

Genetic characterization of ticks and tick-borne prokaryotic pathogens of urban stray dogs in Potchefstroom, South Africa

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DEDICATION

This thesis is dedicated to my parents, Anita and Willie, and my grandparents, Elizabeth and Henry. For their inspiration and support.

“We are all scientists, trying to make sense of the stars inside us.”

-Christopher Poindexter

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ABSTRACT

Ticks are well documented for their ability to transmit pathogenic organisms of medical, veterinary and economical importance mostly to a variety of animal hosts and humans. Ticks harbour various members of bacteria, archaea, and eubacteria and several eukaryotic organisms, including fungi, nematodes, and protozoa, collectively referred to as the microbiome. Associations between the ticks and the microbiota affect several aspects of the ticks such as their vectorial competence, survival, and reproductive fitness. The bacterial composition of ticks varies due to a lot of factors, including but not limited to the tick's life cycle, host ecology, and the absence or presence of bacterial pathogens. Therefore, this study aimed at conducting a systematic review focusing on the global prevalence of *R. sanguineus* and *H. elliptica*, as well as the tick-borne pathogens they harbour. This study also sought to identify the tick species and tick-borne pathogens infesting dogs collected from the Potchefstroom Animal Welfare Society (PAWS). Lastly this study aimed to identify the bacterial communities of dog blood and ticks from the PAWS in comparison to colony ticks from gnotobiotic hosts.

For the systematic review, the Scopus, ScienceDirect, PubMed, and Web of Science databases were searched for English journal articles which were assessed by following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. This systematic review was registered on PROSPERO under the following ID: CRD42022327372. Of the studies included in the systematic review, 247 and 19 articles collected and identified *R. sanguineus* and *H. elliptica* respectively, whereas 15 articles managed to collect and identify both tick species. In total 121 articles were included in the systematic review that reported the presence of haemoparasite infections within *R. sanguineus* or *H. elliptica* ticks. Respectively, 119 and 2 of the aforementioned articles reported the presence of tick-borne pathogens within *R. sanguineus* and *H. elliptica* ticks respectively. In addition, only 1 article reported the presence of tick-borne pathogens within both *R. sanguineus* and *H. elliptica* ticks. The prevalence of the haemopathogens was similar to the prevalence of their tick hosts, thus indicating the potential of tick-borne pathogens to spread and inhabit new niches. Articles included in the systematic review reported the presence of *R. sanguineus* and *H. elliptica* ticks collected from companion animals, vegetation, livestock, humans, as well as several wildlife species. The tick species identification method in the included articles of the systematic review was done employing morphological identification (n=222), as well as a combination of morphological identification and conventional PCR (n=29). Articles that detected tick-borne pathogens within *R. sanguineus* used molecular techniques (n=139) as well as morphological techniques (n=5). Articles that detected tick-borne pathogens within *H. elliptica* used molecular techniques (n=2). There were 64 countries that

reported the presence of *R. sanguineus*, whereas *H. elliptica* was only reported in 6 countries. It is considered that *R. sanguineus* has a global prevalence, mainly in regions that are tropical or subtropical. *H. elliptica* was reported in South Africa and countries with close proximity to South Africa. The close proximity between ticks and travelling hosts or migratory hosts (such as birds), is one of the main contributors to the global prevalence of *R. sanguineus* and the prevalence of *H. elliptica* in the neighbouring countries of South Africa.

In this study tick specimens (n=300) and blood (n=50) were collected from naturally infected dog hosts (n=50) residing at the PAWS located in Potchefstroom of the North West Province, South Africa. In addition, eggs were also collected (n=6 egg batches) from ticks that laid eggs after collection. Tick specimens were morphologically identified and genetically using PCR *CO1* and *18S rRNA* assays, whilst genus or species specific PCR assays were performed for detection of various bacterial pathogens of veterinary importance from tick pools (n=81), egg batches (n=6), and dog blood (n=50). The PCR amplicons were Sanger sequenced and aligned using the nucleotide Basic Local Alignment Search Tool (BLASTn) for confirmation of detected pathogen identity. In this study, two tick species were identified namely *R. sanguineus* with an overall abundance of 70.7% (212/300) and *H. elliptica* with an overall abundance of 29.3% (88/300) based on morphological key features and DNA analysis. For the *CO1* gene all of the tick specimens morphologically identified as *H. elliptica* were 96.89 to 97.74% similar to *H. elliptica* gene sequences on the NCBI database. On the other hand, the tick species morphologically identified as *R. sanguineus* were 97.20 to 99.84% similar to *R. sanguineus* gene sequences on the NCBI database. For the *18S rRNA* gene all of the tick specimens morphologically identified as *H. elliptica* were 99.26 to 99.63% similar to *H. elliptica* gene sequences on the NCBI database. On the other hand, the tick species morphologically identified as *R. sanguineus* were 98.53 to 99.90% similar to *R. sanguineus* gene sequences on the NCBI database. None of *E. canis* or *A. phagocytophylum* were detected by PCR from DNA of ticks, egg batches, or dog blood samples from this study. The overall occurrence of *C. burnetii* within pooled tick DNAs was 9.88% (8/81). The *C. burnetii* were harboured by 8.82% (3/34) of *H. elliptica* ticks and 10.64% (5/47) of *R. sanguineus* ticks. The overall occurrence of *C. burnetii* within egg batches was 16.67% (1/6). The *C. burnetii* was present in 25% (1/4) *H. elliptica* egg batches. One of the PCR products was successfully sequenced and BLASTn search results revealed that the aforementioned gene sequence was 96.89 to 100% similar to *C. burnetii* sequences on the NCBI database. The overall occurrence of *Rickettsia* spp. within tick pools was 6.17% (5/81). *Rickettsia* spp. were harboured by 11.76% (4/34) of *H. elliptica* ticks and 2.13% (1/47) of *R. sanguineus* ticks. The overall occurrence of *Rickettsia* spp. within egg batches was 16.67% (1/6). *Rickettsia* spp. were present in 25% (1/4) *H. elliptica* egg batches. The BLASTn results revealed that two of the *Rickettsia* spp.

were 98.75 to 100% similar to *R. conorii* gene sequences on the NCBI database. Another two *Rickettsia* spp. sequences. BLASTn search results revealed that they were 97.18 to 99.70% similar to *R. massiliae* gene sequences on the NCBI database. In addition, one *R. sanguineus* tick pool was co-infected with both *C. burnetii* and *Rickettsia* spp.

Lastly, this study used Illumina next-generation sequencing targeting the V3 and V4 regions of the 16S rRNA gene to determine bacterial microbiota from *R. sanguineus* (n=1) and *H. elliptica* (n=1), dog blood samples from which ticks were collected. The microbiota of these ticks and dog blood was compared with that of colony reared ticks. Dominant bacterial phyla were identified as Proteobacteria (54.02%), Bacteroidetes (18.03%), and Actinobacteria (11.76%). The dominant bacterial classes were identified as Gammaproteobacteria (41.61%), Bacteroidia (17.93%), Alphaproteobacteria (11.20%), and Actinobacteria (11.11%). Dominant orders were identified as Betaproteobacteriales (14.78%) and Coxiellales (14.40%). Dominant bacterial families were identified as Coxiellaceae (14.40%) and Burkholderiaceae (12.12%). The dominating bacterial genera were identified as *Coxiella* (14.40%). Additionally, at class and genus levels the relative abundance between the *R. sanguineus* and *H. elliptica* of the colony ticks from gnotobiotic hosts were similar, whereas the relative abundances of the *R. sanguineus* and *H. elliptica* ticks and their natural dog hosts were similar indicating similarities between the species richness, whereas the relative abundance between the colony ticks from gnotobiotic hosts and the ticks collected from naturally infected dog hosts was different indicating differentiation between the species richness. The results of the study indicate the presence of several bacterial communities harboured by and shared between *R. sanguineus* and *H. elliptica* ticks from naturally infected dog hosts, dog blood, and *R. sanguineus* and *H. elliptica* ticks from gnotobiotic hosts. The aforementioned bacterial communities are taxonomically similar among all the sample types but the relative abundances of the bacterial communities of both the dog blood and ticks from natural dog hosts differ compared to the ticks from gnotobiotic hosts, specifically among the *Rickettsia* spp. and *Coxiella* spp. that were very high in the tick specimens collected from gnotobiotic hosts. Additionally, several bacterial genera detected in the sample types were of medical, veterinary, and ecological importance.

The systematic review has provided consolidated insight on the prevalence of *R. sanguineus*, *H. elliptica*, and their tick-borne pathogens, as well as other associated subjects, such as the expansion of ticks and their tick-borne pathogens to non-endemic areas, the effects of co-infections, and tick identification and parasite detection methods. The results of this study indicate that dogs admitted to PAWS are infected by *R. sanguineus* and *H. elliptica* ticks which are harbouring tick-borne pathogens responsible for causing zoonotic diseases.

Keywords: Ticks, *Rhipicephalus sanguineus*, *Haemaphysallis elliptica*, *Coxiella burnetii*, *Rickettsia* spp., next-generation sequencing, bacterial communities

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CHAPTER 1

INTRODUCTION

1.1 Study background

Ticks are medically, economically and veterinary important pests on a worldwide scale (Uilenberg & Jongejan, 2004; Manjunathachar *et al.*, 2014). Ticks can transmit pathogenic organisms to their hosts when taking a blood meal. This in turn causes health issues on livestock, wild animals, and companion animals (Uilenberg & Jongejan, 2004; Magnarelli, 2009; Dantas-Torres *et al.*, 2012). Certain tick species have a broad host range, whereas other tick species prefer to infest specific hosts. Ticks that prefer to feed on specific hosts may infest humans in the absence of favorable hosts. This in turn may cause health issues in humans through the transmission of tick-borne pathogens by causing tick paralysis, or the toxins released by ticks may cause the host to have allergic reactions (Edlow & McGillicuddy, 2008; Magnarelli, 2009; Dantas-Torres *et al.*, 2012; van Nunen, 2018). The aforementioned may be as humans often are in close association to livestock and animals, especially companion animals, as well as the fact that ticks are well adapted to live in urban environments (Bethony *et al.*, 2006; O'Haire, 2010; Uspensky, 2014). It was estimated that the domestic dogs reached a population of 87.6 million by 2017 in South Africa (Otranto *et al.*, 2017). As time progresses the population of dogs may increase and so will the ticks infesting said dogs along with the associated tick-borne pathogens (Manjunathachar *et al.*, 2017).

Rapid increases in the populations of ticks are a result of average annual temperature increases as well as seasonal fluctuations (Manjunathachar *et al.*, 2017; Rochlin & Toledo, 2020). Ixodid ticks are well adjusted to endure intense environmental conditions brought forth by warm climates (Baylis, 2017; Rochlin & Toledo, 2020). Due to the survival strategies of ticks, they can inhabit non-endemic areas or establish new niches when they are introduced to new areas. The abovementioned is possible when ticks attach to migrating hosts or when hosts travel long distances (Hoogstraal *et al.*, 1961; Bjöersdorff *et al.*, 2001; Wall *et al.*, 2017). Often when ticks infected with pathogens are introduced to new areas, co-infections of hosts with tick-borne pathogens in that area may occur (Moutailler *et al.*, 2016; Cutler *et al.*, 2020). Co-infections are often problematic as clinical signs portrayed by the infected hosts are difficult to diagnose or may result in a misdiagnosis. This requires more advanced or specific diagnostic techniques, like serological tests or molecular techniques (Moniuszko *et al.*, 2014; Dunaj *et al.*, 2018). Molecular techniques, such as PCR and DNA sequencing (used in this study) allow for specific detection and identification of tick-borne pathogens (Wall, 2016).

Ticks also harbour several bacterial communities, which are collectively referred to as the microbiome (Narasimhan & Fikrig, 2015; Narasimhan *et al.*, 2021;). The composition of the microbiome of ticks may influence the tick in several ways, such as vector competence (Adegoke *et al.*, 2020; Bonnet & Pollet, 2021). The microbiome composition is influenced by factors such as environmental conditions, the presence or the absence of tick-borne pathogens, or the ticks developmental stage (Narasimhan & Fikrig, 2015; Adegoke *et al.*, 2020; Narasimhan *et al.*, 2021). Since ticks spend the majority of their time seeking hosts, attaching to said host, taking blood meals, and developing to different developmental stages until they reach the adult developmental stage, changes in the microbiome composition are inevitable (Magnarelli, 2009; Aivelo *et al.*, 2019). Furthermore, identification of the bacterial communities and determination of the interactions between the tick host and bacterial communities may aid the understanding between the tick-host-pathogen interaction complex (de la Fuente *et al.*, 2016; Bonnet *et al.*, 2018). Detection of the ticks bacterial communities is challenging as certain bacterial species are hard to cultivate outside of the tick host and require culture-independent techniques (Su *et al.*, 2012; Bonnet *et al.*, 2017). Next-generation sequencing, such as Illumina MiSeq sequencing (which is used in this study), is one of the techniques that is applied for the detection and subsequent identification of bacterial communities (Greay *et al.*, 2018). To date, there is limited information available on ticks, their associated tick-borne pathogens, and the bacterial communities of dog ticks in South Africa, particularly in Potchefstroom.

1.2 Study justification

The tick species infesting dogs in South Africa include *Rhipicephalus sanguineus* and *Heamaphysalis elliptica* (Horak *et al.*, 2017, 2018). These tick species may be responsible for the transmission of several pathogenic organisms, including but not limited to *Anaplasma phagocytophylum*, *Babesia* spp., *Coxiella burnetii*, *Hepatozoon canis*, and *Rickettsia* spp. to their hosts (Santander, 2017; Horak *et al.*, 2017, 2018; Rochlin & Toledo, 2020). This study sought to provide more information on dog ticks by characterizing ticks collected from dogs admitted to the Potchefstroom Animal Welfare Society, commonly referred to as PAWS. This study also sought to provide information on tick-borne pathogens of dogs that are of medical, veterinary and economic importance. In addition, this study will provide and insight on the bacterial community composition of dog ticks.

1.3 Aim

The study aimed to conduct systematic review on distribution of *Rhipicephalus sanguineus* and *Haemaphysallis elliptica* dog ticks. Furthermore, this study sought to collect and identify ticks, detect tick-borne pathogens and document bacterial communities from both host and ticks of stray dogs admitted at the Potchefstroom Animal Welfare Society (PAWS).

1.4 Objectives

- To conduct systematic review on global or continental distribution of *Rhipicephalus sanguineus* and *Haemaphysallis elliptica* dog ticks.
- To characterize ticks morphologically by microscopic examination and by amplification of the ITS2, CO1, and 18S rRNA genes using PCR and sequencing.
- To identify tick-borne pathogens associated with dog ticks by using DNA-based PCR assays.
- To determine bacterial microbiota in dog ticks, host dog blood and colony reared dog ticks using a next-generation sequencing platform.

1.5 Hypothesis

Hypothesis 1: Ticks carry more pathogens than their dog hosts.

Hypothesis 2: Ticks naturally infesting dogs have larger bacterial populations than colony ticks.

1.6 Thesis outline

Chapter 1: INTRODUCTION

This chapter contains a short introduction of the relevance of this research project, as well as the aim, objectives and hypothesis of the study project.

Chapter 2: Literature review

The literature review includes a detailed overview of Ixodid ticks, specifically focussing on ticks infesting dogs in South Africa. Information on the tick-borne pathogens is also captured in this chapter, with emphasis on *A. phagocytophylum*, *C. burnetii*, *E. canis*, and *Rickettsia* species. The principles of the molecular methods used in this research study to identify ticks and detect tick-borne pathogens are also described in this chapter. In addition, the role of microbial communities,

next-generation sequencing, and computational methods to determine the bacterial communities are also reviewed.

Chapter 3: Systematic review of the distribution of dog ticks: *Rhipicephalus sanguineus* and *Hemaphysalis elliptica* as well as tick-borne pathogens they harbour

This chapter contains a systematic review that was conducted with data from published articles to better comprehend the epidemiology of *R. sanguineus* and *H. elliptica* and their associated tick-borne pathogens. Subgroup analysis was also done to determine the possible factors that may influence distribution studies, including identification or detection technique used, study period, geographical location studied, sample size, study outcome, or disease frequency.

Chapter 4: Identification of ticks infesting dogs from the Potchefstroom Animal Welfare Society and the pathogens they harbour

This chapter contains information on the morphological and molecular identification, and phylogenetic analysis of ticks infesting dogs that were admitted to PAWS. In addition, this chapter contains the phylogenetic analysis of tick-borne pathogens that were detected from dog ticks, as well as the associated dogs by using their blood samples.

Chapter 5: Bacterial microbiota detected in ticks infesting dogs, dog blood and colony reared dog ticks by next-generation sequencing

This chapter captures information on the bacterial communities of different organs of naturally infected ticks of dogs admitted to PAWS. This chapter also contains information on bacterial communities of dog blood, associated with their ticks. In addition, this chapter also captures information on bacterial communities of colony dog ticks from germ-free hosts.

Chapter 6: Conclusion and recommendations

This chapter concludes on observed data generated in this research study as well as recommendations for future studies.

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CHAPTER 2

LITERATURE REVIEW

2.1 Ixodid ticks

2.1.1 Systematics

Taxonomy of ticks possibly originated in 1758 when Linnaeus described several tick species in the tenth edition of *Systema Naturae*. During the late 1700s and late 1800s tick taxonomy remained vague even though there were several new species descriptions (Dantas-Torres, 2018). Modern tick taxonomy began with the works of Carl Ludwig Koch, during the 19th century, as well as works from Louis George Neumann, during the late 19th and early 20th century (Horak, 2009; Dantas-Torres, 2018). More than 4000 tick species were described during the past 250 years, albeit many of these species are invalid (Horak, 2009).

Ticks are arthropods classified under the order of Ixodida. Furthermore, ticks consist of four different tick families. These families include the Ixodidae, Argasidae, Nuttalliellidae, and extinct Deinocrotonidea (Parola & Raoult, 2001; Guglielmone *et al.*, 2010; Dantas-Torres, 2018). One of the two major families, the Ixodidae are commonly referred to as the hard ticks because of the presence of a sclerotized dorsal plate. This family is of medical, ecological, and veterinary significance. Furthermore, the Ixodidae is made up of two major groups, namely Metastriata and Prostriata (Walker, 1991; Parola & Raoult, 2001). Currently, there are approximately 731 species in this family (Dantas-Torres, 2018). The second major family, the Argasidae are commonly referred to as soft ticks due to the presence of a flexible cuticle. Furthermore, the Argasidae family is divided into two subfamilies, namely Ornithodorinae and Argasinae (Parola & Raoult, 2001; Guglielmone *et al.*, 2010). Currently, there are approximately 216 species in this family (Dantas-Torres, 2018). The Argasid and Ixodid families have major differences regarding morphological features and life cycles (Parola & Raoult, 2001). The Nuttalliellidae family represents a single species, namely *Nuttalliella namaqua* (Parola & Raoult, 2001; Dantas-Torres, 2018). This species has been documented in Southern and Eastern Africa and shares morphological features of tick species belonging to the Ixodidae and Argasidae families (Parola & Raoult, 2001; Mans *et al.*, 2011; Dantas-Torres, 2018). The Deinocrotonidea family is a newly described, extinct family, consisting of a single species, namely *Deinocroton draculi* (Dantas-Torres, 2018). This family was proposed when adult ticks were discovered on fossil material of Burmese amber from Myanmar from the Cretaceous era, of approximately 99 million years ago (Peñalver *et al.*, 2017). This tick species shares several of the same morphological features of *N. Namaqua* (Peñalver *et al.*, 2017;

Dantas-Torres, 2018). Dantas-Torres (2018) suggests that *D. draculi* and *N. namaqua* share a common ancestor and this ancestor may represent the primordial ancestor of all present tick evolutionary lineages.

2.1.2 Morphological features

Morphological features of ixodid ticks are illustrated in Figures 2.1 and 2.2. Ticks are acarines with body sizes ranging from 2 to 30 mm (Parola & Raoult, 2001). During the adult developmental stage ticks are generally described as small or large. Ticks also differ in size; an example of this is that the largest *Haemaphysalis* ticks may be smaller than the smallest *Amblyomma* tick (Horak *et al.*, 2018). During the adult and nymphal developmental stages, ticks have 4 pairs of legs, whereas ticks in the larval developmental stages have 3 pairs of legs (Parola & Raoult, 2001; Horak *et al.*, 2018). Legs are segmented appendages that are designated as the tarsus, metatarsus, tibia, genu, femur, trochanter, and coxa from distal to proximal (Sonenshine *et al.*, 2009; Horak *et al.*, 2018). Coxae represent the first leg segments and are observed as four sets of sclerotized structures located on the ventral idiosomal surface. Coxal spurs spread from the coxae posterior margins (Horak *et al.*, 2018). The males of certain tick species have the presence of the caudal process present on the posterior margins as a medial projection (Sonenshine *et al.*, 2009; Horak *et al.*, 2018). Idiosoma is the section of the tick's body that does not include the legs and capitulum (Hoskins, 1991; Horak *et al.*, 2018). In some *Rhipicephalus* species, an anterior process, also known as an anterior projection, of coxa I are present where it dorsally extends past the scapula (Sonenshine *et al.*, 2009; Horak *et al.*, 2018). Ixodidae ticks differ from insects in the sense that they lack antennae, and the bodies are not distinctively divided by a head, thorax, and abdomen (Hoskins, 1991; Parola & Raoult, 2001).

The capitulum consists of the chelicerae, basis capituli, hypostome, and the palps, which all form part of the anterior movable section of the body (Sonenshine & Roe, 2014; Horak *et al.*, 2018). Chelicerae are dorsally located to the hypostome and are observed as paired cutting mouthparts (Hoskins, 1991; Horak *et al.*, 2018). Basis capituli consists of the basal section of the capitulum that the mouthparts are attached to. Hypostomes are observed as the medioventral mouthparts on the basis capitulum used to attach the tick to a host that contains the denticles located ventrally to the chelicerae between the palps (Hoskins, 1991; Sonenshine & Roe, 2014). Palps is a pair of connected appendages anterolaterally parallel to the hypostome located on the basis capituli consisting of different segments where segment I am proximal to segment IV that is distal (Parola & Raoult, 2001; Sonenshine *et al.*, 2009). Ticks belonging to the Ixodes genus have lateral projections or bulges, known as auriculae, present on the anterolateral margins located on the basis capituli surface (Dantas-Torres, 2018; Horak *et al.*, 2018). Cornua is a pair of projections

located on the basis capituli dorsal posterior margin (Horak *et al.*, 2018). Carinae is a pair of elevated strips alongside the anterolateral scutal margins present on female ticks belonging to the *Ixodes* genus (Parola & Raoult, 2001; Horak *et al.*, 2018). Porose areas are pairs of indented areas located on the basis capituli dorsal surface present on female ixodid female ticks (Hoskins, 1991; Horak *et al.*, 2018). Denticles are ventrally located on the hypostome surface (Sonenshine & Roe, 2014; Horak *et al.*, 2018). Genital aperture is observed as the genital organs external opening, anteriorly located on the ventromedial line behind the basis capituli (Dantas-Torres, 2018; Horak *et al.*, 2018). The genital apron is only present in female ticks and is observed as the flap that covers the genital aperture opening. The genital apron has several appearances, including a broad or narrow U-shape or V-shape (Parola & Raoult, 2001; Horak *et al.*, 2018).

