCCTCCGAGTAAGCCGGGCTGCCCGCAGACCGGGCGCTCGAA
CACGCGATGGTGCCGTGCCCCTCCGGGCTCCGCCGCACGAACAGGGAACGTTCGCGC
GCGGAGCGGGGAGGTGAGGGGCGCGCCCGATATTGCGGTTCGCTGCGTACGCGGCC

TTCGGGGAGCACTGCACGGCGACTCGCCGCGAAGCGGAGAGTGTCTCCCGCTCGAGT
TGGCGAAACGTTGGCGAAGCTTCAGGCGTTCTCGTCGTAGTCCGCCGTCGGTCCAAGT
GCTTCGCAGTTCCCGTCCCGTTCAAAAACTGGGCCACTCCAGTTGGGGCGGGGGCGA
CGCTACACGAGACGATGCCTCTCGCCAGGCTGCGGGCCGCCCTCGCGGCGGCGACC
GGCCTCGGCGGTGTTTGGGCTTTCGACACTGTCGTTTATCACGCAACTGCTCGGACGA
CGCACGCGCGCAGCGGGATACCGCTTGCCAGCCTCGTGAAGATGTGGCCCTGTACAG
GGTTGCGGGCGCACTTGGTAGGGCGTCGTACTCGGTTCGCGATGGGTTTACGAACGT
GTCCCGTCACTTCCACGTCACACCGGTTGTGCGCCGCACGCGTGCAGCGGGGAAGCC
GATTGCCAGCCTTGTGAAGATGTGGCCCTGTACAGGGTTGCGGGCGCACTGTGGTAG
GGCGC

>Seq12 [organism=*Rhipicephalus evertsi evertsi*] [R1] *ITS2 internal transcribed spacer 2 ribosomal RNA* gene cds; partial sequence

TCACATATCAAGAGAGACTTCGGCGCACAAGGGAACGTGGGCCGTCGACTCGTTTTGA
CCGCGTCGGCATCACGGACAGCACGTTGAACGCTGCAGCAAGCGCCAACGGCCGCTC
GAAGGCGAGACGGCGGCGACCGTCGTGCCGGAGCCCGACCGAAACGGGGGCGACCG
ACTGCATTGAGGTTATGGCACCAACAGAGACCGCCGCAGGACTTTGAATCGGAAGAAA
GCCTGCAGGGAAAGTGCGGCCGAGGTTGCGGACTCCTCTCTGCGACCGGGAGCGCG
AAATCTTGCGAGAGACACGGACGCGCAACTTTTAACGCACGGTGAACACGGGGAGCGA
AAGCCGGCCAGCGAAGCTCCTCCAGCCGTGCGCGAAGTGCGCGGGATCGCATCTTTT
CTTGCGCCTGTTGCCCTCCGAGTAAGCCGGGCTGCCCGCAGACCGGGCGCTCGAACA
CGCGATGGTGCCGTGCCCCTCCGGGCTCCGCCGCACGAACAGGGAACGTTCGCGCG
CGGAGCGGGGAGGTGAGGGGCGCGCCCGATATTGCGGTTCGCTGCGTACGCGGCCT
TCGGGGAGCACTGCACGGCGACTCGCCGCGAAGCGGAGAGTGTCTCCCGCTCGAGTT
GGCGAAACGTTGGCGAAGCTTCAGGCGTTCTCGTCGTAGTCCGCCGTCGGTCCAAGT
GCTTCGCAGTTCCCGTCCCGTTCAAAAACTGGGCCACTCCAGTTGGGGCGGGGGCGA
CGCTACACGAGACGATGCCTCTCGCCAGGCTGCGGGCCGCCCTCGCGGCGGCGACC
GGCCTCGGCGGTGTTTGGGCTTTCGACACTGTCGTTTATCACGCAACTGCTCGGACGA
CGCACGCGCGCAGCGGGATACCGCTTGCCAGCCTCGTGAAGATGTGGCCCTGTACAG
GGTTGCGGGCGCACTTGGTAGGGCGTCGTACTCGGTTCGCGATGGGGTTTACGAACG
TGTCCCGTCACTTCCACGTCACACCGGTTGTGCGCCGCACGCGTGCAGCGGGGAAGC
CGATTGCCAGCCTTGTGAAGATGTGGCCCTGTACAGGGTTGCGGGGCGCWCTTGGTA
GGGCGC