Ixodid ticks are distinguished by the presence of a scutum or a conscutum. The scutum is a shield or sclerotized plate that covers about a third of the dorsal surface of ixodid tick larvae, nymphs, and adult females located posteriorly to the capitulum. Conscutum is present in male ixodid ticks, also observed as a shield or sclerotized plate that covers the dorsal surface located posteriorly to the capitulum (Arthur, 1949; Horak *et al.*, 2018). The alloscutum is present on female ticks as well as ticks that are in the early developmental stages. This is the section of the body wall posteriorly located to the scutum that expands during engorgement (Dillinger & Kesel, 2002; Horak *et al.*, 2018). Certain male tick species have the appearance of a pseudoscutum which is the anterior portion of the conscutum. Due to the smoothness or punctuation pattern, it has the same appearance as the scutum of female ticks (Patterson & Winn, 2003; Horak *et al.*, 2018). The integument is the cuticle or the external covering of the body (Dantas-Torres, 2018; Horak *et al.*, 2018). Eyes are observed in certain tick ixodid tick species as paired structures located on the scutum or conscutum on or near the anterolateral margins. Eyes vary and may be edged with punctations, or be flat, beady, or convex. The orbited are described as the groove that surrounds the eyes of some ixodid tick species (Leonovich, 2015). The eyes do not give a thorough perception of the ticks surrounding environment instead several peripheral sensory organs on the legs, mouthparts, body, and Haller's organ is used. Haller's organ is the sensory organ structure of the tick observed on the tarsus 1 sub-apical dorsal surface. These sensory organs enable communication between ticks and aid in the location of hosts (Parola & Raoult, 2001; Horak *et al.*, 2018). The overlaying, the base colour is an enamel-like colour pattern of the scutum or conscutum and other structures and is known as the ornate or ornamentation. Inornate is the part of the scutum or conscutum and the other structures that lack colour patterns (Horak *et al.*, 2018). Marginal grooves are paired grooves posteriorly located to the eyes along the conscutum lateral margins. These grooves may be long and encircle one or multiple festoons, or they may be shorter and restricted to the lateral margins, or they may be complete and outline all the festoons

(Sonenshine & Roe, 2014; Horak *et al.*, 2018). Lateral grooves are present in certain ixodid tick species and are observed as paired grooves. These grooves are located on the scutum or the conscutum, posteriorly expanding from the cervical pits outer angles and outlines the cervical fields outer margins (Hoskins, 1991; Horak *et al.*, 2018). In specific tick species, a pair of cervical grooves may be present on the scutum or conscutum. Cervical grooves outline the cervical fields inner margins and expand posteriorly from inside of the cervical pits (Sonenshine & Roe, 2014; Horak *et al.*, 2018). Cervical pits are observed as a pair of indentations located directly behind the anterior emargination which is observed as an indentation among the scapulae at the attachment base of the basis capituli located anteriorly in the scutum or conscutum (Latif, 2013; Sonenshine & Roe, 2014; Horak *et al.*, 2018). Cervical fields are observed as a pair of variously shaped areas anteriorly located on the scutum or conscutum surface. Generally, the cervical fields are slightly sunken and surrounded by the lateral and cervical grooves (Parola & Raoult, 2001; Horak *et al.*, 2018). Paramedial grooves or posterolateral grooves are paired indents adjacent to the posteromedial groove located in the conscutum present on certain male ixodid tick species. Posteromedial groove is present in some male ticks and is observed as a central indent located in the conscutum ending at the central festoon (Parola & Raoult, 2001; Sonenshine & Roe, 2014; Horak *et al.*, 2018). Festoons are present in some tick species and are observed as various rectangular areas posteriorly located on the body margin. The aforementioned are evident in non-engorged larvae, nymphs, and females, as well as males (Latif, 2013; Horak *et al.*, 2018). The anus is medially and ventrally situated, located posteriorly to the last pair of legs (Parola & Raoult, 2001; Horak *et al.*, 2018). Adanal plates are a pair of sclerotized structures, larger than the accessory adanal plates, present on certain species of male ticks, and are laterally located to the anus (Sonenshine *et al.*, 2009; Horak *et al.*, 2018). Accessory adanal plates are a pair of sclerotized structures present on certain species of male ticks and are laterally located to the adanal plates (Hoskins, 1991; Horak *et al.*, 2018). Sub-anal plates are paired sclerotized structures present in the males of *Hyalomma* species located posteriorly to the adanal plates (Sonenshine *et al.*, 2009; Horak *et al.*, 2018). The anal groove is a semi-circular curve on the integument located directly posteriorly to the anus of nearly all genera, excluding *Ixodes* where the groove is located anteriorly to the anus (Hoskins, 1991; Horak *et al.*, 2018).

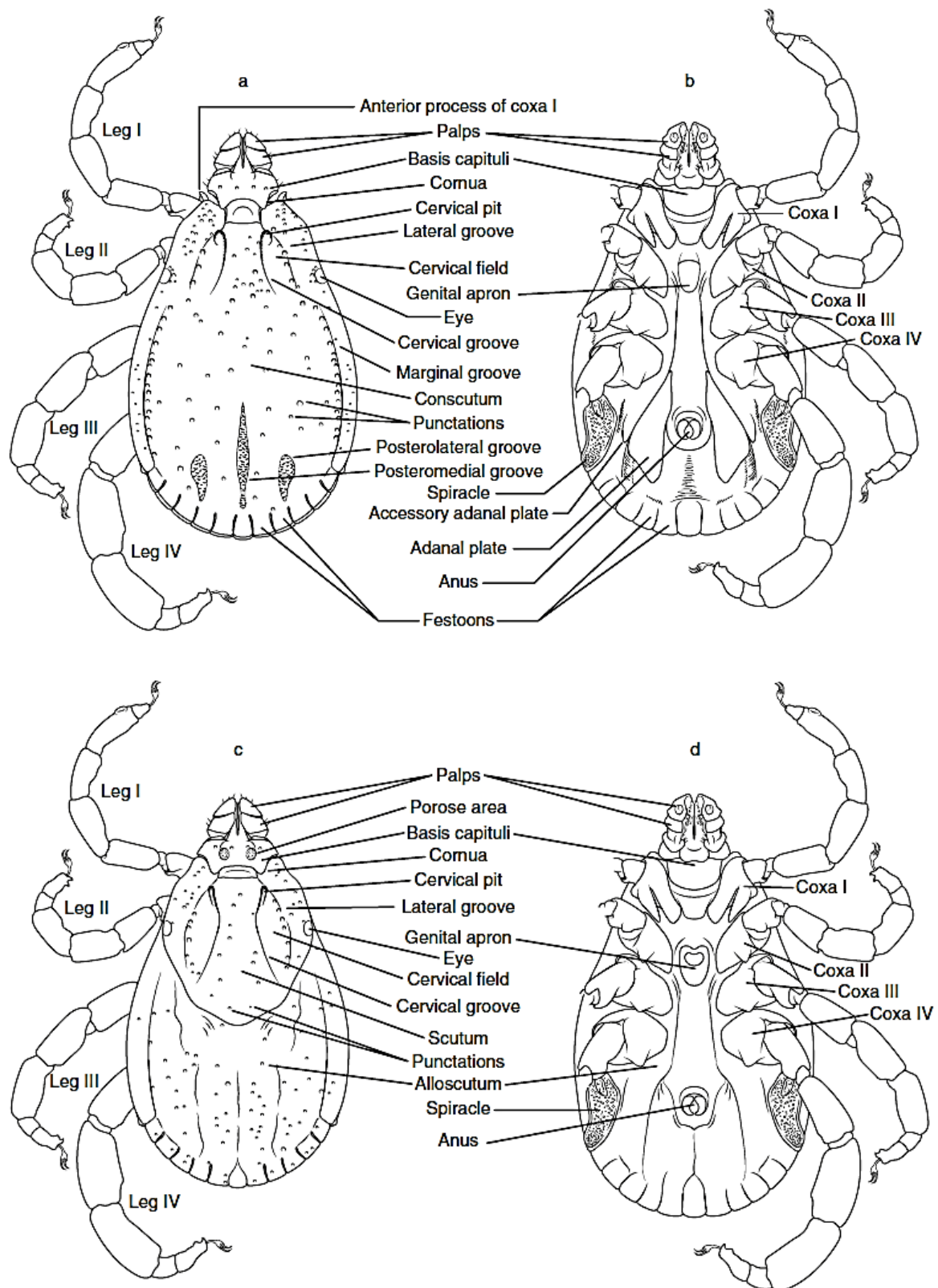


Figure 2-1: Ixodid ticks morphological features adapted from Horak *et al.* (2018). Dorsal (a) and ventral (b) view of male adult ticks. Dorsal (c) and ventral (d) view of female adult ticks

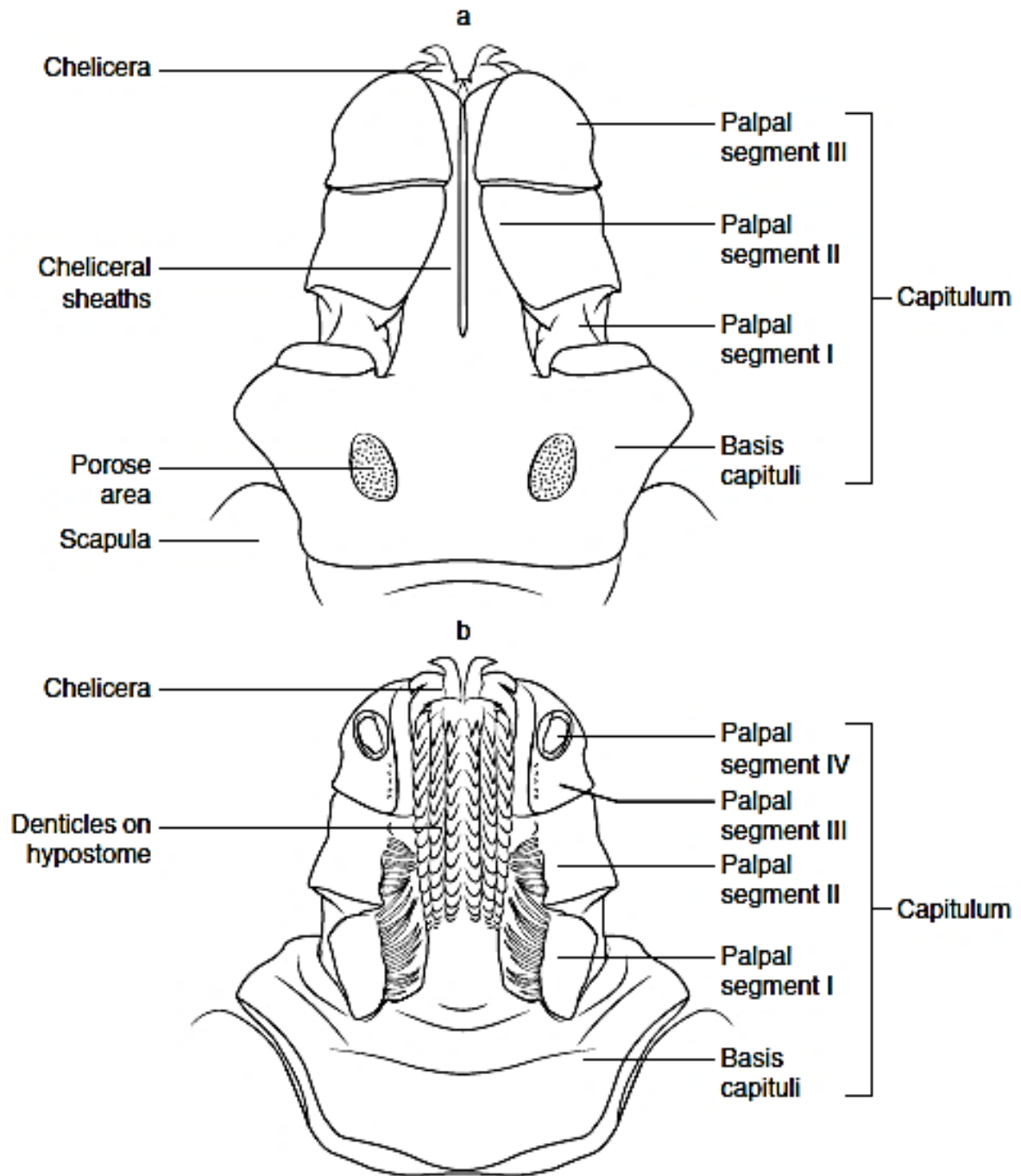


Figure 2-2: Dorsal (a) and ventral (b) view of ixodid tick capitulum (Horak *et al.*, 2018)

2.2 Life cycle and ecology

Completion of the ixodid ticks' life cycle may range from 6 months to 6 years depending on several factors. These factors include but are not limited to the tick species, as well as environmental conditions, such as relative humidity, temperature, and photoperiod (Klompen *et al.*, 1996; Parola & Raoult, 2001; Krige *et al.*, 2019). In general, the ixodid tick life cycle consists of a three-host

cycle with several developmental stages. These stages include the larval, nymphal, and adult developmental stages (Figure 2.3). During each stage, the ticks search for a suitable host, attach to said host and feeds for several days (Waladde *et al.*, 1996; Parola & Raoult, 2001; Kocan *et al.*, 2007). Feeding periods depend on several factors including, but not limited to the life stage of the ticks and environmental conditions (Parola & Raoult, 2001; Uilenberg & Jongejan, 2004). Once the tick is replete it detaches from the host and finds an environment where it digests the blood meal, molts, and enters the next developmental stage, alternatively it can enter a state of diapause where metabolic activity is reduced and development is delayed (Klompen *et al.*, 1996; Parola & Raoult, 2001). The aforementioned is where ticks are normally present in or on vegetation and, depending on the species, in urban environments or human-made structures (Parola & Raoult, 2001; Leal *et al.*, 2020). The time spend attached to a host, the ticks are referred to as parasitizing ticks, whereas the stage is referred to as on-host ecology. When a tick is detached from a host it is referred to as a questing tick and this stage is referred to as off-host ecology (Parola & Raoult, 2001; Leonovich, 2015).

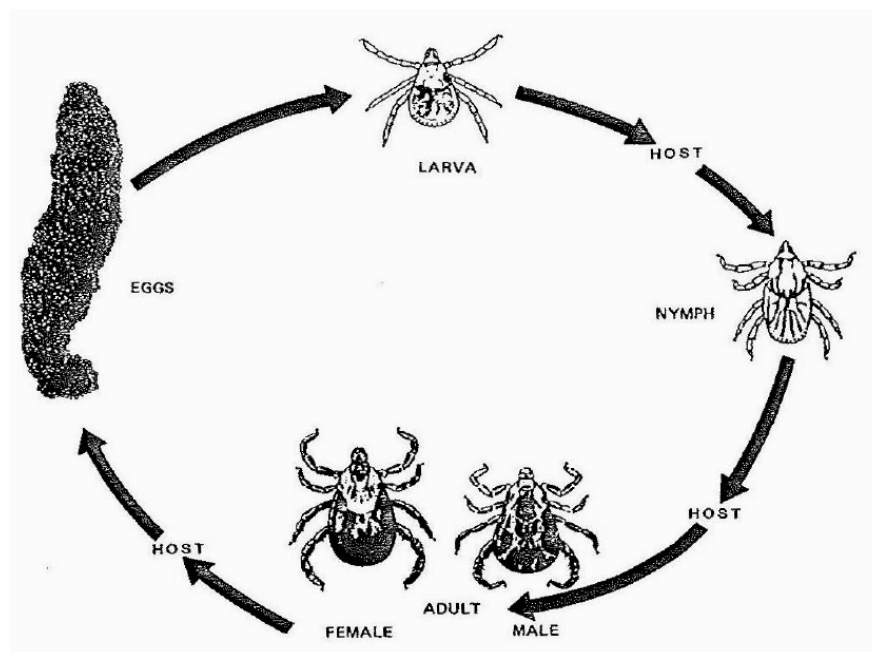


Figure 2-3: Life cycle of ixodid ticks (Varela-stokes *et al.*, 2009)

2.2.1 Questing ticks (off-host ecology)

Ticks spend most of their life cycle, which is more than 90%, unattached from the host in open environments, forests, or meadows (Parola & Raoult, 2001; Leonovich, 2015). Most ticks are exophilic and sensitive to dehydration and are more common in areas with vegetation, especially woodlands and grasslands, or in areas with adequate shelter including human-made structures,

such as crevices and cracks in the walls of human homes (Parola & Raoult, 2001; Leal *et al.*, 2020). Each tick species has its optimal biotype and environmental conditions it requires to thrive and in turn, this determines their geographical distributions (Klompen *et al.*, 1996; Parola & Raoult, 2001).

When environmental conditions are favourable ticks that are seasonally active search for suitable hosts (Parola & Raoult, 2001; Tahir *et al.*, 2020). Ticks are extremely receptive to stimuli that reveal the presence of hosts. These stimuli include chemical stimuli, like changes in carbon dioxide (CO₂) and ammonia (NH₃), as well as changes in body temperatures of warm-blooded hosts, humidity, phenols, airborne vibrations, and aromatic chemicals (Klompen *et al.*, 1996; Parola & Raoult, 2001). Exophilic ticks display two different host-seeking strategies, namely the ambush strategy and hunter strategy. When using the ambush strategy ticks climb up on vegetation with their front legs held out and wait for hosts to pass and use their front legs to attach. When using the hunter strategy, the ticks emerge from their habitat when a host appears, run towards and attach to their hosts (Parola & Raoult, 2001; Leal *et al.*, 2020). Some tick species make use of both strategies (Parola & Raoult, 2001). Other ixodid ticks are endophilic and exhibit a third strategy where they remain hidden in burrows or hosts nests and wait for their hosts to arrive (Leonovich, 2015).

2.2.2 Parasitic ticks (on-host ecology)

Generally, Ixodid ticks have three different life stages, namely the larval, nymphal, and adult stages (Parola & Raoult, 2001; Kocan *et al.*, 2007; Tahir *et al.*, 2020). Immature ixodid ticks detach from the host to a suitable environment to molt to the next developmental stage (Parola & Raoult, 2001; Nava & Guglielmone, 2013). During each developmental stage, the tick feeds once and detaches from the host (Parola & Raoult, 2001; Kocan *et al.*, 2007; Nava & Guglielmone, 2013). The adult ticks usually mate on the host (Parola & Raoult, 2001; Carroll, 2002). Adult male ticks only feed for short periods or skip feeding entirely, whereas engorged adult female ticks detach from the host to digest the blood meal and lay eggs (Parola & Raoult, 2001; Léger *et al.*, 2015). Depending on the species adult female ticks lay from 400 to more than 20 000 eggs in sheltered environments (Parola & Raoult, 2001). Metastriate female ticks may stay attached to the host until mating. In contrast, Prostriate ticks feed on a different host during each developmental stage. Certain male Prostriate ticks, including *R. sanguineus*, may attach to several hosts during the adult developmental stage to locate female ticks to mate with (Cabezas-cruz *et al.*, 2018). Pheromones are essential when it comes to the behaviour of ticks and additionally aid ticks to track their mates and potential hosts. In particular, assembly pheromones, that enable ticks to

find each other, as well as sex pheromones that attract male ticks to female ticks and encourage mounting, are of vital importance during mating (Parola & Raoult, 2001; Carroll, 2002).

2.3 Infection of the reservoir and accidental hosts

There are a lot of theories concerning the specificity of ticks towards their hosts (Horak *et al.*, 2018). Hoogstraal & Aeschlimann (1982) indicated that numerous tick species only infest specific hosts, any other observed associations between ticks and hosts may be a result of incidental infections. Incidental infections referring to feeding ticks detach from a host and incidentally attach to another available host. Alternatively, Klompen *et al.* (1996) suggested that the ecological and biogeographical specification of the tick itself determine tick-host specificity. Klompen *et al.* (1996) further explain that a false observation of the tick-host specificity may be a result of incomplete sampling and that the likelihood of incidental infections will increase as sampling increases.

Cumming (1998) suggested that ticks infest different host groups based on the developmental stage. In general, ticks infest small mammals during the immature developmental stages and infest larger mammals during the later developmental stages. This may be due to ticks in the immature developmental stage that require to sustain a substantial population, whereas the hosts of ticks in the adult developmental stage are widely distributed (Cumming, 1998; Horak *et al.*, 2018). Horak *et al.* (2018) suggested that tick-host associations may be a result of ticks adapting to changing environmental conditions and available hosts.

2.4 Zoonosis

In the absence of specific hosts, ixodid ticks may feed on other hosts, including humans. In the case where infected ticks feed on humans the tick-borne disease-causing organisms, may be transmitted to the human host. This means that the pathogenic organisms that normally infect animal hosts may infect human hosts, in turn, affecting the human population. This is generally referred to as zoonosis (Wall, 2016). Zoonotic diseases are of global concern as novel human infections often emerge or known animal infections re-emerge. Zoonotic diseases originating from companion animals are of particular concern, due to the close association between companion animals and humans (Betancur *et al.*, 2015; Wall, 2016). Often companion animals are in contact with arthropods that exist in their environment. This is a risk to human health as companion animals may become infected or bring infected ticks into homes, leading to human infections using direct infections or infected ticks attaching to the human carers of the companion animals (Ostfeld *et al.*, 2014; Wall, 2016).

2.5 Control strategies

To date, there is no feasible control strategy for ticks and their management remains challenging (Ghosh *et al.*, 2007). Management of diseases affecting humans and animals caused by tick-borne pathogens is for the most part focussed on strategies to manage or eliminate tick populations, though this is not feasible over a large geographical area (Sonenshine *et al.*, 2004). Synthetic chemical and botanical acaricides are the most commonly used control method, such as spraying tick-infected areas or dipping animals in acaricides, but ticks have become somewhat resistant due to the indiscriminate use of certain acaricides (Yessinou *et al.*, 2016). Other control strategies include the daily screening and removal of ticks on companion animals and livestock, the management of landscape vegetation close to living spaces to limit the habitat of ticks and to minimize the recruitment of wildlife, as well as avoiding tick-infected areas (Esteve-Gassent *et al.*, 2016). Alternatively, once a host is infected drug treatments are available and several vaccinations are available depending on the responsible pathogenic organisms (Silva *et al.*, 2021).