>Seq13 [organism=*Rhipicephalus evertsi evertsi*] [R2] *ITS2 internal transcribed spacer 2 ribosomal RNA* gene cds; partial sequence

TCACATATCAAGAGAGACTTCGGCGCACAAGGGAACGTGGGCCGTCGACTCGTTTTGA
CCGCGTCGGCATCACGGACAGCACGTTGAACGCTGCAGCAAGCGCCAACGGCCGCTC
GAAGGCGAGACGGCGGCGACCGTCGTGCCGGAGCCCGACCGAAACGGGGGCGACCG
ACTGCATTGAGGTTATGGCACCAACAGAGACCGCCGCAGGACTTTGAATCGGAAGAAA
GCCTGCAGGGAAAGTGCGGCCGAGGTTGCGGACTCCTCTCTGCGACCGGGAGCGCG
AAATCTTGCGAGAGACACGGACGCGCAACTTTTAACGCACGGTGAACACGGGGAGCGA
AAGCCGGCCAGCGAAGCTCCTCCAGCCGTGCGCGAAGTGCGCGGGATCGCATCTTTT
CTTGCGCCTGTTGCCCTCCGAGTAAGCCGGGCTGCCCGCAGACCGGGCGCTCGAACA
CGCGATGGTGCCGTGCCCCTCCGGGCTCCGCCGCACGAACAGGGAACGTTCGCGCG
CGGAGCGGGGAGGTGAGGGGCGCGCCCGATATTGCGGTTCGCTGCGTACGCGGCCT
TCGGGGAGCACTGCACGGCGACTCGCCGCGAAGCGGAGAGTGTCTCCCGCTCGAGTT
GGCGAAACGTTGGCGAAGCTTCAGGCGTTCTCGTCGTAGTCCGCCGTCGGTCCAAGT
GCTTCGCAGTTCCCGTCCCGTTCAAAAACTGGGCCACTCCAGTTGGGGCGGGGGCGA
CGCTACACGAGACGATGCCTCTCGCCAGGCTGCGGGCCGCCCTCGCGGCGGCGACC
GGCCTCGGCGGTGTTTNGGGCTTTCGACACTGTCGTTTATCACGCAACTGCTCGGACG
ACGCACGCGCGCAGCGGGATACCGCTTGCCAGCCTCGTGAAGATGTGGCCCTGTACA
GGGTTGCGGGCGCACTTGGTAGGGCGTCGTACTCGGTTCGCGATGGGTTTACGAACG
TGTTCCCGTCACTTTCCACGTCACACCGGTTGTGGCGCCGCACGCGTGCAGCGGGGA
AGCCGATTGCCAGCCTTGTGAAGATTGTGGCCCTGTACAGGGTTGCGGGCGCACTTGG
TAGGGCGC

>Seq14 [organism=*Rhipicephalus evertsi evertsi*] [R3] *ITS2 internal transcribed spacer 2 ribosomal RNA* gene cds; partial sequence

TCACATATCAAGAGAGACTTCGGCGCACAAAGGGAACGTGGGCCGTCGACTCGTTTTG
ACCGCGTCGGCATCACGGACAGCACGTTGAACGCTGCAGCAAGCGCCAACGGCCGCT
CGAAGGCGAGACGGCGGCGACCGTCGTGCCGGAGCCCGACCGAAACGGGGGGCGAC
CGACTGCATTGAGGTTATGGCACCAACAGAGACCGCCGCAGGACTTTGAATCGGAAGA
AAGCCTGCAGGGAAAGTGCGGCCGAGGTTGCGGACTCCTCTCTGCGACCGGGAGCGC
GAAATCTTGCGAGAGACACGGACGCGCAACTTTTAACGCACGGTGAACACGGGGAGC
GAAAGCCGGCCAGCGAAGCTCCTCCAGCCGTGCGCGAAGTGCGCGGGATCGCATCTT
TTCTTGCGCCTGTTGCCCTCCGAGTAAGCCGGGCTGCCCGCAGACCGGGCGCTCGAA
CACGCGATGGTGCCGTGCCCCTCCGGGCTCCGCCGCACGAACAGGGAACGTTCGCGC
GCGGAGCGGGGAGGTGAGGGGCGCGCCCGATATTGCGGTTCGCTGCGTACGCGGCC

TTCGGGGAGCACTGCACGGCGACTCGCCGCGAAGCGGAGAGTGTCTCCCGCTCGAGT
TGGCGAAACGTTGGCGAAGCTTCAGGCGTTCTCGTCGTAGTCCGCCGTCGGTCCAAGT
GCTTCGCAGTTCCCGTCCCGTTCAAAAACTGGGCCACTCCAGTTGGGGCGGGGGCGA
CGCTACACGAGACGATGCCTCTCGCCAGGCTGCGGGCCGCCCTCGCGGCGGCGACC
GGCCTCGGCGGTGTTTGGGCTTTCGACACTGTCGTTTATCACGCAACTGCTCGGACGA
CGCACGCGCGCAGCGGGATACCGCTTGCCAGCCTCGTGAAGATGTGGCCCTGTACAG
GGTTGCGGGCGCACTTGGTAGGGCGTCGTACTCGGTTCGCGATGGGTTTACGAACGT
GTCCCGTCACTTCCACGTCACACCGGTTGTGCGCCGCACGCGTGCAGCGGGGAAGCC
GATTGCCAGCCTTGTGAAGATGTGGCCCTGTACAGGGTTGCGGGCGCACTTGGTAGG
GCGC

>Seq15 [organism=*Rhipicephalus evertsi evertsi*] [gmf3*] ITS2 internal transcribed spacer 2 ribosomal RNA* gene cds; partial sequence

TCACATATCAAGAGAGACTTCGGCGCACAAGGGAACGTGGGCCGTCGACTCGTTTTGA
CCGCGTCGGCATCACGGACAGCACGTTGAACGCTGCAGCAAGCGCCAACGGCCGCTC
GAAGGCGAGACGGTGGCGACCGTCGTGCCGGAGCCCGACCGAAACGGGGGCGACCG
ACTGCATTGAGGTTATGGCACCAACAGAGACCGCCGCAGGACTTTGAATCGGAAGAAA
GCCTGCAGGGAAAGTGCGGCCGAGGTTGCGGACTCCTCTCTGCGACCGGGAGCGCG
AAATCTTGCGAGAGACACGGACGCGCAACTTTTAACGCACGGTGAACACGGGGAGCGA
AAGCCGGCCAGCGAAGCTCCTCCAGCCGTGCGCGAAGTGCGCGGGATCGCATCTTTT
CTTGCGCCTGTTGCCCTCCGAGTAAGCCGGGCTGCCCGCAGACCGGGCGCTCGAACA
CGCGATGGTGCCGTGCCCCTCCGGGCTCCGCCGCACGAACAGGGAACGTTCGCGCG
CGGAGCGGGGAGGTGAGGGGCGCGCCCGATATTGCGGTTCGCTGCGTACGCGGCCT
TCGGGGAGCACTGCACGGCGACTCGCCGCGAAGCGGAGARTGTCTCCCGCTCGAGTT
GGCGAAACGTTGGCGAAGCTTCAGGCGTTCTCGTCGTAGTCCGCCGTCGGGTCCAAG
TGCTTCGCAGTTCCCGTCCCGTTCAAAAACTGGGCCACTCCAGTTGGGGCGGGGGCG
ACGCTACACGAGACGATGCCTCTCGCCAGGCTGCGGGCCGCCCTCGCGGCGGCGAC
CGGCCTCGGCGGTGTTTGGGCTTTCGACACTGTCGTTTATCACGCAACTGCTCGGACG
ACGCACGCGCGCAGCGGGATACCGCTTGCCAGCCTTGTGAAGATGTGGCCCTGTACA
GGGTTGCGGGCGCACTTGGTAGGGCGTCGTACTCGGTTCGCGATGGGTTTACGAACG
TGTCCCGTCACTTCCACGTCACACCGGTTGTGCGCCGCACGCGTGCAGCGGGGAAGC
CGATTKCCAGCCTTGTGAAGATGTGGCCCTGTACAGGGTTGCGG

8.10. Tick identification: *18S ribosomal RNA* gene sequences

>Seq1 [organism=*Rhipicephalus evertsi evertsi]* [RE7] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