2.6 Tick species infesting dogs in South Africa

2.6.1 *Rhipicephalus sanguineus* (Latreille, 1806)

Commonly known as the kennel tick, brown dog tick, as well as the pantropical tick (Pegram *et al.*, 1987; Walker *et al.*, 2000; Latif, 2013; Horak *et al.*, 2018). Previously described by Walker *et al.* (2000) and Latif (2013). Dantas-Torres (2018) suggests that this species requires a redescription and neotype deposition and further research is required to establish the taxonomic position of ticks that resemble *R. sanguineus* but differ in terms of genetics or morphology. Previously reported within the African continent in South Africa, Botswana, Namibia, Swaziland, and Mozambique (Anatriello *et al.*, 2010; Gray *et al.*, 2013; Horak *et al.*, 2018). Horak *et al.* (2018) suggested that this species is so widely distributed that the tick is endemic in any area where domestic dogs are kept in confinement. This tick species transmits several pathogenic organisms, including *Ehrlichia canis* responsible for canine ehrlichiosis or tropical pancytopenia, *Babesia vogeli* and *B. canis* responsible for canine babesiosis, *Hepatozoon canis* responsible for canine hepatozoonosis, and *Rickettsia conorii* responsible for rickettsiosis in dogs and tick-bite fever in humans (Dantas-Torres & Otranto, 2015; Anatriello *et al.*, 2010; Horak *et al.*, 2018).

Rhipicephalus sanguineus ticks are classified as specialists where all the developmental stages feed on the species *Canis familiaris*, better known as domestic dogs (Walker *et al.*, 2000).

Although these ticks have a host preference for dogs these ticks were previously reported to infest humans. These infections are relatively low and only occur as a result of the close associations between humans and domestic dogs (Goddard, 1989; Dantas-Torres & Otranto, 2015). These ticks were also previously collected from domestic cats, cattle, horses, and goats. These collections were also considered accidental infections due to the close association between infected dogs, other domestic animals and livestock (Horak *et al.*, 2018).

Rhipicephalus sanguineus is a three-host species that follows a four-stage life cycle (Anatriello *et al.*, 2010), whereby, more than one life cycle may be completed annually (Horak *et al.*, 2018). This tick species is adapted to survive in human-made structures, including domestic dwellings, sheds, and kennels, where the female ticks may lay eggs underneath dogs' bedding or in the surrounding structures in crevices and cracks (Dantas-Torres, 2010). Molding of engorged larvae or nymphs takes place in the same locations, where these ticks move up and down the structures to mold after detaching from the current host or to find a new host (Dantas-Torres, 2010; Horak *et al.*, 2018). Heavy infestations may occur where dogs are kennelled or caged (Little *et al.*, 2007). Jacobs *et al.* (2004) studied the life cycle stages periods of *R. sanguineus* in the laboratory at different temperatures and relative humidities. Here, adult female ticks feed for 7 to 14 days. The next pre-oviposition takes place for respective periods of 5 and 21 days at 25°C with 90% relative humidity and 15°C with 40% relative humidity. Larvae hatch at respective periods of 19 and 72 days at 30°C with 90% relative humidity and 15°C with 90% relative humidity. Subsequently, the larvae feed for 4 to 6 days. Nymphs pre-mold at respective periods of 10 and 37 days at 30°C with 90% relative humidity and 15°C with 90% relative humidity. Nymphs then feed for 4 to 6 days. Adults then pre-mold for respective periods of 15 and 45 days at 30°C with 90% relative humidity and 20°C with 70% relative humidity. Under ideal conditions in a location such as a domestic dwelling or kennels the life cycle of ticks may be completed in 9 weeks (Dantas-Torres, 2008; Horak *et al.*, 2018). Annually, two or more life cycles may be completed in regions with summer rainfall, where the climate in the summer is warm or hot and where there may be frost during the winter. In these regions, the ticks enter diapause as engorged nymphs and enter the next life stage as adults during spring. In regions that have winter rainfall one or sometimes two life cycles may be completed annually (Horak *et al.*, 2018).

2.6.2 *Haemaphysalis elliptica* (Koch, 1844)

Haemaphysalis elliptica, commonly known as the southern African yellow dog tick (Apanaskevich *et al.*, 2007; Latif, 2013). Previously described as *Rhipistoma ellipticum* by Koch (1844), was subsequently placed under the *Haemaphysalis* genus and synonymized as *Haemaphysalis leachi*

originating from North and East Africa by Neumann in 1897 (Horak *et al.*, 2018). This species was re-described by Apanaskevich *et al.* (2007) and reinstated as *H. elliptica*.

The *H. elliptica* is endemic to South Africa and was previously reported in Swaziland, Botswana, Namibia, and Mozambique. This tick is distributed in the eastern regions in South Africa within the Grassland and Savanna biomes, as well as Fynbos and Thicket biomes in the southern part of South Africa. It was also previously recorded in the western region of the Northern Cape, within the Succulent Karoo biome, and in Swaziland, within the Grassland and Savanna biome, as well as in Mozambique, Namibia close to the Angolan border, as well as the eastern and southern regions, as well as the Okavango delta of Botswana. This tick is common in Zimbabwe, excluding the west of the country (Latif, 2013; Horak *et al.*, 2018). The *H. elliptica* is known to transmit *Babesia rossi* that is the causative agent of babesiosis in domesticated dogs (Horak *et al.*, 2018).

The *H. elliptica* is classified as a specialist tick that infest hosts belonging to the order Carnivora. In general, the immature tick stages infest two distinct host groups, namely carnivores or rodents. Within the first host group, the ticks prefer rodent hosts, such as mice and rats. The second host group ticks prefer carnivore hosts, usually the same hosts that the adults infest (Latif, 2013; Horak *et al.*, 2018). Horak *et al.* (1987), Horak (1995), Bryson *et al.* (2000), Horak *et al.* (2001), and Horak & Matthee, (2003) suggests that *H. elliptica* is non-existent or occurs in low numbers in highly populated urban environments where only a few rodents are present, whereas these ticks thrive in peri-urban environments with big gardens, farms, and small holdings where rodents occur in large numbers. The *H. elliptica* adult ticks were previously collected from domestic animals including dogs, cats, sheep, and goats, as well as wild carnivores, birds, and humans (Latif, 2013; Horak *et al.*, 2018). Questing *H. elliptica* ticks also occur in higher numbers on vegetation with taller grass swards and a thicker ground cover that incidentally is the perfect habitat for rodents (Schroder & Reilly, 2013; Horak *et al.*, 2018).

The *H. elliptica* is classified as a three-host species that follows a four-stage life cycle (Latif, 2013; Horak *et al.*, 2018). Questing ticks search for suitable hosts, in this case, carnivores, from the vegetation. In general, equal numbers of both female and male adult questing ticks are often reported on vegetation. In the case of parasitic ticks, the males outnumber the females. This skewed comparison is the result of male ticks that stay on the hosts for longer periods, whereas females usually spend a week on the host where they engorge, detach, and oviposition (Schroder & Reilly, 2013; Horak *et al.*, 2018). Ticks that are in the early developmental stages are rarely reported from the vegetation. This may be as a result of questing immature ticks that search for rodent hosts in environments such as soil surfaces, burrows, or rodent holes (Horak *et al.*, 1987, 2001, 2018). The duration of the developmental stages often depends on different factors, such

as temperature and relative humidity. Free-living ticks often enter diapause and have extended periods of developmental stages in colder temperatures, such as 10-15°C, whereas, developmental stages are shorter in warmer temperatures, such as 25-30°C (Horak *et al.*, 2018). During a bloodmeal of female adult tick, initial engorgement takes place slowly and approximately 24 hours before detaching from the host, where it rapidly engorges (Latif, 2013; Horak *et al.*, 2018). Jacobs *et al.* (2004) determined that *H. elliptica* females feed for a period of 7 to 14 days. The female adults pre-oviposition for a period of respectively 4 or 12 days at 30°C with a relative humidity of 90% or 15°C with a relative humidity of 40%. Oviposition then lasts for 15 days. This is followed by an incubation period of respectively 16 or 67 days at 30°C with a relative humidity of 90% or 15°C with a relative humidity of 40%. After incubation, the ticks enter the larval developmental stage where the ticks feed for 3 to 6 days. Afterward, the nymphs pre-molt for respectively 14 or 192 days at 30°C with a relative humidity of 90% or 15°C with a relative humidity of 40%. The nymphs then feed for 4 to 6 days. The adults then pre-molt for respectively 13 or 41 days at 30°C with a relative humidity of 90% or at 15°C with a relative humidity of 40%.

2.7 Tick-borne pathogens transmitted by dog hosts in South Africa

Several arthropods are important vectors of pathogens in domestic animals due to their feeding behaviour (Piesman & Beard, 2012; Wall, 2016; Boulanger *et al.*, 2019). Ticks are classified as obligate hematophagous arthropods that parasitize four vertebrate classes (mammals, reptiles, birds and amphibians) in all the regions of the world (Brown *et al.*, 2005; Wall, 2016; Boulanger *et al.*, 2019). After mosquitoes, ticks are responsible for the transmission of the most pathogens to animals and humans on a global scale (Domingos *et al.*, 2013; Wall, 2016). Ticks have different behaviours regarding feeding and blood digestion habits compared to other insects. This in turn may influence pathogen transmission and acquisition (Willadsen & Jongejan, 1999; Cabezas-cruz *et al.*, 2018). In particular, digestion is a lengthy process. With each location, attachment, and feeding on a new host ticks may acquire pathogens from or transmit pathogens to the hosts (Cabezas-cruz *et al.*, 2018; Madison-Antenucci *et al.*, 2020). Ticks act as vectors of pathogens in one of two ways, namely biological or mechanical transmission. With the biological transmission, the pathogens are transmitted using infected ticks transmitting the pathogens while feeding. With the mechanical transmission the pathogens are acquired by ticks, normally on the mouthparts or other locations of the ticks body and the ticks may infect new hosts (Wall, 2016).

Ticks may become infected with pathogens using transovarial or transstadial transmission. During transstadial transmission, ticks become infected with pathogens between their developmental stages when they ingest blood from infected hosts, where transovarial transmission occurs

between generations through eggs (Wall, 2016; Madison-Antenucci *et al.*, 2020). The majority of ticks that are important vectors of diseases in companion animals are three-host species (Süss *et al.*, 2008; Wall, 2016). The combination of the movement between hosts during the developmental stages of the ticks as well as several tick species that lack host specificity when it comes to feeding preferences make these ticks vital disease vectors (Willadsen & Jongejan, 1999; Wall, 2016). The habitats of ticks are also important since they spend the majority of their life cycle off of hosts. This is where ticks are exposed to potentially infected wild animals, where infected ticks may, in turn, infect domestic animals or humans at a later stage (Pfäffle *et al.*, 2013; Wall, 2016). Ticks have several characteristics that contribute to the enhancement of their vectoral potential. These characteristics include feeding for extended periods and painless bites that go unnoticed for long periods (Parola & Raoult, 2001; Pfäffle *et al.*, 2013).

The coexistence of multiple pathogens within a tick vector, otherwise known as coinfections, is a common occurrence (Fritz, 2009; Cabezas-cruz *et al.*, 2018). Coinfections may influence the host's risk of infection, as well as the vectors transmission and acquisition of other pathogenic microorganisms (Gilbert, 2010; Domingos *et al.*, 2013; Cabezas-cruz *et al.*, 2018). When several pathogenic organisms share a reservoir host direct interactions between the pathogens are possible or indirect interactions are possible through host immune-mediation or when competing for similar resources (Foil *et al.*, 2004; Cabezas-cruz *et al.*, 2018). Interactions between pathogens within the host are so intense that certain mechanisms of a pathogen inside the host or inside a population cannot be fully comprehended without understanding the other coexisting pathogens (Gayle & Ringdahl, 2001; Cabezas-cruz *et al.*, 2018). The pathogens that are transmitted by ticks infesting dogs in South Africa are summarised in Table 2.1.

Table 2-1: Summary of pathogens transmitted by ticks infesting dogs in South Africa

Tick-borne pathogen	Primary vector	Primary host	Associated disease	Geographical distribution	References
<i>Ehrlichia canis</i>	<i>R. sanguineus</i>	Canids	Canine monocytic ehrlichiosis	Worldwide, excluding Australia	Gayle & Ringdahl (2001), Shaw <i>et al.</i> (2001), Chomel (2011), and Wall (2016)
<i>Anaplasma phagocytophylum</i>	<i>R. sanguineus</i> , <i>Ixodes persulcatus</i> group, <i>I. scalpularis</i> , <i>I. ricinus</i> and <i>I. pacificus</i>	Humans and mammals	Human Granulocytic Anaplasmosis and Canine Granulocytic Anaplasmosis	The United States of America and Europe	Shaw <i>et al.</i> (2001), Chomel (2011), Wall (2016), and Madison-Antenucci <i>et al.</i> (2020)
<i>Babesia canis</i>	<i>R. sanguineus</i> , <i>Dermacentor reticulatus</i> and <i>D. marginatus</i>	Dogs	Canine babesiosis	Tropical, as well as semitropical worldwide distribution	Shaw <i>et al.</i> (2001), Chomel (2011) and Wall (2016)
<i>Babesia vogeli</i>	<i>R. sanguineus</i>	Dogs	Canine babesiosis	Tropical, as well as semitropical worldwide distribution	Shaw <i>et al.</i> (2001), Chomel (2011), and Wall (2016)
<i>Babesia rossi</i>	<i>H. elliptica</i>	Dogs	Canine babesiosis	Southern Africa	Shaw <i>et al.</i> (2001), Chomel (2011), and Wall (2016)
<i>Coxiella burnetii</i>	Several tick species	Humans and several mammals	Q-fever	Worldwide distribution	Wall (2016) and Boulanger <i>et al.</i> (2019)

Table 2-1: (continue)

Tick-borne pathogen	Primary vector	Primary host	Associated disease	Geographical distribution	References
<i>Rickettsia</i> spp.	<i>R. sanguineus</i> (responsible for the spread of <i>R. conorii</i> and <i>R. conorii caspia</i>) and <i>R. pumilio</i> (responsible for the spread of <i>R. conorii caspia</i>)	Humans (<i>R. conorii</i> and <i>R. conorii caspia</i>) and dogs (<i>R. conorii caspia</i>)	Mediterranean spotted fever (associated with <i>R. conorii</i>) and Asrakhan fever (associated with <i>R. conorii caspia</i>)	<i>R. conorii</i> and <i>R. conorii caspia</i> are endemic to Asia and Africa, where <i>R. conorii</i> is also endemic to Europe	Gayle & Ringdahl (2001), Shaw <i>et al.</i> (2001), Chomel (2011), Wall (2016), and Boulanger <i>et al.</i> (2019)
<i>Hepatozoon canis</i>	<i>R. sanguineus</i> , <i>Amblyomma maculatum</i> and <i>H. longicornis</i>	Dogs	Hepatozoonosis	Africa, the Middle East, Eastern Asia, southern Europe, and the southern United States of America	Shaw <i>et al.</i> (2001), Chomel (2011), and Wall (2016)

2.7.1 *Anaplasma phagocytophylum*

2.7.1.1 Background and systematics of *Anaplasma phagocytophylum*

Anaplasma phagocytophylum is a rickettsial organism, causing acute febrile disease, mainly infecting the host neutrophils and are transmitted by ticks (Woldehiwet, 2010). Originally this pathogen was described in sheep originating from Scotland during the early 1930s. The *A. phagocytophylum* responsible for anaplasmosis in dogs was first reported during the 1980s from North America, Sweden, and Switzerland. In cats, granulocytotropic anaplasmosis was first reported during the late 1990s in Sweden. Since then several case reports demonstrated that the infection exists in northern temperature zone countries (Dumler *et al.*, 2005; Parola *et al.*, 2005). With the improvement of gene sequencing techniques used to examine *A. phagocytophylum*, it has been renamed and reclassified numerously. Formerly, this organism was named *Ehrlichia phagocytophila*, *Ehrlichia phagocytophylum*, *Ehrlichia equi*, *Cytoecetes phagocytophilia*, and also referred to as human granulocytic *Ehrlichia* agent (Parola *et al.*, 2005; Harrus *et al.*, 2016). The taxonomic classification of *A. phagocytophylum* is summarised in Table 2.2 (Dehhaghi *et al.*, 2019).

Table 2-2: Taxonomic classification of *A. phagocytophylum*

Taxonomic classification	
Domain	Bacteria
Phylum	Proteobacteria
Class	Alphaproteobacteria
Order	Rickettsiales
Family	Ehrlichiaeae
Genus	<i>Anaplasma</i>
Species	<i>A. phagocytophylum</i>

2.7.1.2 Life cycle, bacteriology, and pathogenesis of *Anaplasma phagocytophylum* infections

Anaplasma phagocytophylum is an obligate intracellular gram-negative organism that primarily infects cells originating from bone marrow. They are located in host cells and occur within membrane-bound vacuoles in the cytoplasm. Multiplication of organisms occurs using binary fission and ultimately form morulae or large inclusion bodies. In circulating neutrophils the cytoplasmic inclusions may be distinguished for 1 to 2 weeks if the host remains untreated (Dumler *et al.*, 2005; Parola *et al.*, 2005). Reservoir hosts of *A. phagocytophylum* include rodents, as well as wild and domestic ruminants, such as sheep and deer. The agricultural and local natural landscape determines the predominant reservoir host (Woldehiwet, 2010). Primarily the *Ixodes* tick species serve as *A. phagocytophylum* vectors and are transmitted using transstadial transmission. Ticks feed on several vertebrate animals enabling the transmission of *A. phagocytophylum* to numerous host species. Within 24 to 48 hours of feeding transmission of the pathogen from vector to host occurs (Harrus *et al.*, 2016; Boulanger *et al.*, 2019).

When ticks attach to the host to feed, *A. phagocytophylum* enters the dermis through tick-bite immunization (Fritz, 2009; Harrus *et al.*, 2016). For primary replication it is unknown whether *A. phagocytophylum* invades precursor myeloid cells or mature cells, its suggested targets are hematopoietic cells, endothelial cells, and megakaryocytes (Harrus *et al.*, 2016; Boulanger *et al.*, 2019). The *A. phagocytophylum* adheres and binds to molecules on the target cell membrane, such as sLe^x and PSGL-1, where they infect neutrophils. The pathogens then enter the neutrophil by endocytosis (Carlyon & Fikrig, 2003; Harrus *et al.*, 2016). Generally, the pathogen is observed in peripheral blood, within eosinophils, as well as mature granulocytes primarily in neutrophils. The *A. phagocytophylum* multiplies in cytoplasmic vacuoles after endocytosis (Chomel, 2011; Harrus *et al.*, 2016). This pathogen has several virulence factors including the capability to prevent degradation of vacuoles containing *A. phagocytophylum* by avoiding lysosome fusion with the aforementioned *Anaplasma*-containing vacuoles (Brown *et al.*, 2005; Harrus *et al.*, 2016). Co-infections of *Anaplasma* with several *Borrelia*, *Babesia*, *Bartonella*, *Ehrlichia*, *Rickettsia*, and arboviral species are possible in animals as well as humans. These co-infections may cause pathological and clinical irregularities, fluctuating from asymptomatic infection to mortality (Carlyon & Fikrig, 2003; Dumler *et al.*, 2005). It was previously demonstrated that ticks infected with both *Borrelia burgdorferi* and *A. phagocytophylum* transmit both pathogens to hosts as effectively as ticks infected with a single pathogen. Granulocytotropic anaplasmosis is often reported in geographical areas where borreliosis are endemic, as *A. phagocytophylum* and *B. burgdorferi* share similar vectors and reservoir hosts (Carlyon & Fikrig, 2003; Harrus *et al.*, 2016).

2.7.1.3 Clinical characteristics, diagnosis and treatment of *Anaplasma phagocytophylum* infections

Dogs diagnosed with granulocytotropic anaplasmosis usually have a history of anorexia and lethargy. Reluctance to move, fever as well as occasional splenomegaly, and phadenomegaly are also clinical signs of *A. phagocytophylum* infections (Dumler *et al.*, 2005; Madison-Antenucci *et al.*, 2020). Sometimes more localized signs are present respectively in the gastrointestinal, respiratory, and musculoskeletal systems depicted by clinical signs such as diarrhea, coughing, and lameness (Brown *et al.*, 2005; Dumler *et al.*, 2005). Multi-organ failure, shock, or haemorrhage may indicate systematic manifestations (Dumler *et al.*, 2005; Chomel, 2011). Generally, mild and subclinical infections are often depicted by sero-epidemiological data (Dumler *et al.*, 2005; Boulanger *et al.*, 2019). The disease is also depicted distinguished by mild to severe leucopenia and thrombocytopenia (Dumler *et al.*, 2005; Stanneck *et al.*, 2012).

The *A. phagocytophylum* infections are often considered when patients present an acute febrile illness during seasons when ticks seek hosts in endemic geographical locations (Woldehiwet, 2010; Stuen *et al.*, 2013). Diagnosis may also be presented by examinations of Wright's-stained blood smears, but *Anaplasma* spp. may be difficult to detect as the morulae are tough to detect or are often scarce (Dumler *et al.*, 2005; Stuen *et al.*, 2013). Diagnosis is also done by using *A. phagocytophylum*-specific primers in PCR assays of blood, tissue, cerebrospinal fluid, and synovial fluid samples (Stuen *et al.*, 2013; Madison-Antenucci *et al.*, 2020). Serological tests used for diagnosis include ELISA, immunoblot analysis, or indirect IFAT using anti-*A. phagocytophylum* IgG antibody assessment (Brown *et al.*, 2005; Stuen *et al.*, 2013). Diagnosis may also be done by using *in-vitro* culture of *Anaplasma*, but are limited to certain laboratories (Chomel, 2011; Stuen *et al.*, 2013).

Doxycycline is considered to be the most effective and first choice of treatment for cats and dogs diagnosed with granulocytotropic anaplasmosis. Numerous intracellularly active antibiotics, such as tetracyclines, are susceptible to *in vitro* *A. phagocytophylum* (Wiebe, 2015). To date, no vaccines are available for protection or post-exposure prophylaxis (Harrus *et al.*, 2016). Avoiding tick-infected areas or applying residual acaricidal products are the most reliable way to prevent *A. phagocytophylum* infections. For animals spot-on liquid, spray, or collar formulations to repel ticks may be used (Uilenberg & Jongejan, 2004). During blood transfusions routine screening is recommended as blood from donors may be infected with *A. phagocytophylum* (Woldehiwet, 2010; Stuen *et al.*, 2013; Harrus *et al.*, 2016).

2.7.1.4 Zoonotic potential of *Anaplasma phagocytophylum* infections

The extensive feeding behaviour of ticks allows the transmission of *A. phagocytophylum* to several vertebrate species including humans as previously reported in Europe. It was indicated by analysing seroprevalence data that the infection is minor and self-resolving (Betancur *et al.*, 2015; Harrus *et al.*, 2016). Human granulocytotropic anaplasmosis common clinical signs are often displayed by headache and fever. Infections are also often displayed by non-specific symptoms including stiffness, arthralgia, malaise, and myalgia (Dumler *et al.*, 2005; Harrus *et al.*, 2016). Respectively, other clinical signs include confusion, non-productive cough, as well as vomiting, nausea, and diarrhea displaying the involvement of the central nervous, respiratory and gastrointestinal systems (Stuen *et al.*, 2013; Harrus *et al.*, 2016). On occasion, skin rashes were reported where host inflammatory response or involvement of coexisting pathogens was suggested (Woldehiwet, 2010; Harrus *et al.*, 2016). Severe complications of human granulocytotropic anaplasmosis include death, prolonged fever, adult respiratory distress syndrome, shock, opportunistic infections, seizures, rhabdomyolysis, acute kidney injury, and pneumonitis (Chomel, 2011; Betancur *et al.*, 2015).

2.7.2 *Coxiella burnetii*

2.7.2.1 Background and systematics of *Coxiella burnetii*

Coxiella burnetii is a gram-negative, spore-forming, obligate intracellular bacterium (Baca & Paretsky, 1983; Pfeffer *et al.*, 2016). The spores are highly resistant to environmental conditions (Van den Brom *et al.*, 2015; Pfeffer *et al.*, 2016). The taxonomic classification of *C. burnetii* is summarised in Table 2.3 (Saini & Gupta, 2021).

Table 2-3: Taxonomic classification of *C. burnetii*

Taxonomic classification	
Domain	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Legionellales
Family	Coxiellaceae
Genus	<i>Coxiella</i>
Species	<i>C. burnetii</i>

2.7.2.2 Life cycle, bacteriology, and pathogenesis of coxiellosis

Transmission of *C. burnetii* occurs in several ways. In the wild, the pathogen is transmitted by arthropods, generally by ticks using transstadial and transovarial transmission (Baca & Paretsky, 1983; Van den Brom *et al.*, 2015). Ticks have an important role in the natural conservation of *C. burnetii* but they are not important vectors when it comes to human and animal infections (Baca & Paretsky, 1983; Kazar, 2005). The *C. burnetii* may be spread between hosts when the spores are spread into the environment through infected fluids, including urine, milk, uterine or vaginal secretions as well as ingestion of infected tissues, such as placental material, or contaminated food or milk (Kazar, 2005; Van den Brom *et al.*, 2015).

The *C. burnetii* are highly contagious to such an extent that a single organism is required to cause infection (Parola & Raoult, 2001; Van den Brom *et al.*, 2015). Multiplication mainly occurs in the regional lymph nodes where bacteraemia occurs for 5 to 7 days. Afterward, *C. burnetii* are constricted to the host mammary glands or the placenta of pregnant female animals (Fritz, 2009; Van den Brom *et al.*, 2015). Transient bacteraemia may occur where infection may be located in the liver, reproductive tract, spleen, bone marrow, or several other organs as a result of haematogenous spread. As a result of the above-mentioned, granulomatous lesions, located in the liver and bone marrow, may form (Van den Brom *et al.*, 2015; Boulanger *et al.*, 2019).

In pregnant animals, *C. burnetii* may persist following the initial acute phase. During the initial pregnancy period up to 13 weeks, the organisms may be located in the bone marrow, nodes,

kidneys, liver, and intestine. The placenta, amniotic, as well as other fluids, becomes infected with the organism (Kazar, 2005; Van den Brom *et al.*, 2015). When the animal gives birth, contaminated *C. burnetii* aerosols are released into the environment. At this stage, antibodies multiply, most likely the IgG antibodies will increase. In the following pregnancies, these organisms will be either be released to a lesser extent or will not be released. In addition, this organism may be present in the milk of animals after birth (Baca & Paretsky, 1983; Van den Brom *et al.*, 2015).

2.7.2.3 Clinical characteristics, diagnosis, treatment, and control of *Coxiella burnetii* infections

In domestic animals, including in dogs and cats, the bacterium is responsible for the subclinical infection. In humans, *C. burnetii* infections are associated with Q-fever where the clinical signs include hepatitis, fever, myalgia, arthralgia, and respiratory signs (Parola & Raoult, 2001; Eldin *et al.*, 2017).

Diagnosis of infections is determined by using molecular techniques, such as using PCR and species-specific primers (Fritz, 2009; Eldin *et al.*, 2017). Serological tests demonstrating raising antibody titres may also be used to confirm *C. burnetii* infections (Brown *et al.*, 2005; Eldin *et al.*, 2017). Tissue biopsy samples are also used for diagnosis by using immunohistochemical techniques (Kazar, 2005; Eldin *et al.*, 2017).

The *C. burnetii* infections are susceptible for 2 to 4 weeks with potentiated sulphonamides, fluoroquinolones, or macrolides, such as azithromycin or erythromycin. A combination of fluoroquinolones and doxycycline with rifampicin is also efficient for treatment. A combination of chloramphenicol and tetracycline is generally used for clinical infections in cats (Wiebe, 2015).

Since *C. burnetii* has a tropism for the placenta and uterus it is released in exceedingly high doses in the environment when infected ruminants deliver or miscarry. For this reason personal protective equipment, including masks and gloves, should be outfitted (Kazar, 2005; Pfeffer *et al.*, 2016). Areas with potential infections should be avoided, such as where arthropods or spore-containing aerosols may be spread (Uilenberg & Jongejan, 2004). Currently, there are several vaccines available for the prevention of *C. burnetii*. The inactivated *C. burnetii* vaccines, sometimes also referred to as Coxevac, are commercially available to use for sheep, and clinical trials demonstrated that it is effective for goats from birth to the age of 16 months. The Q-vax *C. burnetii* vaccines are commercially available used for livestock and it is commercially available for the use of humans in Australia, where it is primarily given to laboratorians, farmers, as well as

abattoir workers (Kersh *et al.*, 2013; Scholzen *et al.*, 2019). There is however no licensed vaccine for dogs or cats (Pfeffer *et al.*, 2016).

2.7.2.4 Zoonotic potential of *Coxiella burnetii* infections

In general, domestic ruminants are regarded as reservoirs for human infections. However, several domestic and wild animals may be infected with *C. burnetii* (Betancur *et al.*, 2015). Cats infected with *C. burnetii* are regarded as vital reservoirs for human coxiellosis. The aforementioned may be as a result of *C. burnetii* that is often present in the vaginal area of healthy cats living in endemic areas of the pathogen and as a result contact between humans and infected expectant cats may pose a risk factor (Fritz, 2009; Pfeffer *et al.*, 2016; Boulanger *et al.*, 2019). In previous studies, cross-reactivity was reported between *Bartonella henselae* and *C. burnetii* in humans (Baca & Paretsky, 1983; Pfeffer *et al.*, 2016). Reports of *C. burnetii* infections in dogs are limited. However, it is suggested that dogs may be implicated during *C. burnetii* outbreaks. In Canada, there was a case where a dog was reportedly responsible for *C. burnetii* infection between family members (Buhariwalla *et al.*, 1996). Other cases of *C. burnetii* infections in dogs were respectively reported in the Netherlands and Brazil utilizing PCR and using culture depended techniques (Pfeffer *et al.*, 2016).

2.7.3 *Ehrlichia canis*

2.7.3.1 Background and systematics of *Ehrlichia canis*

Ehrlichia canis is a small, obligatory intracellular, coccoid, pleomorphic, gram-negative bacterium (Rikihisa, 1991; Harrus *et al.*, 2016). The *E. canis* is the disease-causing agent of canine monocytic ehrlichiosis or CME, formerly known as tropical canine pancytopenia, primarily infecting domestic dogs and members of the Canidae family. Globally, CME is recognized as a significant canine disease (Shaw *et al.*, 2001; Harrus *et al.*, 2016). The infection potential of CME of cats and the associated disease is still to be fully understood (Harrus *et al.*, 2016). Transmission occurs by *R. sanguineus* ticks, using transstadial transmission, or by blood transfusions, when infected blood is given to hosts (Rikihisa, 1991; Harrus *et al.*, 2016). Ticks become infected during the larvae and nymphal developmental stages when feeding occurs on infected dogs or hosts become infected when bacteriaemic ticks feed to reach to nymphal or adult developmental stages. Infected ticks that entered diapause still can transmit the pathogen to hosts once environmental conditions are favourable and diapause ended (Parola & Raoult, 2001; Harrus *et al.*, 2016). Geographical distribution and occurrence of *E. canis* infections are dependent on vector distribution. Respectively, most cases of CME are reported during the summer and spring seasons and have a global distribution, as *R. sanguineus* ticks favour warm temperatures and

are globally distributed (Brown *et al.*, 2005; Harrus *et al.*, 2016). The taxonomic classification of *E. canis* is summarised in Table 2.4 (Garrity *et al.*, 2004).

Table 2-4: Taxonomic classification of *E. canis*

Taxonomic classification	
Domain	Bacteria
Phylum	Proteobacteria
Class	Alphaproteobacteria
Order	Rickettsiales
Family	Ehrlichiaeae
Genus	<i>Ehrlichia</i>
Species	<i>E. canis</i>

2.7.3.2 Life cycle, bacteriology, and pathogenesis of ehrlichiosis

The *E. canis* organisms cluster together, termed morulae, where they invade the circulating monocytes intracytoplasmically (Parola & Raoult, 2001; Waner, 2008). Upon infection, CME has an incubation period of 8 to 20 days. During the incubation period, *E. canis* infiltrate the bloodstream and lymphatics. Subsequently, they locate and settle in the macrophages, primarily in the liver and spleen, where they duplicate, utilizing binary fusion. Infected macrophages then distribute and enable the infection to other organ systems (Arolaa *et al.*, 2005; Waner, 2008). An acute, subclinical and chronic phase may follow the incubation period (Waner, 2008; Fritz, 2009). The acute phase lasts a period of 1 to 4 weeks. During this phase, recovery is possible considering dogs receive suitable treatment. Improperly treated or dogs left completely untreated move into the subclinical phase (Chomel, 2011; Waner, 2008). During this phase, hosts may be frequent carriers of this pathogen for months or even years. After the subclinical phase, some infected dogs develop the chronic phase of CME, whereas others instinctively recover. The reasons and leading factors remain unclear why not all infected dogs enter the chronic phase. The diagnosis during the chronic phase is critical with the possibility of death due to secondary infection or haemorrhage (Waner, 2008; Chomel, 2011).

Auto-agglutination tests and positive Coombs indicate that several immunological mechanisms are implicated in the disease pathogenesis using antibody production and complement proteins binding to erythrocyte membranes (Waner, 2008; Fritz, 2009). Another immunological mechanism includes the presence of platelet-bound antibodies that are possibly involved in thrombocytopenia pathogenesis, resulting in a reduction in the lifespan of platelets and CME thrombocytopenia (Parola & Raoult, 2001; Waner, 2008). Dogs that were experimentally and naturally infected with *E. canis* displayed circulating immune complexes in the dog's sera (Parola *et al.*, 2005; Waner, 2008).

2.7.3.3 Clinical characteristics, diagnosis, treatment, and control of *Ehrlichia canis* infections

All dog breeds are susceptible to *E. canis* (Rikihisa, 1991; Chomel, 2011). CME infections are exhibited by several clinical signs. Clinical signs are influenced by different factors including, host immunity, dog breed, *E. canis* strain pathogenicity potential, and presence of other pathogens that may result in co-infections (Shaw *et al.*, 2001; Chomel, 2011). In general, the non-specific signs include weight loss, anorexia, depression, tachypnoea, pyrexia, and lethargy (Fritz, 2009; Chomel, 2011). Specific clinical signs include splenomegaly, lymphadenomegaly, infrequent epistaxis, as well as ecchymoses and petechiae of the mucus membranes and skin (Parola *et al.*, 2005; Chomel, 2011). Other less frequently reported clinical signs include dyspnoea, oculonasal discharge, and vomiting (Parola & Raoult, 2001; Chomel, 2011). Additional clinical signs that may be present during the chronic severe form of CME include emaciation, pale mucus membranes, as well as peripheral oedema mainly of the scrotum and hind limbs (Shaw *et al.*, 2001; Chomel, 2011). Renal failure, interstitial pneumonia, as well secondary protozoal and bacterial infections may also be present during the chronic severe form (Rikihisa, 1991; Chomel, 2011). During the acute and chronic phases of CME ocular signs were previously reported, including corneal oedema, iridal petechiae, conjunctivitis, as well as hyphaema, and panuveitis. Hyperviscosity and gammopathy may cause retinal detachment and subretinal haemorrhage resulting in blindness (Parola & Raoult, 2001; Chomel, 2011). Neurological signs may also transpire throughout the acute and chronic phases of CME including paresis, ataxia, hyperesthesia, seizures, cranial nerve deficits, as well as vestibular signs. In addition, neurological signs may be endorsed by extensive monocytic and lymphoplasmacytic infiltration, gliosis, and perivascular cuffing demonstrating meningoencephalitis or meningitis. Infrequently, dogs displaying neurological signs may have the presence of morulae in the cerebrospinal fluid (Parola *et al.*, 2005; Chomel, 2011).

CME diagnosis is based on clinical-pathological findings, clinical presentation, and compatible history in along with PCR, serology, or *in vitro* culture methods (Chomel, 2011; Harrus & Waner,

2011). During the acute phase of infections, haematology and blood smears may be used to visualize the intracytoplasmic *E. canis* morulae in monocytes (Rikihisa, 1991; Harrus & Waner, 2011). The use of an immunofluorescent antibody test, commonly referred to as IFAT, is considered the standard serology test to detect the presence of *E. canis* and for the titration of antibodies (Parola & Raoult, 2001; Harrus & Waner, 2011). The *E. canis* IgG antibody detection is also possible by using conventional enzyme-linked immunosorbent assays, also known as ELISA (Parola *et al.*, 2005; Harrus & Waner, 2011). Respectively, conventional PCR and sequencing are used for the detection and characterization of *E. canis* DNA (Rikihisa, 1991; Harrus & Waner, 2011). Real-time PCR (qPCR) permits the quantitative analysis of particular DNA (Parola & Raoult, 2001; Harrus & Waner, 2011). More recently, veterinary diagnoses are used utilizing point-of-care loop-mediated isothermal amplification (LAMP) methods (Parola *et al.*, 2005; Harrus & Waner, 2011). Co-infections of *E. canis* with *Babesia* sp. or *Hepatozoon canis* are common thus the microscopic examination of blood smears, PCR, or serological screening is often used to determine the presence of co-infecting pathogens (Shaw *et al.*, 2001; Harrus & Waner, 2011).

The *E. canis* infections are treated by using doxycycline, whereas tetracycline hydrochloride, minocycline, oxytetracycline, and chloramphenicol are also reportedly successful. A combination of Imidocarb dipropionate and doxycycline was also previously reported to treat CME. Using low doses of oxytetracycline were previously reported as a prophylactic measure to be used in endemic areas (Wiebe, 2015). The *E. canis* antibody titres may still be present for months or even years. The presence of high antibody titres for prolonged periods may indicate treatment failure or an unusual immune response, whereas continuing reductions in the gammaglobulin concentrations indicate elimination of the organisms. The *E. canis* antibodies do not protect the host from future CME infections and reinfections after successful treatment remains a possibility (Harrus & Waner, 2011; Harrus *et al.*, 2016). An attenuated *E. canis* strain originating from Israel was determined to potentially serve as an effective vaccine but commercially no anti-*E. canis* vaccines are on the market (Harrus *et al.*, 2016). To date, the management of infected ticks serves as the best preventive measure of *E. canis* infections (Parola & Raoult, 2001; Uilenberg & Jongejan, 2004).

2.7.3.4 Zoonotic potential of *Ehrlichia canis* infections

The *E. canis* was suspected of human infections, between the years of 1987 to 1991, but the cause of the human monocytic ehrlichial disease was later determined as *Ehrlichia chaffeensis* (Rikihisa, 1991; Harrus *et al.*, 2016). Even though *E. canis* is not regarded as a zoonotic agent, a

human in Venezuela was infected with an *E. canis*-like agent, indicating that *E. canis* zoonotic potential still needs to be fully understood (Harrus *et al.*, 2016).

2.7.4 *Rickettsia* species

2.7.4.1 Background and systematics of *Rickettsia* species

Rickettsia spp. is an obligate intracellular, gram-negative microorganism (Parola *et al.*, 2005; Harrus *et al.*, 2016). Members of the *Rickettsia* genus are transmitted by ticks, mites, and fleas and are globally distributed where infected vectors are present. Several *Rickettsia* spp. are transmitted by ticks through transstadial and transovarial transmission (Harrus *et al.*, 2016; Schroeder *et al.*, 2016). *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever, also known as RMSF, is the most pathogenic and well-known member of the *Rickettsia* spotted fever group, also known as SFG. Infections of *R. rickettsii* are conceivably fatal and humans, as well as dogs diagnosed with RMSF, are equally affected. In cats, the naturally occurring disease has not been reported. Although several tick species are implicated to spread RMSF, *R. sanguineus* are considered as important vectors in the transmission of *Rickettsia* (Chomel, 2011; Boulanger *et al.*, 2019). *Rickettsia conorii*, which is the causative agent of Mediterranean spotted fever or Boutonneuse fever, is similar to *R. rickettsii* and is present in areas of Africa, Europe, and Asia. The *R. conorii* are transmitted mainly by ticks belonging to the *Rhipicephalus* genus and the main animal reservoir animals of this pathogen are rodents and dogs. Infected dogs often appear to be asymptomatic but they serve as reservoir hosts for human infections and may assist the transportation of infected ticks (Parola *et al.*, 2005; Chomel, 2011). Other significant SFG rickettsiae include *R. africae* (the causative agent of African tick bite fever), *R. australis* (the causative agent of Queensland tick typhus), *R. honei* (the causative agent of Flinder's Island spotted fever), *R. japonica* (the causative agent of Japanese spotted fever), *R. sibirica* (the causative agent of North Asian tick typhus), as well as *R. slovaca*, *R. mongolotimonae* and *R. helvetica* (associated with unnamed European rickettsioses). All of the SFG rickettsiae mentioned above are known to infect humans, are transmitted by arthropods (Chomel, 2011; Boulanger *et al.*, 2019). The taxonomic classification of *Rickettsia* is summarised in Table 2.5 (Ogata & Claverie, 2005).

Table 2-5: Taxonomic classification of *Rickettsia*

Taxonomic classification	
Domain	Bacteria
Phylum	Proteobacteria
Class	Alphaproteobacteria
Order	Rickettsiales
Family	Rickettsiaceae
Tribe	Rickettsieae
Genus	<i>Rickettsia</i>

2.7.4.2 Life cycle, bacteriology, and pathogenesis of rickettsiosis

Upon inoculation into the body, *Rickettsia* spp. goes into the bloodstream, infects the endothelial cells, and replicates. As the pathogens cause endothelial necrosis to spread and infect new endothelial cells, extensive vasculitis occurs (Parola *et al.*, 2005; Schroeder *et al.*, 2016). Fibrinolytic and coagulation pathways are stimulated when a vascular injury occurs (Azad & Beard, 1998; Schroeder *et al.*, 2016). Coagulatory mechanisms may be a result of platelet consumption, whereas immune-mediated mechanisms may be from platelet destruction (Nicholson *et al.*, 2010; Schroeder *et al.*, 2016). Cases that are potentially severe or left untreated for extended periods may result in numerous focal necrosis of organ systems, like the kidneys, skin, heart, and brain, with end-arterial circulation (Shaw *et al.*, 2001; Schroeder *et al.*, 2016). Infected humans usually experience organ failure more commonly than dogs (Fritz, 2009; Schroeder *et al.*, 2016). Oedema formation is usually a result of intravascular fluids leaking into extravascular fluid spaces caused by vascular injury. Cardiorespiratory or progressive mental depression may be a result of tissues, such as the central nervous system, that accumulate fluids leading to sizable brain oedema (Parola *et al.*, 2005; Schroeder *et al.*, 2016).

2.7.4.3 Clinical characteristics, diagnosis, treatment, and control of *Rickettsia* species infections

Clinical signs of *Rickettsia* spp. infections in dogs are often displayed by maculopapular rashes on exposed skin and the ears, fever, petechial lesions on the mucus membranes, tremors,

lethargy, scleral injection, and appetite loss (Parola *et al.*, 2005; Nicholson *et al.*, 2010). During the spring and summer seasons, when the weather is more preferable for ticks, most dogs seem to develop this illness. The aforementioned are less evident in areas that have lower altitude (Azad & Beard, 1998; Parola *et al.*, 2005). One of the most consistent clinical signs of *Rickettsia* spp. infection is a developing fever. After exposure to infected ticks, fever develops within days and is generally associated with mental dullness, lethargy, and inappetence (Parola *et al.*, 2005; Schroeder *et al.*, 2016). Infected animals may experience difficulty in rising, standing, and ultimately be hesitant to walk as gaits stiffen, as demonstrated by myalgia and arthralgia (Shaw *et al.*, 2001; Parola *et al.*, 2005). Lymphadenomegaly may be evident in peripheral lymph nodes (Arolaa *et al.*, 2005; Parola *et al.*, 2005). Subcutaneous oedema and hyperaemia may develop mucosal surfaces (Philippe Parola & Raoult, 2001; Parola *et al.*, 2005). In the case of severe infections dermal necrosis arises (Arolaa *et al.*, 2005; Parola *et al.*, 2005). Infected male dogs may develop scrotal oedema (Parola *et al.*, 2005; Fritz, 2009). In exposed skin areas a macropapular skin rash may be present and may spread from the ears to the trunk and limbs (Shaw *et al.*, 2001; Parola *et al.*, 2005). Unlike humans, infected dogs hardly develop petechial haemorrhages. In the rare cases that dogs develop petechial haemorrhages, it is located in the mucus membranes of the gums and buccal mucosa (Parola *et al.*, 2005; Nicholson *et al.*, 2010). Infected male dogs may have fine petechiae present on the penile mucosa (Azad & Beard, 1998; Parola *et al.*, 2005). In the conjunctiva petechiae and sclera vascular injection may occur (Parola *et al.*, 2005; Schroeder *et al.*, 2016). Haemorrhages may be present more frequently within the ocular fundus. Severely infected animals may demonstrate overt haemorrhage, disseminated intravascular coagulation, or necrosis of the extremities (Shaw *et al.*, 2001; Parola *et al.*, 2005). Diagnosis delay and lack of treatment may result in animals suffering neurological complications including vestibular dysfunction, seizures, hyperaesthesia, as well as several manifestations dependant on the location of the lesion. After the development of neurological signs, permanent deficits or delayed recovery may occur (Parola & Raoult, 2001; Parola *et al.*, 2005).