CATTATGGTTCCTTAGATCGTTTCTTCCTACTTGGATAACTGTGGCAATTCTAGAGCTAA
TACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCGA
TCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGG-
AATACCGAGGGTAATGATTAAGAGGGACGGACGGGGGCATTCGA

>Seq2 [organism=*Rhipicephalus evertsi evertsi*] [gmf1] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

AGTTATGGTTCCTTAGATCGTTTCTTCCTACTTGGATAAACTGTGGGCCAATTCTAGAGC
TAATACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGAT
CGATCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATG
GCCACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTT
ACTTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGG
AGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTC
CCGGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTA
ATTGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTG
CCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAA
GCTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCG
GACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCG
GTGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTT
GCATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAA
TTTGATTAAGAGGGACGGACGGGGGCATTCGT

>Seq3 [organism=*Rhipicephalus evertsi evertsi*] [gmf3] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

AGTTATGGTTCCTTAGATCGTTTCTTCCTACTTTGGATAACTGTGGCAATTCTAGAGCTA
ATACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCG
ATCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGGTAA
TGATTAAGAGGGACGGACGGGGGCATTCGT

>Seq4 [organism=*Rhipicephalus evertsi evertsi*] [gmm3] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

AGTTATGGTTCCTTAGATCGTTTCTTCCTACTTGGATAACTGTGGCCAATTCTAGAGCTA
ATACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCG
ATCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAAT
GATTAAGAGGGACGGACGGGGGCATTCGT

>Seq5 [organism=*Rhipicephalus sanguineus*] [SAN2] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

AGTTATGGTTCCTTAGATCGTTTCTTCCTACTTGGATAACTGTGGCAATTCTAGAGCTAA
TACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCGA
TCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAAT
GATTAA-GAGGGACGGACGGGGGCATTCTA

>Seq6 [organism=*Rhipicephalus sanguineus*] [SAN3] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

AGTTATGGTTCCTTAGATCGTTTCTTCCTACTTGGATAACTGTGGCAATTCTAGAGCTAA
TACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCGA
TCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAAT
GATTAAGAGGGACGGACGGGGGCATTCTA

>Seq7 [organism=*Rhipicephalus evertsi evertsi*] [RE11] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

CATTATGGTTCCTTAGATCGTTTCTTCCTACTTGGATAACTGTGGCAATTCTAGAGCTAA
TACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCGA
TCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAAA
TGATTAAGAGGGACGGACGGGGGCATTCGA

>Seq8 [organism=*Rhipicephalus evertsi evertsi*] [RE15] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

CATTATGGTTCCTTAGATCGTTTCTTCCTACTTGGGATAACTGTGGCAATTCTAGAGCTA
ATACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCG
ATCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAAA
TGATTAAGAGGGACGGACGGGGGCATTCGA

>Seq9 [organism=*Rhipicephalus evertsi evertsi*] [h6] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

CATTATGGTTCCTTAGATCGTTTCTTCCTACCTTTGGGATAACTGTGGCAATTCTAGAGC
TAATACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGAT
CGATCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATG
GCCACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTT
ACTTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGG
AGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTC
CCGGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTA
ATTGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTG
CCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAA
GCTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCG
GACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCG
GTGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTT
GCATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAA
TGATTAA-GAGGGACGGACGGGGGCATTCGA

>Seq10 [organism=*Rhipicephalus evertsi evertsi*] [h7] *18S ribosomal RNA gene* cds; partial sequence; mitochondrial

CATATGGTTCCTTAGATCGTTTCTTCCTACTTGGATAACTGTGGCAATTCTAGAGCTAAT
ACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCGAT
CGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGCC
ACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTACT
TGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAGC
CTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCG
GCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAATT
GAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCA
GCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAGCT
CGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGGAC
TGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGGTG
CTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTGCA
TGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAAATG
ATTAAAAGGGACGGACGGGGGCATTCGA

>Seq11 [organism=*Rhipicephalus evertsi evertsi*] [h9] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

CCATATGGTTCCTTAGATCGTTTCTTCCTACTTGGGATAACTGTGGCAATTCTAGAGCTA
ATACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCG
ATCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAAT
GATTAAGAGGGACGGACGGGGGCATTCGA

>Seq12 [organism=*Rhipicephalusevertsi evertsi*] [Re1] *18S ribosomal RNA gene* cds; partial sequence; mitochondrial