Diagnosis is done by using tissues for direct immunolabelling. For example, full-thickness skin biopsy samples may be used to determine if *Rickettsia* spp. is present in dermal blood vessels (Azad & Beard, 1998; Schroeder *et al.*, 2016). Molecular detection methods, such as PCR, are also used to determine the presence of *Rickettsia* spp. in blood and tissue samples (Azad & Beard, 1998; Parola *et al.*, 2005). Diagnosis may also be done by microscopic examination, where several organs will have distributed necrotizing vasculitis (Azad & Beard, 1998; Nicholson *et al.*, 2010). Cerebrospinal analysis may reveal neutrophil pleocytosis and protein increases (Azad & Beard, 1998; Parola *et al.*, 2005). Joint fluid analysis generally reveals increases in cell counts where the majority of cells are neutrophils (Azad & Beard, 1998; Parola & Raoult, 2001).

Pulmonary interstitial density increases may be demonstrated by thoracic radiography (Azad & Beard, 1998; Schroeder *et al.*, 2016). The presence of specific IgM and IgG antibodies may be demonstrated by using IFAT (Azad & Beard, 1998; Parola *et al.*, 2005).

A combination of early treatment with suitable antibiotics often results in complete and fast recovery (Behravesh & Massung, 2016; Schroeder *et al.*, 2016). Due to the fatality of the disease and sometimes a delay in diagnosis, it is suggested that empirical treatment should be started if *Rickettsia* spp. infection is suspected (Parola *et al.*, 2005; Behravesh & Massung, 2016). Treatment with tetracycline or doxycycline is highly suggested, especially to prevent the infection to return in dogs. If these antibiotics are not available chloramphenicol may also be used as a last option, as it may result in fatalities in human patients (Wiebe, 2015). There are several vaccines available for *Rickettsia* spp. Including a live vaccine from the attenuated Mandrid E (E) strain of *R. prowazekii*, inactivated vaccines, and live vaccines of *R. typhi* (Osterloh, 2020). Unfortunately, there is no licensed vaccine for dogs to prevent tick-borne rickettsial infections (Behravesh & Massung, 2016). It is highly suggested to wear gloves when removing vectors, especially ticks, from infected animals and cleaning the bite mark (Behravesh & Massung, 2016; Schroeder *et al.*, 2016). Regular checks should be done to determine the presence of ticks, especially when spending time outdoors in known tick areas (Shaw *et al.*, 2001; Behravesh & Massung, 2016). Tick repellents should also be applied and pet ectoparasite control products, including acaricidal shampoo, tick collars, or topical acaricides, may also reduce exposure to ticks (Uilenberg & Jongejan, 2004).

2.7.4.4 Zoonotic potential of *Rickettsia* species infections

Rickettsial infections, especially RMSF, is an important zoonotic disease due to the potential fatality of humans if a diagnosis is delayed or treatment fails (Betancur *et al.*, 2015). For RMSF outbreaks in humans, dogs may act as sentinels. In addition, dogs may also be transporting hosts when they carry infected ticks into human households. Due to the potential of large urban outbreaks, resulting in household clustering and areas infected with potentially infected *R. sanguineus* ticks, RMSF is a public health concern (Behravesh & Massung, 2016; Schroeder *et al.*, 2016). Infection rates of people and dogs are normally parallel as both are exposed to similar environments. A case of household clustering rickettsial infection occurred when two dogs and subsequently their owner were infected and died in a time of three weeks (Behravesh & Massung, 2016).

2.8 Genetic identification of ticks and detection of tick-borne pathogens

2.8.1 Principle of PCR

Polymerase chain reaction (PCR) is a technique that is used for gathering a large quantity of the desired DNA sequence from a DNA sample. The technique is based on *in vitro* replication by targeting specific DNA sequences using DNA polymerase (Ward *et al.*, 2016; Kadri, 2019). If the desired sequence is present the use of PCR will selectively amplify the sequence in very large quantities (Valones *et al.*, 2009; Kadri, 2019). The process of PCR takes place in a thermal cycler where tubes contain reaction mixtures (Templeton *et al.*, 2003; Kadri, 2019). Each reaction mixture consists of the template DNA, the two DNA primers, *Taq* polymerase as well as the dNTPs (the four deoxyribonucleoside triphosphates) in surplus in a buffer solution (Flori *et al.*, 2004; Kadri, 2019). The DNA template contains the portion of the target DNA that needs to be amplified. DNA polymerase is the heat-resistant enzyme that polymerizes the new DNA strands. The primers serve as the initiation site where the DNA polymerase binds to start elongation. Primers are complementary to the 3' end of the sense as well as the antisense strands of the target DNA. The dNTPs are the nucleotides that contain the triphosphate groups and serve as the building blocks that are used where the DNA polymerase synthesizes a new DNA strand from. The buffer solution stabilizes the DNA polymerase and creates an appropriate chemical environment for optimal activity (Wacharapluesadee *et al.*, 2011; Kadri, 2019). The thermal cycler heats and cools the reaction mixtures (Cullen *et al.*, 2001; Kadri, 2019). The process of PCR is illustrated in Figure 2.4. In general, PCR consists of 20 to 40 thermal cycles where the temperatures are repeatedly changed. Each thermal cycle is made up of different stages, namely denaturation, annotation, and elongation (Flori *et al.*, 2004; Kadri, 2019). These cycles are preceded by a single denaturation phase, referred to as initial denaturation, and the cycles are ended by a final single stage for extending DNA products, referred to as final elongation, or for storage for a brief period, referred to as final hold (Valones *et al.*, 2009; Kadri, 2019). During the initial denaturation phase, the temperature is raised to more than 90°C. The denaturation phase is where the thermal cycling starts at temperatures between 94 to 98°C for 20 to 30 seconds (Cullen *et al.*, 2001; Kadri, 2019). During the denaturation phase, the double-stranded DNA is separated into single-stranded DNA by disrupting the hydrogen bonds holding the strands together (Wacharapluesadee *et al.*, 2011; Kadri, 2019). During the annealing phase, the temperature is decreased to between 50 to 65°C for 20 to 40 seconds. This enables the primers to anneal to the single-stranded DNA templates. Each primer consists of a short single-stranded DNA sequence itself. In general, there are two primers in a reaction mixture that complements the two 3' ends of the single-stranded DNA. During the annealing phase, the temperature depends on the primers used, as the temperature is generally 3 to 5°C below the melting temperature (T_m) of the primers. By decreasing the

temperature it allows the reformation of hydrogen bonds between the primers and the template DNA (Templeton *et al.*, 2003; Kadri, 2019). Subsequently, the DNA polymerase binds to the hybrid of the primer and the template DNA and initiates the DNA formation (Ward *et al.*, 2016; Kadri, 2019). During the elongation phase, the temperature is determined by the DNA polymerase, but is generally 72°C, the period of elongation also depends on the DNA polymerase, as well as the size of the target DNA region that needs to be amplified. This is the phase where a new DNA strand is synthesized by the DNA polymerase. The sequences of the new DNA strand are complementary to the sequence of the DNA template (Wacharapluesadee *et al.*, 2011; Kadri, 2019). The new DNA strand is synthesized by adding free dNTPs, within the reaction mixture, in the direction of 5'-to-3', condensing the dNTPs 5'-phosphate group with the 3'-hydroxyl group located at the edge of the elongating DNA strand (Cullen *et al.*, 2001; Kadri, 2019). During each elongation phase, the amount of target DNA sequences increases twofold. This means that with each successive cycle all of the newly synthesized strands, as well as the original template strands, serve as the subsequent round of elongation phase template strands, resulting in an exponential increase of the exact DNA target region (Valones *et al.*, 2009; Kadri, 2019). After the final thermal cycle, there may be an optional single phase of final elongation. During this phase, any residual single-stranded DNA is entirely elongated, at temperatures between 70 to 74°C for a period of 5 to 15 minutes (Ward *et al.*, 2016; Kadri, 2019). This is after a single final holding phase at 4 to 15°C for an infinite period for short-term storage (Wacharapluesadee *et al.*, 2011; Kadri, 2019).

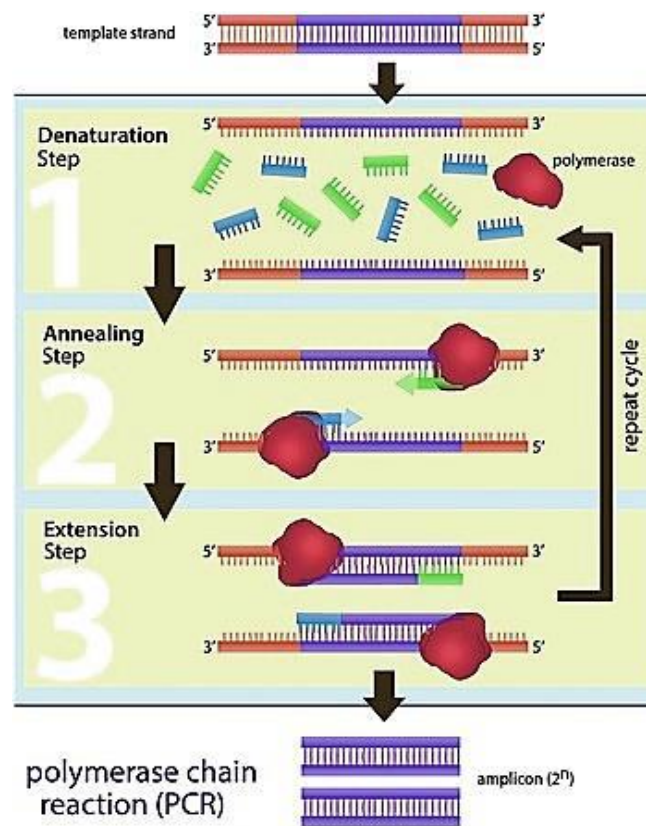


Figure 2-4: Illustration of the PCR process (Hui *et al.*, 2015)

2.8.2 Principle of Sanger Sequencing

Sanger sequencing, generally known as the chain termination method, is used for the determination of the nucleotide bases sequences of a segment of DNA (Valencia *et al.*, 2013; Dey, 2018). Shortly, the Sanger Sequencing consists of several steps illustrated in Figure 2.5. Firstly, the double-stranded DNA is denatured producing two single-stranded DNA strands (Valencia *et al.*, 2013; Tipu & Shabbir, 2015). A DNA primer is added, that compliments the template DNA, and serves as the initial point for DNA synthesis (Valencia *et al.*, 2013; Bleidorn, 2017). Four different polymerase solutions are added, consisting of four different types of dNTPs and one ddNTP, otherwise known as dideoxynucleotide triphosphates. The polymerase adds dNTPs, which are complementary to the template DNA strand, therefore, lengthening the primer (Kieleczawa *et al.*, 2011; Valencia *et al.*, 2013). The determination of the nucleotide type that is integrated into the nucleotide chain is determined by four types of, otherwise known as ddNTPs (ddCTP, ddGTP, ddTTP, and ddATP). These ddNTPs are used for the termination of the synthesis reaction and are identified by distinct fluorescent dyes. Unlike dNTPs, the ddNTPs lacks an oxygen atom on the ribonucleotide, making them unable to connect with the following nucleotide (Valencia *et al.*, 2013; Tipu & Shabbir, 2015). The end DNA product fragments are denatured to form ssDNA (Kieleczawa *et al.*, 2011; Valencia *et al.*, 2013). Subsequently, the

denatured DNA fragments are subjected to gel electrophoresis, where the products are loaded into a gel with four lanes, where the lanes are determined by the diverse terminating nucleotides, and afterward, the sequence of the DNA is determined (Valencia *et al.*, 2013; Dey, 2018).

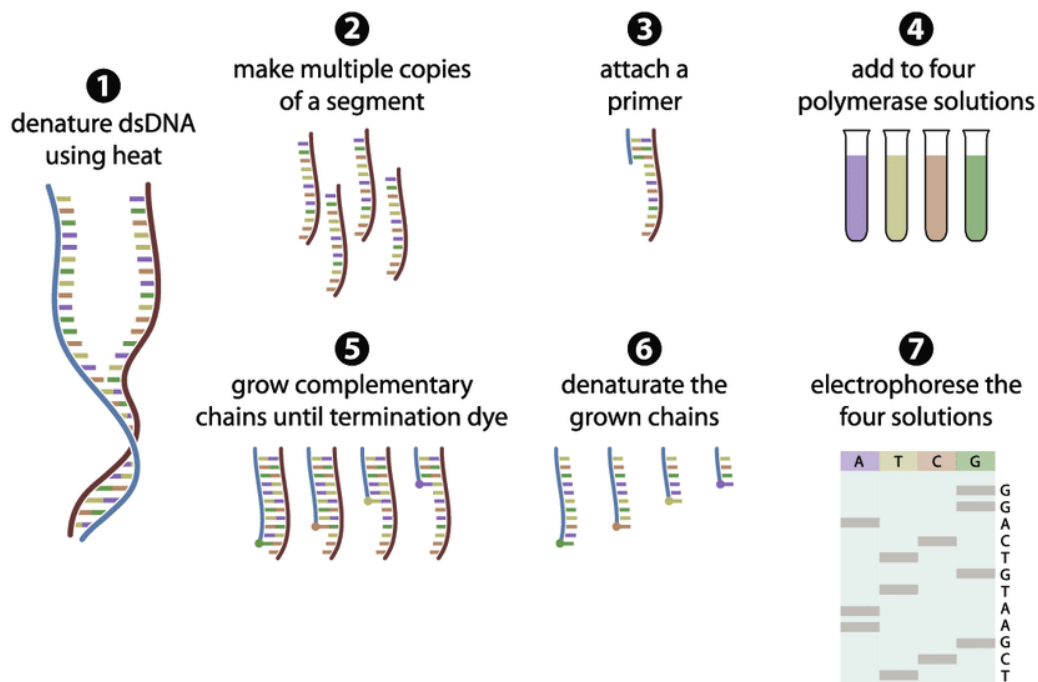


Figure 2-5: Illustration of Sanger sequencing (Gauthier, 2007)

2.8.3 Principle of phylogenetic trees

The construction of phylogenetic trees is used as visual presentations that display the evolutionary relationships between several organisms or other entities that are centered upon genetic or morphological characteristic differences or similarities (Hordijk & Gascuel, 2005; Godini & Fallahi, 2019). Phylogenetic trees show the evolutionary period starting at a common ancestor and ends at the different descendants (Godini & Fallahi, 2019; Price & Yasukawa, 2021). When constructing a rooted phylogenetic tree, as illustrated in Figure 2.6, the edge lengths display the time estimates, whereas the nodes of the different descendants, generally known as the taxonomic units signifies the common ancestor of those descendants (Hordijk & Gascuel, 2005; Godini & Fallahi, 2019). It is not possible to directly observe the internal nodes, which are commonly referred to as the hypothetical taxonomic units (Godini & Fallahi, 2019; Price & Yasukawa, 2021).

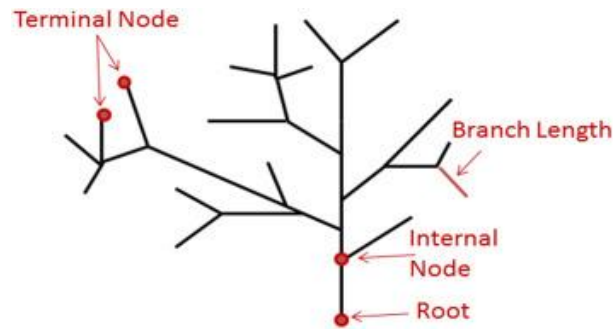


Figure 2-6: Elements of a phylogenetic tree (Sarma *et al.*, 2019)

2.9 Microbiota of ticks

Several arthropods are considered to be pest species and are damaging towards animal and human health, and have negative impacts on forestry and agriculture (Bonnet *et al.*, 2017; Gurung *et al.*, 2019). Ticks, like all other organisms, are associated with microorganisms that impact their ecology, biology, and evolution. These microorganisms include viruses, archaea, bacteria, fungi, and protozoa. Collectively all of these organisms are referred to as the microbiome (Gurung *et al.*, 2019; Adegoke *et al.*, 2020). All the microbiome components may influence the physiological and behavioural traits of the host through metabolic or genetic interactions. In contrast, environmental factors, including vegetation, biogeography, hygrometry, temperature, and night-day cycles, influence the composition of the microbial communities of ticks (Swei & Kwan, 2017; Cabezas-cruz *et al.*, 2018). A diverse microbiota of ticks is an evolutionary strategy to aid the ticks to cope with mechanisms, such as metabolic deficiencies (Cabezas-cruz *et al.*, 2018; Narasimhan *et al.*, 2021). Within the microbiome, several microbial groups are in association with each other and are associated with their host (Gurung *et al.*, 2019; Estrada-peña *et al.*, 2020). Associations between the aforementioned microorganisms and their hosts may have a beneficial, negative, or neutral impact on the hosts fitness and be on a temporary or permanent basis (Gurung *et al.*, 2019; Wu-Chuang *et al.*, 2021). Microorganisms that have a beneficial impact on the host, namely endosymbionts, reside in the cells or tissue of the hosts where they are dependent on the host to provide them with nutrients, and in turn, they provide the host with fitness advantages such as protection from parasites and pathogens, providing the host protection against abiotic factors, disabling host defences, or providing the host with nutrients. On the other hand, microorganisms that have a negative impact on hosts, such as pathogens or parasites, may decrease host viability and may cause physiological difficulties or morbidity decreased viability. Interactions between microbial groups determine the formation and response patterns (Gurung *et al.*, 2019; Narasimhan *et al.*, 2021).

2.9.1 Interactions between ticks and bacterial communities

Considering the different variety of microbial species in association between arthropods, it is vital to focus on interactions beyond the insect host and the associated microbiome (Gurung *et al.*, 2019; Adegoke *et al.*, 2020). Even though the microbiome contains members of other kingdoms that influence arthropods, the majority of studies done on the microbiota of insects, including pests, focus on the bacterial communities (Bonnet *et al.*, 2017; Gurung *et al.*, 2019). By studying a single bacterial species it aids the understanding of the bacterial functions of the organism or possible host interaction mechanisms (Swei & Kwan, 2017; Gurung *et al.*, 2019). It is also important to acknowledge that microorganisms live in different communities interacting with each other and the host (Gurung *et al.*, 2019; Estrada-peña *et al.*, 2020). The above-mentioned are illustrated in Figure 2.7. Ticks should be considered as a mini-ecosystem where interactions between the host and the microbiome are a system, given their complicated potential interactions. Therefore, it is essential to take into consideration the perspective of the community ecology of the insect host (Bonnet *et al.*, 2017; Gurung *et al.*, 2019). Interactions between individual members of a bacterial group, as well as the interactions between different bacterial groups, are in abundance and are significant to study the interactions between microorganism and their hosts, host performance, and pest insect ecology (Gurung *et al.*, 2019; Adegoke *et al.*, 2020).

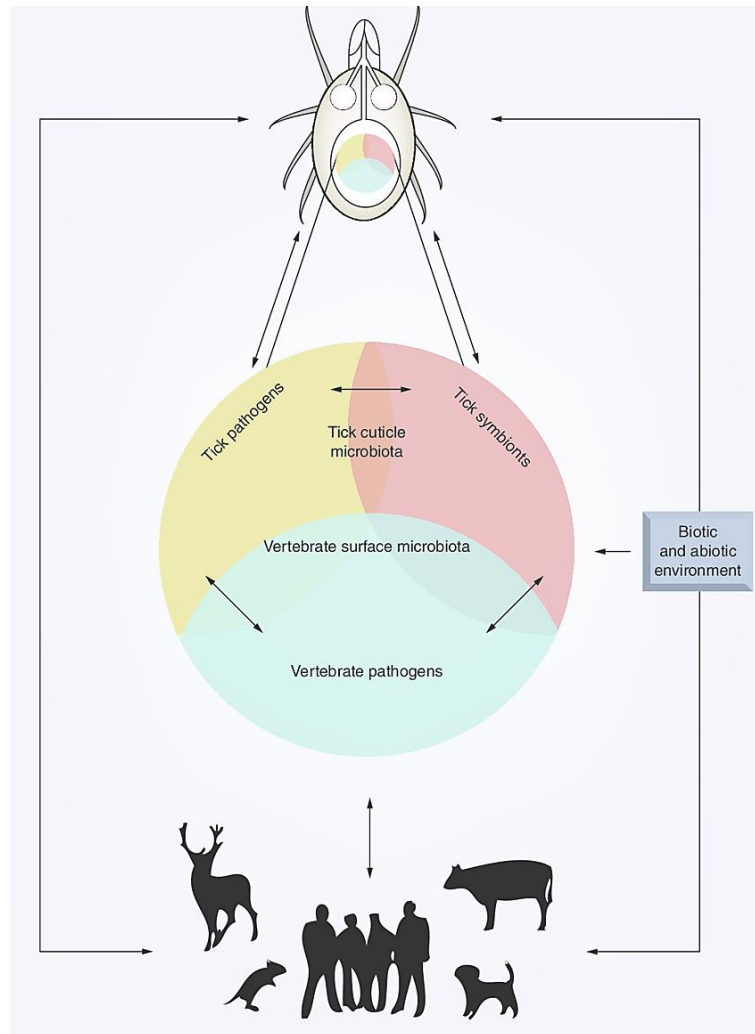


Figure 2-7: Interactions between ticks and their associated hosts, tick-borne pathogens, as well as the microbiome of the ticks (Vayssier-taussat *et al.*, 2015)

2.9.2 Next-generation sequencing technologies

Up until two decades ago research focussed on the detection and interactions between a single bacterial species and their associated host. The focus of these studies was on the microbiome composition and their functional role on the insect biology (Slatko *et al.*, 2018; Gurung *et al.*, 2019). Within the past decade, studies have focussed on the insect microbiome characterization by using next-generation sequencing methods (Besser *et al.*, 2018; Gurung *et al.*, 2019). To determine the bacterial community function and composition it is required to separate the microorganisms from the host insects. The majority of microorganisms are challenging to cultivate outside of the host. For this reason, culture-independent methods are used for the taxonomic classification and characterization of microbial communities. These culture-independent methods include but are not limited to amplicon sequencing (to identify bacterial communities), shotgun

sequencing (to illustrate the microbiome functional potential), and metatranscriptomics (to determine gene expression) (Cabezas-cruz *et al.*, 2018; Gurung *et al.*, 2019). Next-generation sequencing (NGS) technologies have presented new insights into the ecology and dynamics of microbial communities (Cabezas-cruz *et al.*, 2018; Kumar *et al.*, 2019). The most commonly used sequencing techniques are 454 pyrosequencing and Illumina MiSeq sequencing, with the former been the preferred method due to the high amount of sequences produced (Cabezas-cruz *et al.*, 2018; Jagadeesan *et al.*, 2019). The majority of documented tick bacterial community diversity and composition are from 16S *rRNA* gene sequencing from extracted DNA. The 16S *rRNA* gene consists of nine regions, namely V1 to V9. When Illumina MiSeq sequencing is used the second part of the 16S *rRNA* gene is amplified by targeting the V3 to V4 or V3 to V5 or V3 to V6 regions (Besser *et al.*, 2018; Cabezas-cruz *et al.*, 2018). Since bacterial communities' identification is only available to the genus level, when using Illumina MiSeq sequencing, there is a requirement for a straightforward approach of 16S *rRNA* gene-based profiling that covers a larger portion of the gene and delivers a higher taxonomic classification of the tick microbiota identification. One other limitation of DNA-based 16S *rRNA* gene sequencing does not allow the distinction between active cells and non-active (Cabezas-cruz *et al.*, 2018; Jagadeesan *et al.*, 2019). In contrast, RNA-based 16S *rRNA* sequencing can identify metabolically active cells (Cabezas-cruz *et al.*, 2018; Kumar *et al.*, 2019). These limitations influence the capability to investigate how non-pathogenic micro-organisms forming part of the host microbiota interact with those members that are pathogenic (Cabezas-cruz *et al.*, 2018; Slatko *et al.*, 2018).

The steps of next-generation sequencing are summarised in Figure 2.8. Next-generation sequencing-based on sequencing the 16S *rRNA* starts with library construction. A library refers to an assembly of DNA fragments illustrating the sample's sequence diversity. The aforementioned fragments are enhanced from the genomic DNA of the community by using PCR primers similar to the targeted gene or the significant microbial population (Preheim *et al.*, 2013; Besser *et al.*, 2018). Illumina sequencing the molecular construct consists of the amplified genomic DNA, the barcodes (sequences that identifies the sample origin and allows multiplexing), and lastly the sequences that attach the library fragments and the solid matrix, as well as provide the sequencing reaction priming site (Preheim *et al.*, 2013; Jagadeesan *et al.*, 2019). Illumina sequencing produces sequences for both the forward and reverse template strands, generally referred to as paired-end sequencing. This enables short template sequences resequencing providing longer reads enabling improved accuracy (Preheim *et al.*, 2013; Slatko *et al.*, 2018).

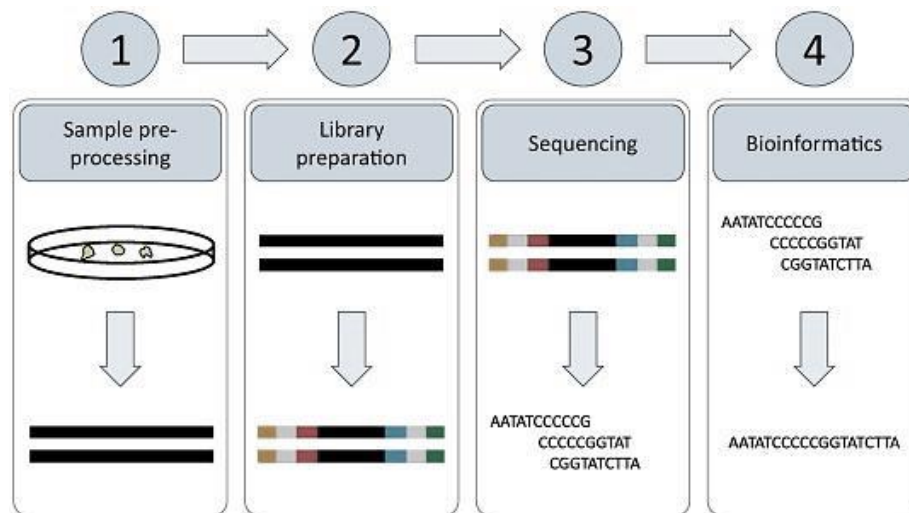


Figure 2-8: Workflow of next-generation sequencing (Hess *et al.*, 2020)

2.9.2.1 Computational methods for determination of bacterial communities

Quality filtering needs to be done to minimize methodological artifacts, sequences need to be arranged into suitable groups for computational analyses, whereas the allocation of a taxonomic label to a group aids biological interpretation. If an error arises at any step of data analysis interpretation of the data will be incorrect (Preheim *et al.*, 2013). There are several different methods for the analysis and grouping of sequence data. Quantitative insight into microbial ecology is referred to as QIIME, and the mothur software packages contain a suite of tools required for 16S *rRNA* analysis (Schloss, 2020).

Metagenomic or amplicon sequencing starts with raw sequence data. For 16S Illumina sequencing, the raw sequence data is in the format of a fastq file. This file contains the DNA sequences as well as their quality scores (Preheim *et al.*, 2013). Firstly, the reads need to be demultiplexed. A unique barcode sequence is attached to an end or both ends of each read. During demultiplexing, the barcode sequences are identified and mapped back to the original samples to verify the original sample of every read. Illumina sequencing normally delivers paired-end reads that are also joined during the demultiplexing step (Hall & Beiko, 2018). After demultiplexing the denoising and clustering steps follow (Bolyen *et al.*, 2019). Sequences are denoised into amplicon sequence variants, generally referred to as ASVs, to eliminate or rectify noisy reads, remove chimeras and singletons, dereplicate sequences, as well as correct marginal sequences errors. The denoising programs included in QIIME 2 are Deblur and DADA2 (Prodan *et al.*, 2020). Sequences are then clustered into operational taxonomic units, referred to as OTUs, to group similar sequences, generally, sequences that are equal to or more than 97% similar, to distinct replicate sequences (Bolyen *et al.*, 2019). There are three different methods for OTU

clustering, namely *de novo*, closed reference, and open reference. The *de novo* clustering method ensures that reads are clustered against each other, without the use of an external reference sequence collection. This method is generally used if there is not a reference sequence collection available to sequence against, such as using a marker gene that is rarely used. The closed reference clustering method clusters reads against a reference sequence database. If any reads are not clustered against the reference sequence in the database they will be excluded from further downstream analysis. This clustering method is used for the comparison of non-overlapping amplicons, like the 16S *rRNA* V2 and V4 regions, as the reference sequences will span the sequenced regions. The open reference clustering method clusters reads against a reference sequence database and any reads that were unclassified are then clustered *de novo*. This is the preferred method for clustering and is not used in the event of comparing non-overlapping regions and in the absence of a reference sequence database (Chen *et al.*, 2013; Bolyen *et al.*, 2019; Barnes *et al.*, 2020). The results of denoising and clustering are representative sequences along with a feature table that is a matrix consisting of the amount of times a feature (ASVs and OTUs) is detected in a sample within the data set. The feature table and representative sequences are then used for further downstream analysis (Bolyen *et al.*, 2019). Some of the most general above-mentioned analyses includes alpha- and beta diversity analysis, taxonomic classification, evaluation of phylogenetic relationships by the alignment of sequences, as well as the measures of the differential abundance of features in experimental groups (Preheim *et al.*, 2013).

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CHAPTER 3

SYSTEMATIC REVIEW OF THE DISTRIBUTION OF DOG TICKS: *RHIPICEPHALUS SANGUINEUS* AND *HAEMAPHYSALIS ELLIPTICA* AS WELL AS TICK-BORNE PATHOGENS THEY HARBOUR

3.1 Introduction

On a global scale the threat posed by ticks, as well as the pathogenic organisms they harbour, is increasing. Ticks transmit tick-borne pathogens that affect the health of humans, livestock, wildlife, and companion animals (Esteve-Gassent *et al.*, 2016; Magnarelli, 2009). In addition, ticks are also responsible for toxic conditions, including allergic reactions, tick paralysis, and irritation (Esteve-Gassent *et al.*, 2016; Jongejan & Uilenberg, 2004). Ticks also contribute to economic losses, such as losses due to hide damage, death of livestock, or mounting medical and veterinary costs of humans and companion animals resulting from diseases of tick-borne pathogens (Jongejan & Uilenberg, 2004; Magnarelli, 2009).

Tick-borne diseases of humans and companion animals have gained increasing interest in research within the last couple of years since the discovery of zoonotic diseases, such as Lyme disease and rickettsiosis (Jongejan & Uilenberg, 2004). Ticks infesting dogs, specifically *R. sanguineus* and *H. elliptica*, with the potential to infect humans are of particular interest. This is due to companion animals that have integral roles when it comes to their association with humans, such as being owned companions, working animals assisting with livestock or disabled humans. Companion animals that are considered stray or community-owned are also in close association with humans (Baneth, 2014; Wall, 2016). Often companion animals are permitted indoor access where they are living in close quarters with humans and outdoor access where they may come into contact with ticks in the environment or wildlife infected with ticks. This poses a risk to human health as companion animals may bring infected ticks into the indoor environment that may attach to human hosts, where stray dogs may be responsible for vector-transmitted infections inside human communities (Jones *et al.*, 2018; Wall, 2016).

The global distributions of ticks are of concern as tick populations are increasing due to changing environmental conditions and their ability to adapt to intense environmental conditions, including unfavourable temperatures and humidity (Esteve-Gassent *et al.*, 2016; Estrada-Peña, 2008). In addition, populations of several tick species are introduced to new areas, by means of migrating or travelling hosts, where the ticks may establish new niches and inhabit non-endemic areas.

Often if the aforementioned happens with ticks infected with tick-borne pathogens, these pathogens are introduced to new areas, resulting in novel or co-infections of that area (Buczek & Buczek, 2020; Esteve-Gassent *et al.*, 2016).

To the best of our knowledge, there is no systematic review, to date, documenting the distribution of *R. sanguineus* and *H. elliptica* ticks, as well as the presence of the tick-borne pathogens these ticks may harbour, even though there are numerous studies documenting the prevalence of one or both of these tick species and/or their tick-borne pathogens. As a result, this systematic review documented the global distribution of *R. sanguineus* and *H. elliptica* and determined the presence of tick-borne pathogens harboured by these ticks.

3.2 Materials and methods

This systematic review was conducted and written using the guidelines specified by the preferred reporting items for systematic reviews and meta-analysis (PRISMA) (Page *et al.*, 2021a; Page *et al.*, 2021b) as illustrated in Figure 3.1. This systematic review was registered on PROSPERO under the following ID: CRD42022327372.

3.2.1 Review questions

This review aimed to identify, obtain, and evaluate relevant English journal articles documenting the prevalence of *R. sanguineus* and *H. elliptica*, as well as the tick-borne pathogens these ticks harbour which were published after the 1st January 1990 up to 1st June 2021 from several scientific databases. The review questions of this systematic review are: “What is the global prevalence of *R. sanguineus* and *H. elliptica*?” and “Globally, what tick-borne pathogens do *R. sanguineus* and *H. elliptica* harbour?”

3.2.2 Search strategy and selection criteria

Literature was searched from Scopus, ScienceDirect, PubMed, and Web of Science databases. Each search was limited to open-access English journal articles. Keywords used were “Ticks”, “dog”, “canine”, “*Canis familiaris*”, “*Rhipicephalus sanguineus*”, “brown dog tick”, “kennel tick”, “pantropical dog tick”, “*Haemaphysalis elliptica*”, “*Haemaphysalis leachi*”, “yellow dog tick”. Searches on the databases either used the above-mentioned keywords alone or combined by using the “AND” and/or “OR” operators. After searching each database the article titles were downloaded and exported to the reference management software Mendeley (Sungur & Seyhan, 2013).

3.2.3 Exclusion and inclusion criteria

After the journal articles were exported to Mendeley the duplicated journal articles were removed. Subsequently, the titles and then abstracts of the journal articles were screened for studies that were relevant to the review questions. The full-text screening was then carried out to identify eligible studies that adhered to the specific criteria. Studies were only deemed eligible if (1) respectively, the source type and document type was a journal article, (2) the journal articles were open access, (3) journal articles were published in English, (4) the articles were published from the 1st January 1990 to 1 June 2021, (5) relevant tick species (*R. sanguineus* and/or *H. elliptica*) were identified in the study, (6) ticks were identified to species level, (7) study locations were specified or spatial data were included, (8) the number of ticks collected during the study was specified, (9) the sampled hosts were specified and enumerated, (10) a minimum of 50 ticks were collected, (11) the study was not an incidence study, and (12) the study was not a case-control, experimental study, or a review.

3.2.4 Quality assessment (risk of bias)

Assessments of the quality of the included studies were done using the Joanna Briggs Institute (JBI) critical appraisal tool for prevalence studies (Munn *et al.*, 2015) as illustrated in Appendix 3A that considers 9 aspects of the studies, where a score of 1 was assigned for adhering to each aspect. A score of 5 or above was considered a low risk of bias, whereas a score below 5 was considered a high risk of bias (Azene *et al.*, 2020).

3.2.5 Data collection and analysis

Data extracted from eligible studies included the author's surnames, year of publication, period the study was conducted, diagnostic method of tick identification, host type and number of hosts sampled, the total number of ticks collected, the prevalence of *R. sanguineus* and/or *H. elliptica*, as well as the presence, species, prevalence, and detection method of tick-borne pathogens in *R. sanguineus* and/or *H. elliptica* ticks. Extracted data were recorded into a spreadsheet. All steps carried out during the systematic review, including eligibility, inclusion, quality assessment, and extraction of data, was primarily done by an individual reviewer. In cases where data was not made available in the article but was available as supplementary material, the supplementary material was downloaded and included during the screening process. No further attempts were made regarding contacting the authors of journal articles for additional or unpublished data.

3.3 Results

3.3.1 Study selection

After searching the databases there were a total of 2841 journal articles (Figure 3.1). The search results were then imported to the Mendeley reference manager and the duplicate articles were identified and removed. A total of 571 duplicate articles were removed, leaving 2270 articles that were subsequently used for title screening. After title screening 527 articles were removed, leaving a total of 1743 articles that were used for abstract screening. There were 1199 articles removed after screening the abstracts, where 544 articles remained and were subsequently examined for eligibility by full-text screening. Of the aforementioned articles 290 were removed as these articles focused on irrelevant tick species (n=30), the ticks were not identified to species level (n=7), the number of the ticks collected during the study was not specified (n=93), the type of host sampled and/or the number of sampled hosts was not specified (n=81), less than 50 ticks were collected during sample collections (n=51), an article based on an incidence study (n=1), and lastly, articles that were based on case controls, experimental studies or reviews (n=27). After screening articles for eligibility, 254 studies were included in the systematic review.

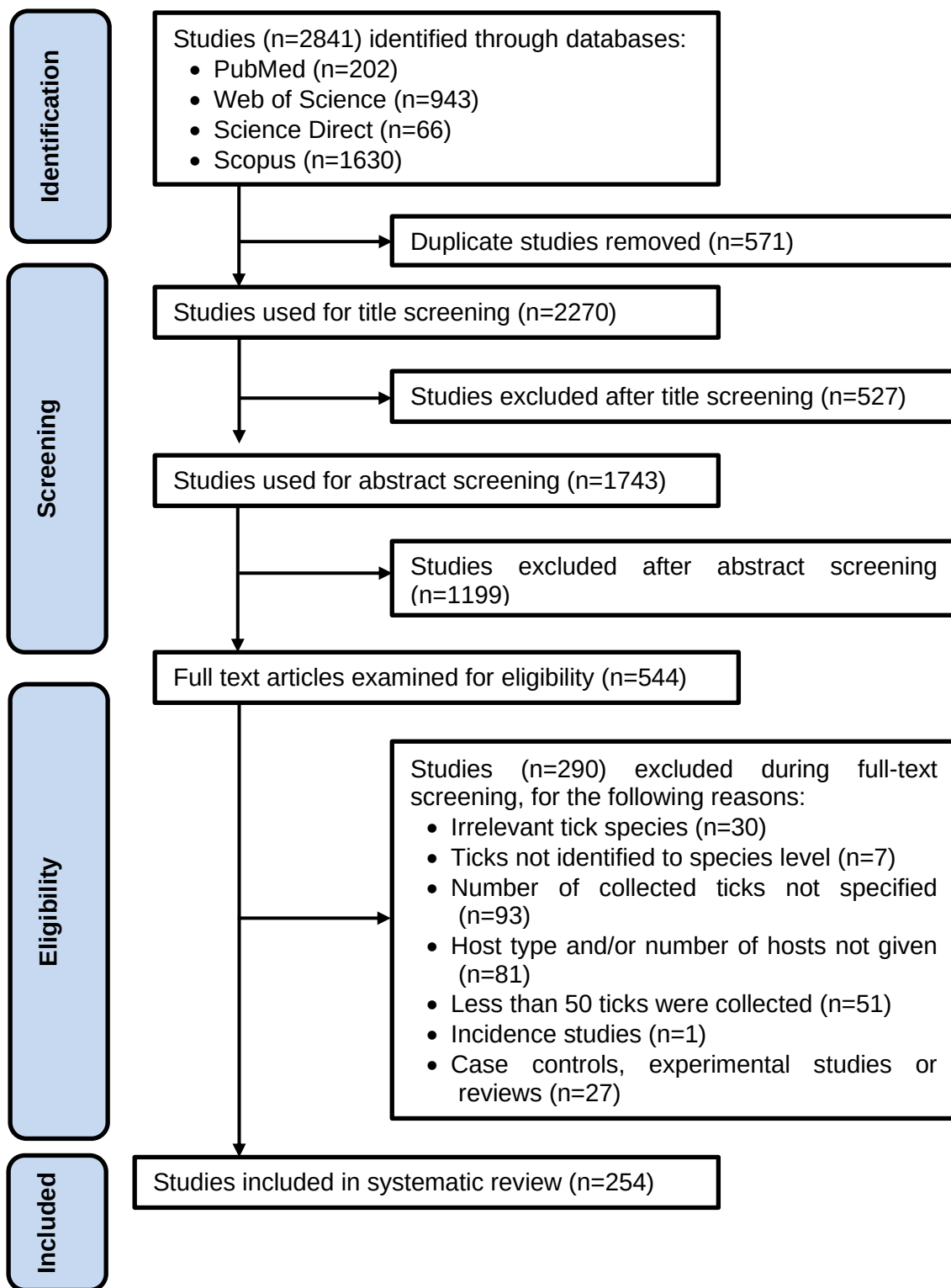


Figure 3-1: Flow diagram illustrating the eligible studies that were identified during the selection process by following the PRISMA guidelines.

3.3.2 Study characteristics

For the first review question concerning the global prevalence of *R. sanguineus* and *H. elliptica*, the study characteristics included the authors of each article and year of publication, the country where the study was conducted, identification method of ticks, sampled hosts and number of hosts sampled, the total number of ticks collected during sampling, as well as the prevalence of *R. sanguineus* and/ or *H. elliptica* (Table 3.1). For the second review question concerning the tick-borne pathogens that *R. sanguineus* and/ or *H. elliptica* harbour on a global scale, the study characteristics included the authors of each article and year of publication, the geographical location of the study where the infected ticks were collected, the tick-borne pathogens detected in *R. sanguineus* and/ or *H. elliptica* ticks, as well as the haemoparasite detection method (Table 3.2).

3.3.2.1 Global distribution of *R. sanguineus* and/or *H. elliptica*

Of the studies included in the systematic review, respectively 247 and 19 articles collected and identified *R. sanguineus* and *H. elliptica*, whereas 15 articles managed to collect and identify both tick species (Table 3.1). Of the above-mentioned studies, *R. sanguineus* ticks were collected from Brazil (n=50), Italy (n=21), United States (n=13), Turkey (n=11), China (n=9), Mexico (n=9), Taiwan (n=9), Iran (n=8), South Africa (n=7), Malaysia (n=6), Nigeria (n=6), Algeria (n=5), Spain (n=5), Argentina (n=4), Australia (n=4), Cuba (n=4), Japan (n=4), Romania (n=4), Chile (n=3), Colombia (n=3), Costa Rica (n=3), France (n=3), Israel (n=3), Mozambique (n=3), Philippines (n=3), Thailand (n=3), Tunisia (n=3), Ethiopia (n=2), Germany (n=2), Greece (n=2), India (n=2), Malta (n=2), Panama (n=2), Peru (n=2), Portugal (n=2), Russia (n=2), Serbia (n=2), Uganda (n=2), Ukraine (n=2), Vietnam (n=2) Benin (n=1), Cameroon (n=1), Croatia (n=1), Democratic Republic of the Congo (n=1), Egypt (n=1), Georgia (n=1), Indonesia (n=1), Iraq (n=1), Ireland (n=1), Jordan (n=1), Laos (n=1), Mali (n=1), Mauritius (n=1), Netherlands (n=1), Nevis (n=1), New Caledonia (n=1), Nicaragua (n=1), Pakistan (n=1), Palestine (n=1), Saint Kitts (n=1), Singapore (n=1), South Korea (n=1), Sri Lanka (n=1), Sudan (n=1), and Uruguay (n=1) (Figure 3.2 and Figure 3.3). On the other hand, *H. elliptica* ticks were collected from South Africa (n=9), Nigeria (n=4), Mozambique (n=3), Benin (n=1), Ethiopia (n=1), and Uganda (n=1). Both tick species were reported from studies in South Africa (n=5), Nigeria (n=4), Mozambique (n=3), Benin (n=1), Ethiopia (n=1), and Uganda (n=1) (Figure 3.2 and Figure 3.4).