AGTTATGGTTCCTTAGATCGTTTCTTCCCTACTTGGGATAACTGTGGCAATTCTAGAGCT
AATACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATC
GATCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATG
GCCACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTT
ACTTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGG
AGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTC
CCGGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTA
ATTGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTG
CCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAA
GCTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCG
GACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCG
GTGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTT
GCATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAA
TGATTAAGAGGGACGGACGGGGGCATTCGA

>Seq13 [organism=*Rhipicephalus microplus*] [Rm1] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

AGTTATGGTTCCTTAGATCGTTTCTTCCTACTTGGATAACTGTGGCAATTCTAGAGCTAA
TACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCGA
TCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAAT
GATTAAAGAGGGACGGACGGGGGCATTCGA

>Seq14 [organism=*Rhipicephalus microplus*] [Rm2] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

AGTTATGGTTCCTTAGATCGTTTCTTCCTACTTGGATAACTGTGGCAATTCTAGAGCTAA
TACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCGA
TCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAAT
GATTAAGAGGGACGGACGGGGGCATTCGA

>Seq15 [organism=*Rhipicephalus appendiculatus*] [Rp1] *18S ribosomal RNA gene* cds; partial sequence; mitochondrial

AGTTATGGTTCCTTAGATCGTTTCTTCCTACTTGGATAACTGTGGCAATTCTAGAGCTAA
TACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCGA
TCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAAT
GATTAAGAGGGACGGACGGGGGCATTCGT

>Seq16 [organism=*Rhipicephalus sanguineus*] [S3] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

AGTTATGGTTCCTTAGATCGTTTCTTCCTACTTGGATAACTGTGGCAATTCTAGAGCTAA
TACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCGA
TCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAAT
GATTAA-GAGGGACGGACGGGGGCATTCGA

>Seq17 [organism=*Rhipicephalus evertsi evertsi*] [R1C] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

CGTTATGGTTCCTTAGATCGTTTCTTCCTACTTGGATAACTGTGGCAATTCTAGAGCTAA
TACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCGA
TCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGGTAA
TGATTAAAGAGGGACGGACGGGGGCATTCGA

>Seq18 [organism=*Rhipicephalus evertsi evertsi*] [R2C] *18S ribosomal RNA* gene cds; partial sequence; mitochondrial

AGTTATGGTTCCTTAGATCGTTTCTTCCTACTTGGATAACTGTGGCAATTCTAGAGCTAA
TACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCGA
TCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAAT
GATTAAGAGGGACGGACGGGGGCATTCGA

>Seq19 [organism=*Rhipicephalus evertsi evertsi*] [R3C] 18S ribosomal RNA gene cds; partial sequence; mitochondrial

AGTTATGGTTCCTTAGATCGTTTCTTCCTACTTGGATAACCTGTGGCAATTCTAGAGCTA
ATACATGCAGTGAGCCTGGAGCCCTTTGGGTAACGGGTGCTTTTATTAGACCAAGATCG
ATCGGGTTTCGGCCCGTATTGTGTGGTGACTCTGGATAACTTTGTGCTGATCGCATGGC
CACGAGCCGGCGACGTTTCTTTCAAGTGTCTGCCTTATCAACTTTCGATGGTAGGTTAC
TTGCTTACCATGGTTGTTACGGGTAACGGAGAATCAGGGTTCGATTCCGGAGAGGGAG
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GGCACGGGGAGGTAGTGACGAAAAATAACAATACGGGACTCTTTTGAGGCCCCGTAAT
TGAAATGAGTACACTCTAAATCCTTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGCTGCTGCGGTTAAAAAG
CTCGTAGTTGGATCTCAGTTCCAGACGAGTAGTGCATCTACCCGATGCGACGGCTCGG
ACTGAACATCATGCCGGTTCTTTCTTGGTGCACTTCATTGTGTGCCTCGAGATGGCCGG
TGCTTTTACTTTGAAAAAATTAGAGTGCTCAACGCAGGCGAGTCGCCTGAATAAACTTG
CATGGAATAATAGAACAAGACCTCGTTTCTGTTCTGTTGGTTTTTGGAATACGAGGTAAT
GATTAAGAGGGACGGACGGGGGCATTCGA