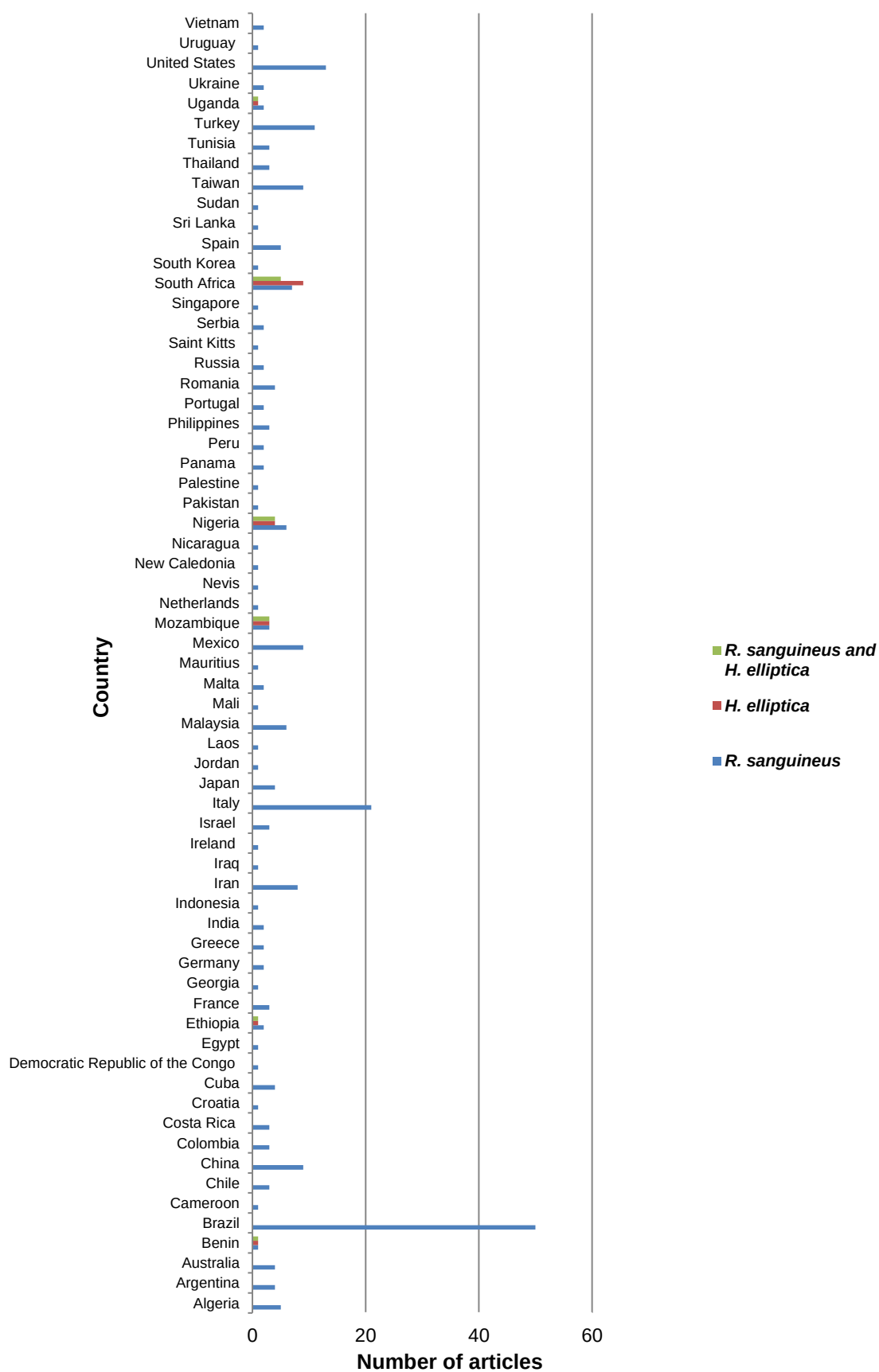


Figure 3-2: Frequency of countries where *R. sanguineus* and/or *H. elliptica* ticks were collected

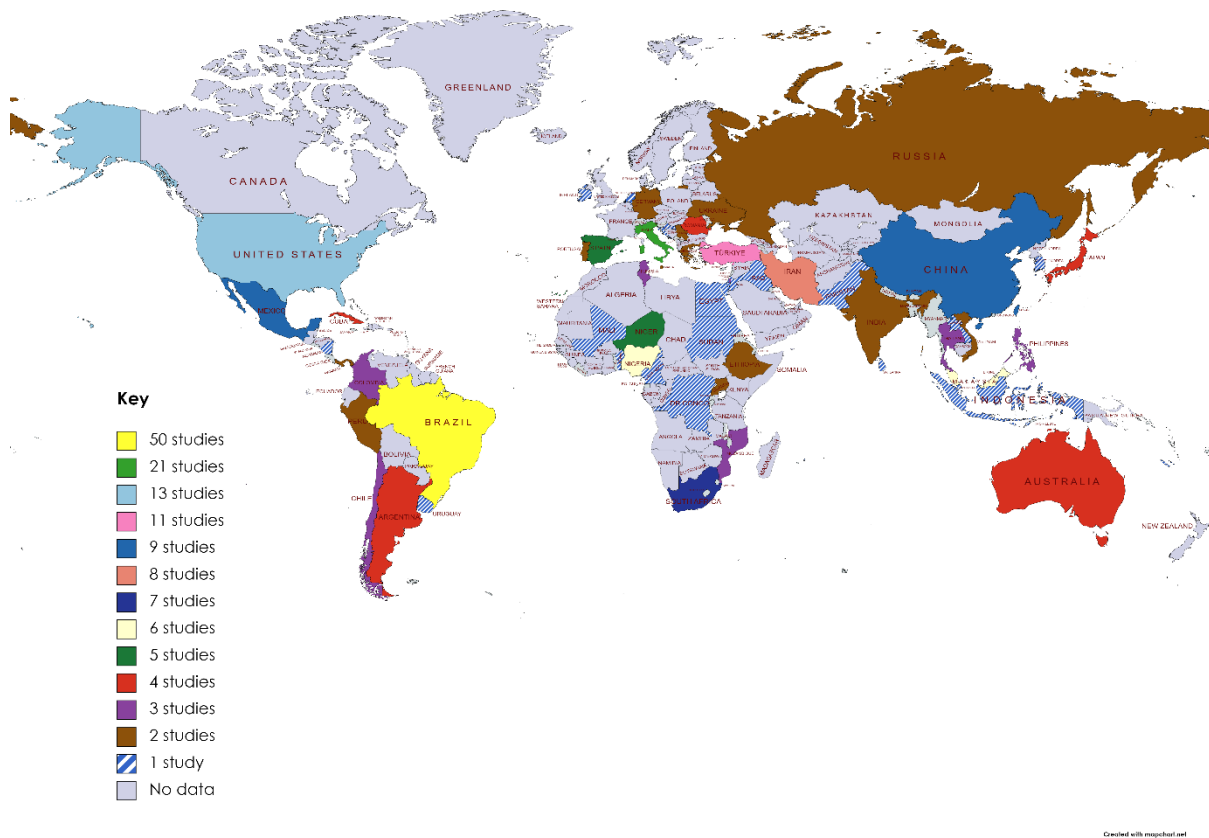


Figure 3-3: World map showing number of articles from different countries which reported the *R. sanguineus* (<https://www.mapchart.net/world.html> (accessed on 12 March 2023))

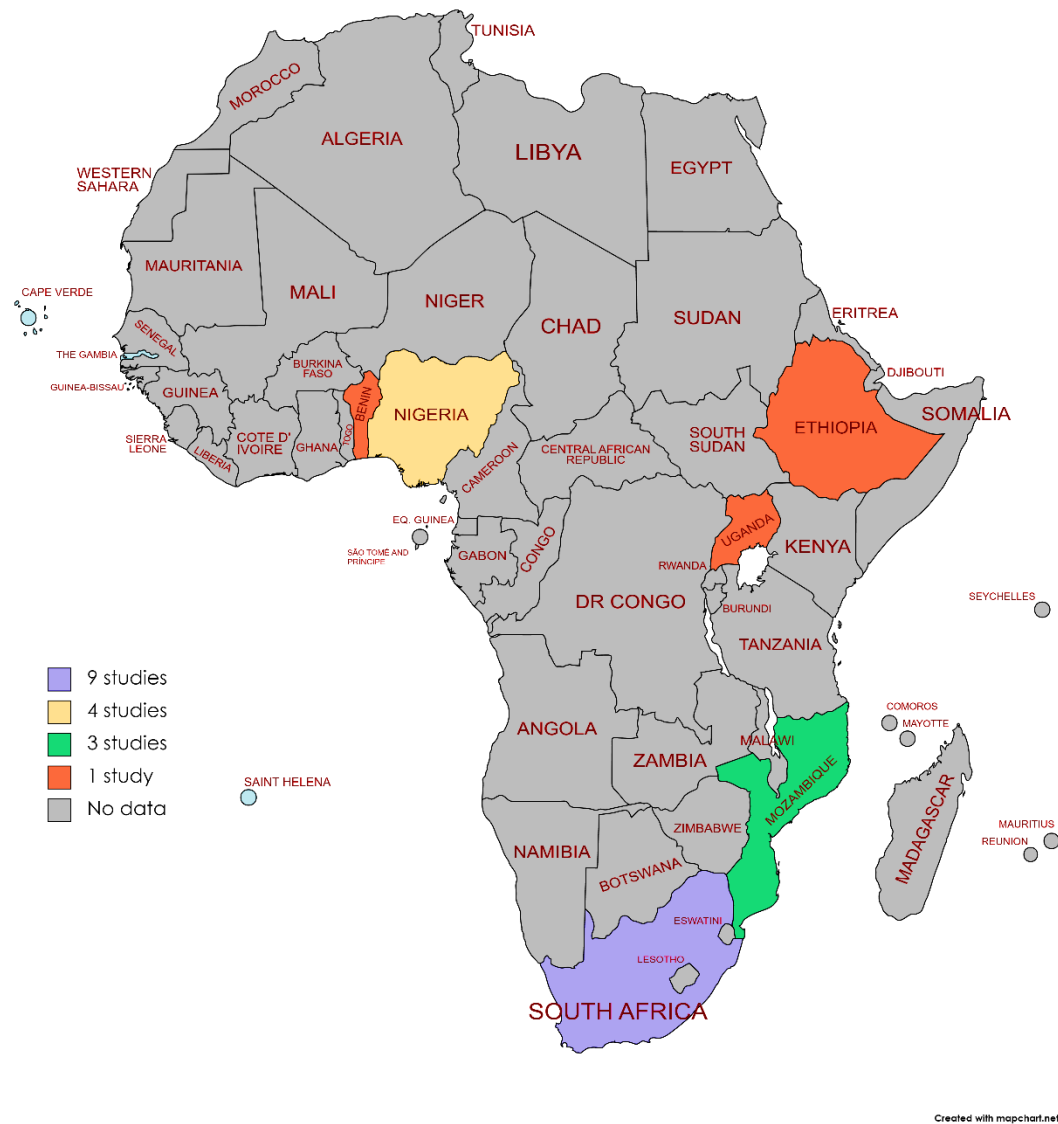


Figure 3-4: World map showing number of articles from different countries which reported the *H. elliptica* (<https://mapchart.net/africa.html>) (accessed on 12 March 2023))

Articles included in this systematic review indicated that tick species identification was mainly done by means of morphological identification (n=222), as well as a combination of morphological identification and conventional PCR (n=29). For the detection of *R. sanguineus* ticks, morphological identification methods (n=218) and a combination of morphological identification and conventional PCR (n=29) were used. On the other hand, *H. elliptica* ticks were identified using morphological identification methods (n=19). Additionally, the 15 articles that reported the presence of both *R. sanguineus* and *H. elliptica* only used morphological identification methods (Figure 3.5).

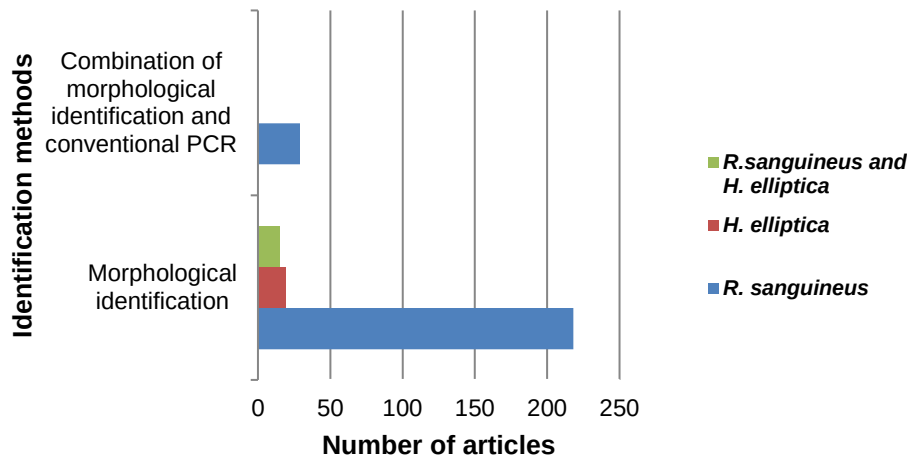


Figure 3-5: Frequency of identification methods of *R. sanguineus* and/or *H. elliptica* ticks

Several studies reported that *R. sanguineus* were collected from domestic dogs (n=213), vegetation (n=23), domestic cats (n=17), goats (n=14), sheep (n=14), cattle (n=9), humans (n=5), deer (n=4), rodents (n=3), hedgehogs (n=3), foxes (n=2), camels (n=2), horses (n=2), wolves (n=2), Black-tailed marmoset (n=1), coyotes (n=1), Croatia (n=1), raccoons (n=1), guinea pigs (n=1), Iberian lynx (n=1), mouflon (n=1), unspecified host (n=1), donkeys (n=1), European pine marten (n=1), Chinese hare (n=1), boars (n=1), ring-necked pheasants (n=1), jackals (n=1), mongoose (n=1), as well as several species of canine hosts (n=1).

Articles that reported the presence of *H. elliptica* were collected from domestic dogs (n=14), vegetation (n=3), cheetahs (n=2), rodents (n=2), jackals (n=1), domestic cats (n=1), caracals (n=1), lions (n=1), and leopards (n=1).

Of the articles that reported the presence of both *R. sanguineus* and *H. elliptica* mentioned that both tick species were collected from domestic dogs (n=14), rodents (n=1), and domestic cats (n=1) (Figure 3.6).

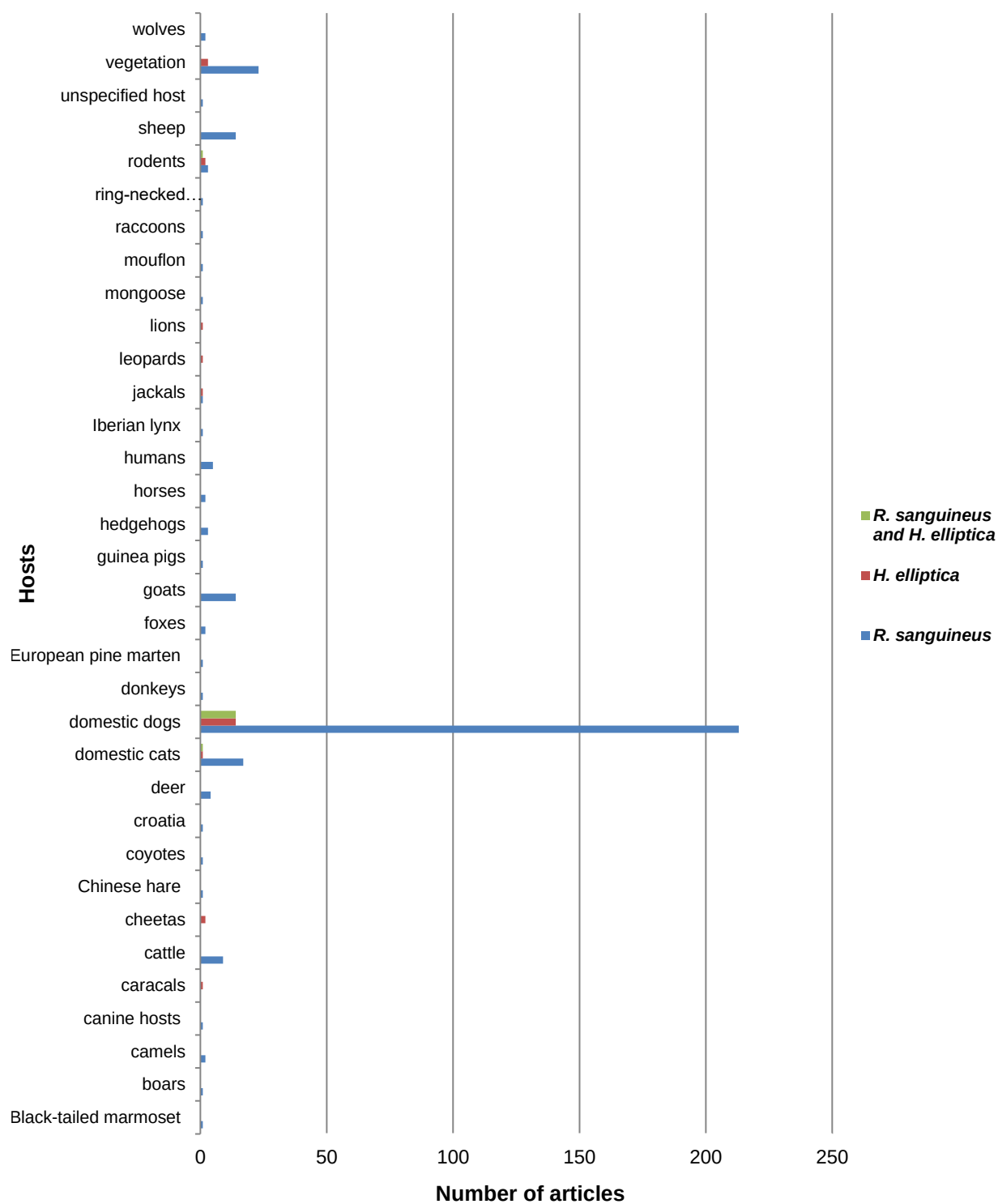


Figure 3-6: Frequency of hosts of *R. sanguineus* and/or *H. elliptica* tick collections

3.3.2.2 Tick-borne pathogens infecting *R. sanguineus* and/or *H. elliptica*

A total 121 articles were included in this systematic review that reported the presence of haemopathogen infections within *R. sanguineus* or *H. elliptica* ticks (Table 3.2). Respectively, 119 and 2 of the aforementioned articles reported the presence of tick-borne pathogens within *R. sanguineus* ticks and *H. elliptica* ticks. Only 1 article reported the presence of tick-borne pathogens within both *R. sanguineus* and *H. elliptica* ticks. Of the aforementioned studies, tick-borne pathogens detected within *R. sanguineus* were from Brazil (n=20), Italy (n=13), Turkey (n=10), China (n=7), Taiwan (n=7), Mexico (n=6), United States (n=6), Malaysia (n=5), Spain (n=4), France (n=3), Philippines (n=3), Algeria (n=2), Australia (n=2), Colombia (n=2), Costa Rica (n=2), Cuba (n=2), Nigeria (n=2), Panama (n=2), Portugal (n=2), Romania (n=2), Russia (n=2), South Africa (n=2), Thailand (n=2), Tunisia (n=2), Vietnam (n=2), Argentina (n=1), Cameroon (n=1), Egypt (n=1), Greece (n=1), Indonesia (n=1), Iran (n=1), Israel (n=1), Laos (n=1), Nicaragua (n=1), Palestine (n=1), Serbia (n=1), Singapore (n=1), and Ukraine (n=1). Of the aforementioned studies, tick-borne pathogens detected within *H. elliptica* were from South Africa (n=1) and Uganda (n=1). The study that reported the presence of tick-borne pathogens within both tick species was from South Africa (n=1) (Figure 3.7).

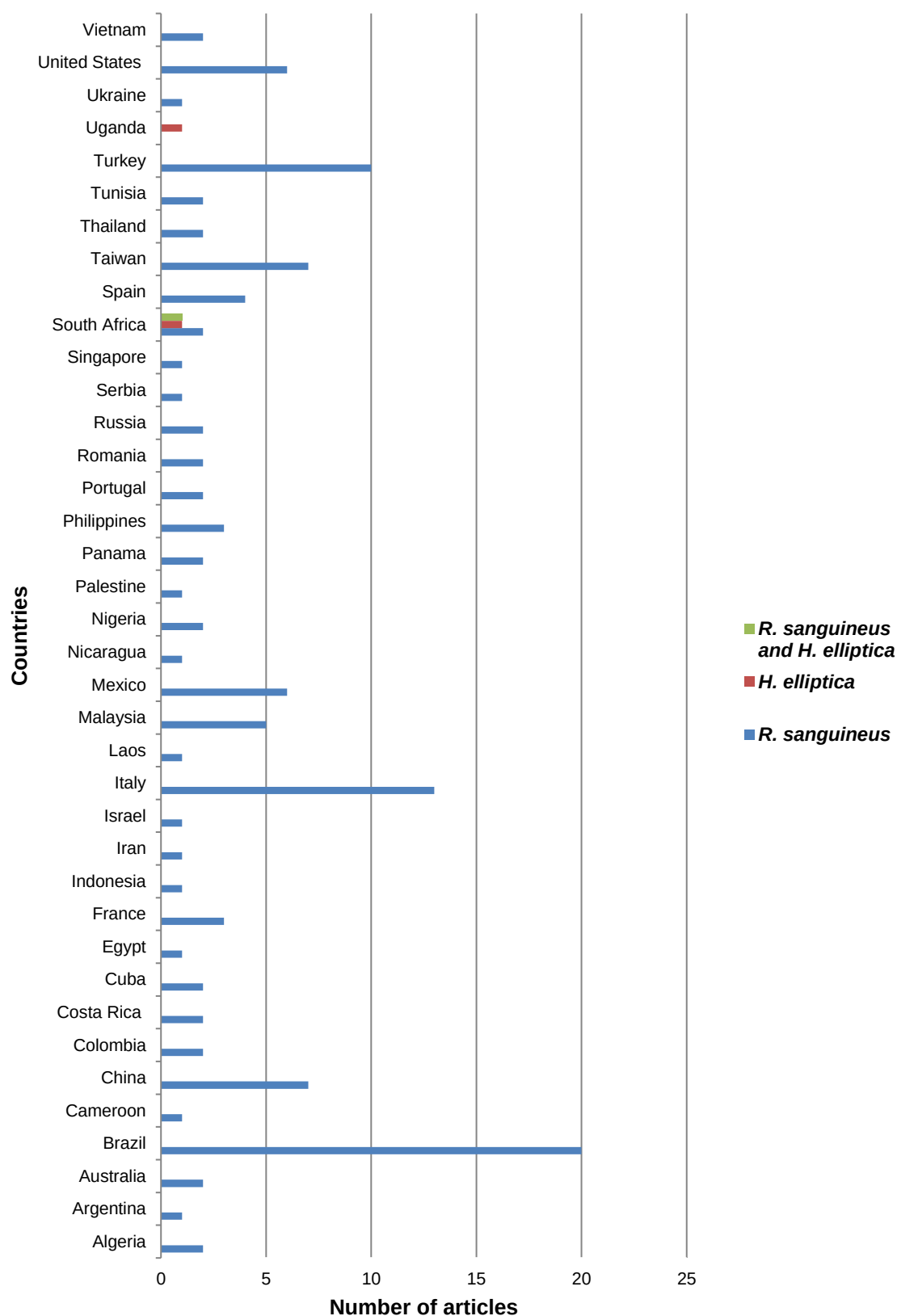


Figure 3-7: Frequency of countries where tick-borne pathogens were detected harboured by *R. sanguineus* and/or *H. elliptica* ticks

The studies identified the tick-borne pathogens infecting *R. sanguineus* ticks as belonging to *Rickettsia* (n=75), *Babesia* (n=36), *Ehrlichia* (n=36), *Anaplasma* (n=33), *Hepatozoon* (n=21), *Leishmania infantum* (n=6), *Theileria* (n=6), *Bartonella* (n=5), *Coxiella* (n=5), *Borrelia* (n=4), *Staphylococcus* (n=3), *Bacillus* (n=2), *Brucella* spp. (n=1), *Acinetobacter iwoffii* (n=1), and *Ehrlichia/Anaplasma* (n=1). Of the 75 articles that identified *Rickettsia* from *R. sanguineus* ticks the following species were reported *Rickettsia* spp. (n=28) *Candidatus Rickettsia asemboensis* (n=1), *C. R. barbariae* (n=2), *C. R. goldwasesrii* (n=1), *R. rickettsii* (n=4), *R. amblyommii* (n=1), *R. asembonensis* (n=2), *R. conorii* (n=8), *R. conorii israelensis* (n=1), *R. massiliae* (n=15), *R. parkeri* (n=1), *R. rhipicephali* (n=1), *R. felis* (n=2), *R. helvetica* (n=1), *R. monacensis* (n=3), *R. raoultii* (n=2), *R. sibirica* subsp. *mongolitimonae* (n=1), and *R. slovaca* (n=1). The 36 articles that identified *Babesia* from *R. sanguineus* were identified as *Babesia* spp. (n=3), *B. canis* (n=2), *B. capreoli* (n=1), *B. capreoli/divergens* (n=1), *B. gibsoni* (n=3), *B. microti* (n=2), *B. rossi* (n=1), *B. venatorum* (n=1), *B. vogeli* (n=19), *B. vulpes* (n=1), and *B. ovis* (n=2). There were 36 articles that identified *Ehrlichia* from *R. sanguineus* and were identified as *Ehrlichia* spp. (n=6), *Ehrlichia* sp. HF (n=1), *E. canis* (n=27), *E. chaffeensis* (n=1), and *E. ewingii* (n=1). The 33 articles that identified *Anaplasma* from *R. sanguineus* were *Anaplasma* spp. (n=6), *A. ovis* (n=2), *A. phagocytophylum* (n=6), and *A. platys* (n=19). There were 21 articles that identified *Hepatozoon* from *R. sanguineus* and were identified as *Hepatozoon* spp. (n=2) and *H. canis* (n=19). The 6 articles that identified *Theileria* from *R. sanguineus* were identified as *Theileria* spp. (n=1), *T. buffeli/sergenti/orientalis* (n=1), *T. cervi* (n=1), *T. equi* (n=1), and *T. ovis* (n=2). The 6 articles that identified *Bartonella* from *R. sanguineus* were reported as *Bartonella* spp. (n=3), *B. rochalimae* (n=1), and *B. clarridgeiae* (n=1). Five articles reported the presence of *Coxiella* of *R. sanguineus* that were identified as *Coxiella* sp. (n=2) and *C. burnetii* (n=3). There were 4 articles that reported *Borrelia* on *R. sanguineus*, that were identified as *Borrelia* spp. (n=1) and *B. burgdorferi* (n=3). The 3 articles that reported *Staphylococcus* from *R. sanguineus* were identified as *S. cohnii* (n=1), *S. epidermidis* (n=1), and *S. sacccapitis* (n=1). The 2 articles that identified *Bacillus* from *R. sanguineus* were identified as *B. clausii* (n=1) and *B. megaterium* (n=1). There were 8 articles reporting co-infections of tick-borne pathogens of *R. sanguineus* including co-infections between *B. gibsoni* and *E. canis* (n=2), *Anaplasma* spp., and *Babesia* spp. (n=1), *B. gibsoni* and *A. platys* (n=1), *E. canis* and *A. platys* (n=1), *E. canis*, *A. platys* and *B. burgdorferi* (n=1), *E. chaffeensis* and *E. canis* (n=1), as well as *R. rickettsii* and *R. rhipicephali* (n=1). The studies identified the tick-borne pathogens infecting *H. elliptica* ticks as belonging to *Rickettsia* (n=2) [*Rickettsia* sp. (n=1) and *R. africae* (n=1)], *B. rossi* (n=1), *Ehrlichia* spp. (n=1), and *Ehrlichia/Anaplasma* (n=1) (Figure 3.8-3.19).

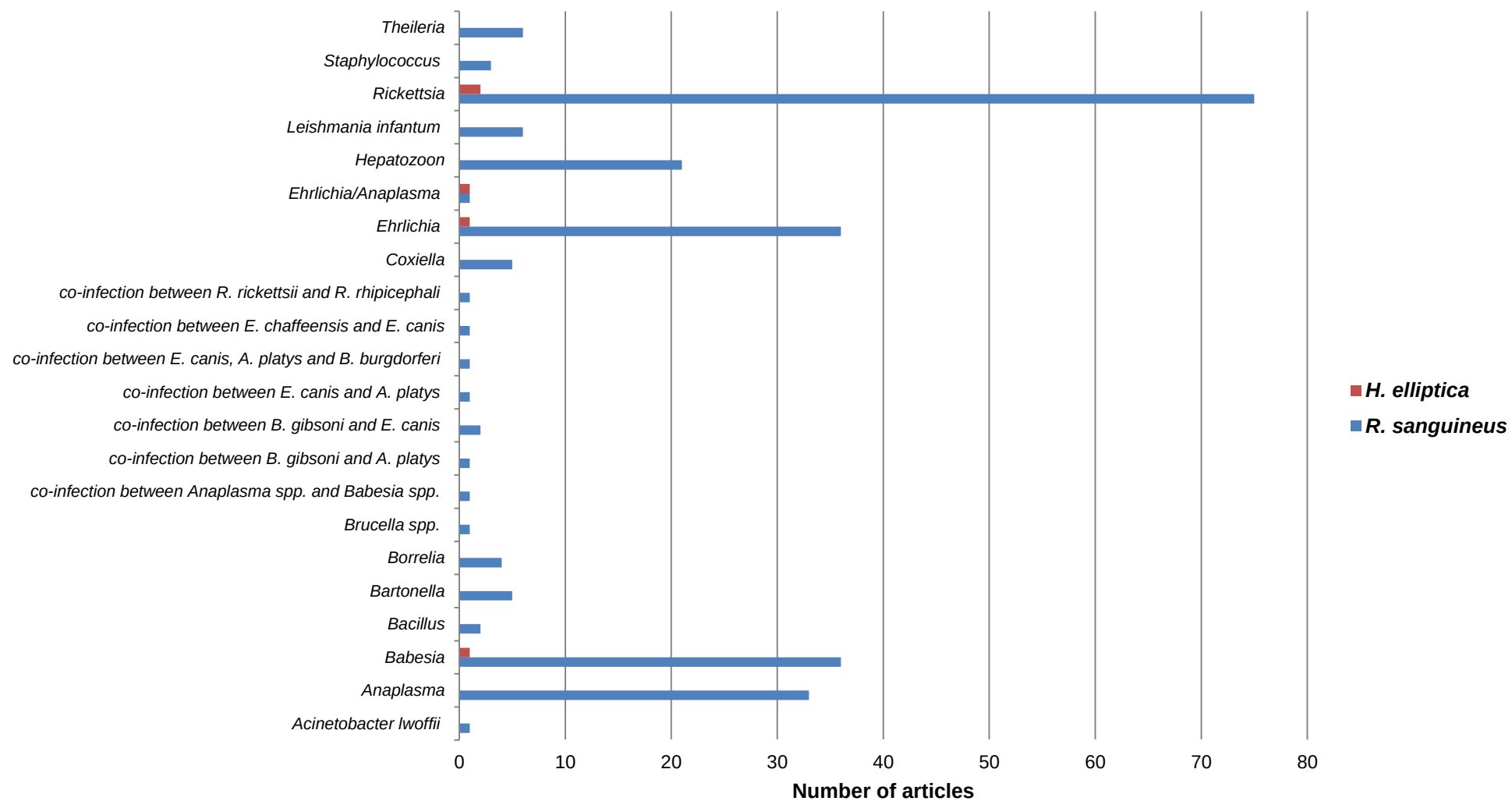


Figure 3-8: Frequency of tick-borne pathogens detected harboured by *R. sanguineus* and/or *H. elliptica* ticks

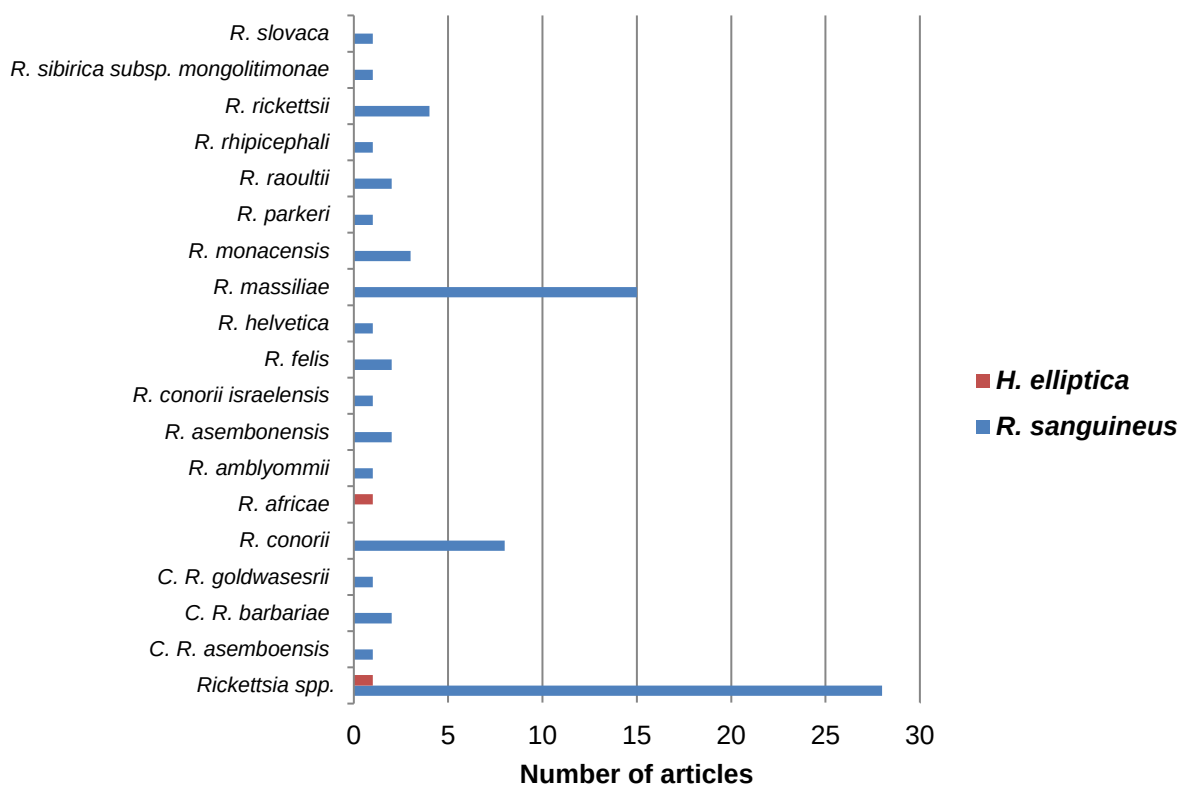


Figure 3-9: Frequency of *Rickettsia* spp. detected

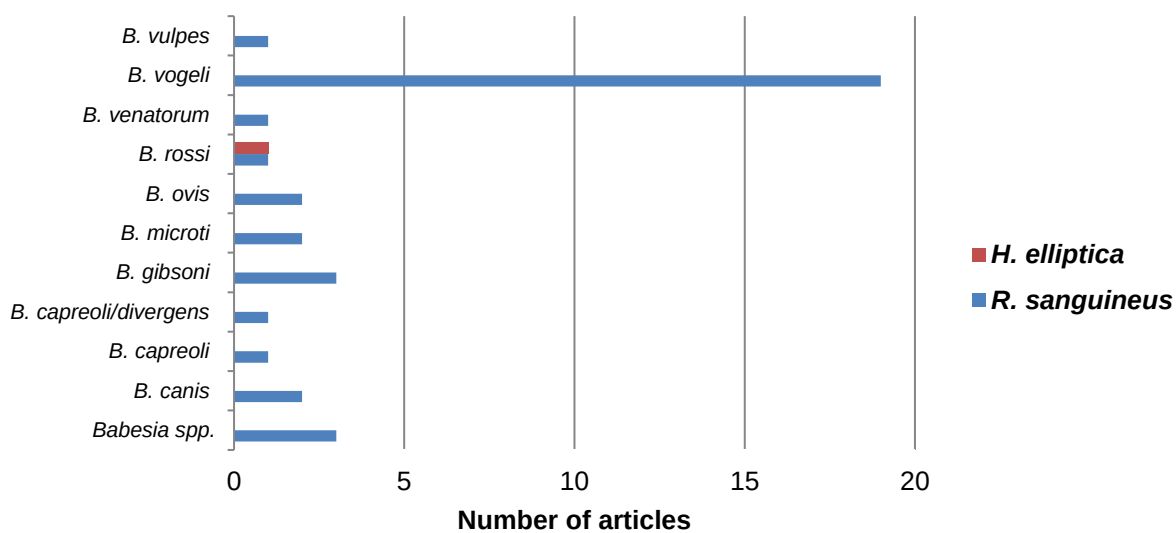


Figure 3-10: Frequency of *Babesia* spp. detected

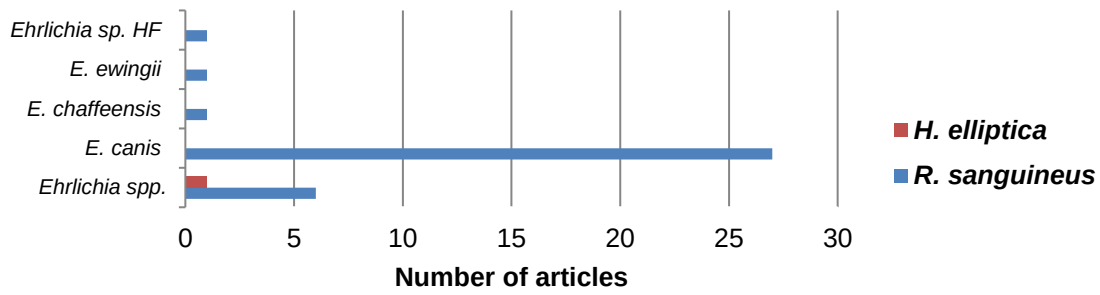


Figure 3-11: Frequency of *Ehrlichia* spp. detected

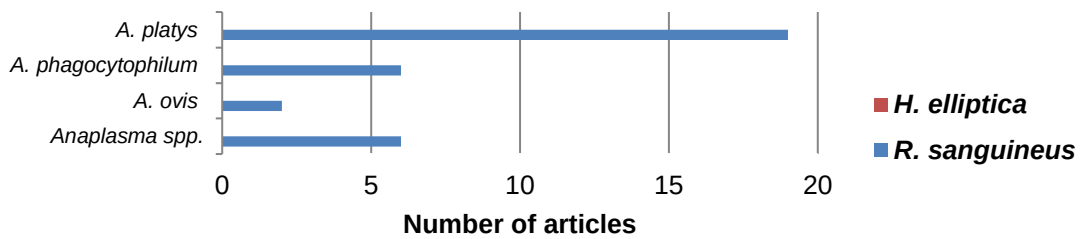


Figure 3-12: Frequency of *Anaplasma* spp. detected

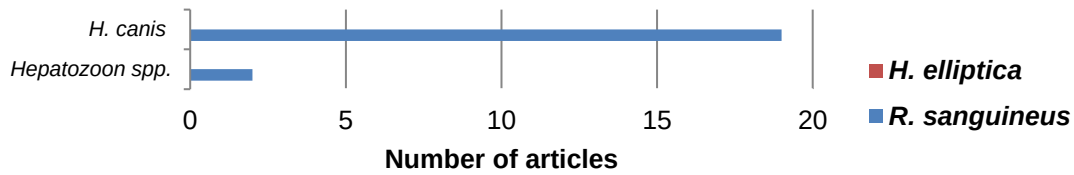


Figure 3-13: Frequency of *Hepatozoon* spp. detected

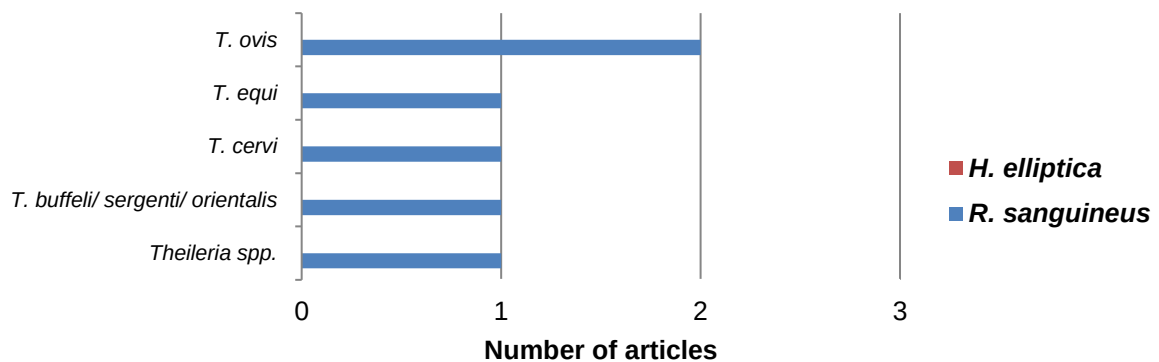


Figure 3-14: Frequency of *Theileria* spp. detected

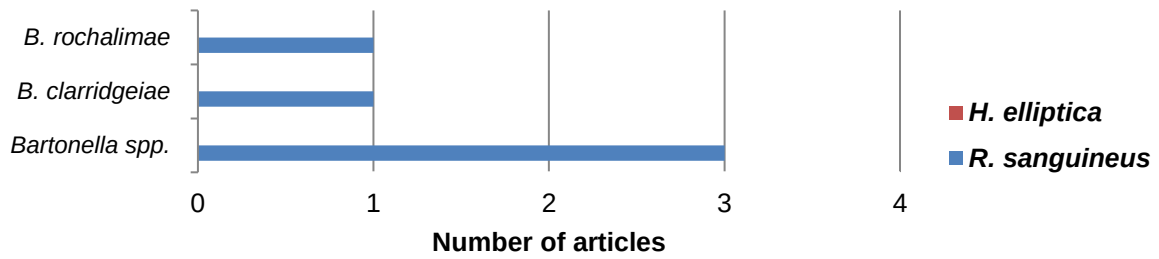


Figure 3-15: Frequency of *Bartonella* spp. detected



Figure 3-16: Frequency of *Coxiella* spp. detected

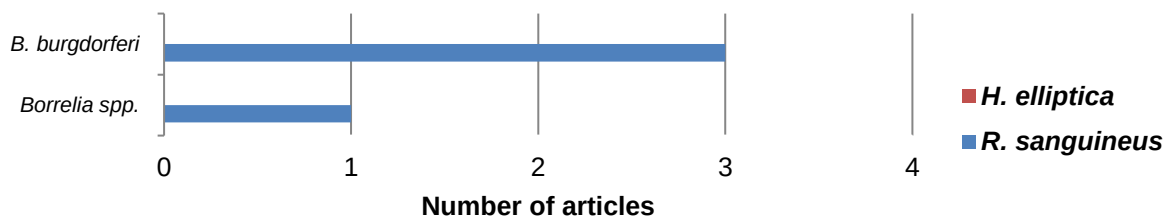


Figure 3-17: Frequency of *Borrelia* spp. detected

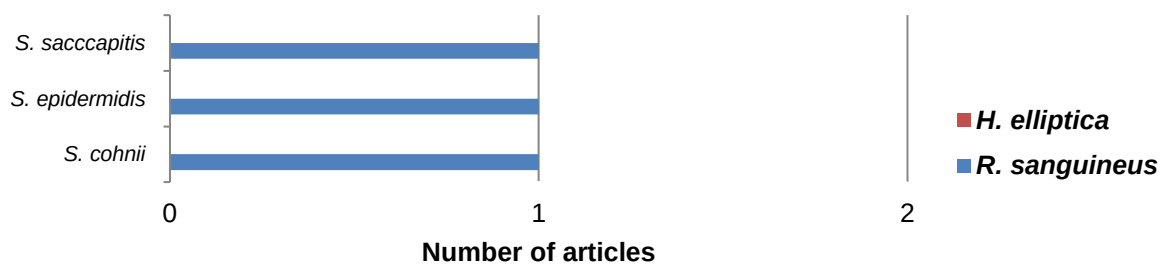


Figure 3-18: Frequency of *Staphylococcus* spp. detected



Figure 3-19: Frequency of *Bacillus* spp. detected

Of the articles included in the systematic review that detected tick-borne pathogens within *R. sanguineus*, the articles used molecular techniques including conventional PCR (n=80), real-time PCR (qPCR) (n=26), nested PCR (n=22), reverse line blot (RLB) hybridization (n=4), heminested PCR (n=1), semi-nested PCR (n=1), indirect immunofluorescence (n=1), multiplex PCR (n=1), end-point PCR (n=1), nPCR (n=1), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (n=1), as well as morphological techniques by microscopic examination (n=4), and bacterial culturing using the streak plate method performed on blood agar, trypticase soy agar, Baird-Parker agar, and Mannitol salt agar (n=1). Of the articles included in the systematic review that detected tick-borne pathogens within *H. elliptica*, the articles used molecular techniques including RLB (n=1) and qPCR (n=1). The article that discovered the presence of tick-borne pathogens in both *R. sanguineus* and *H. elliptica* ticks used RLB (n=1) (Figure 3.20).

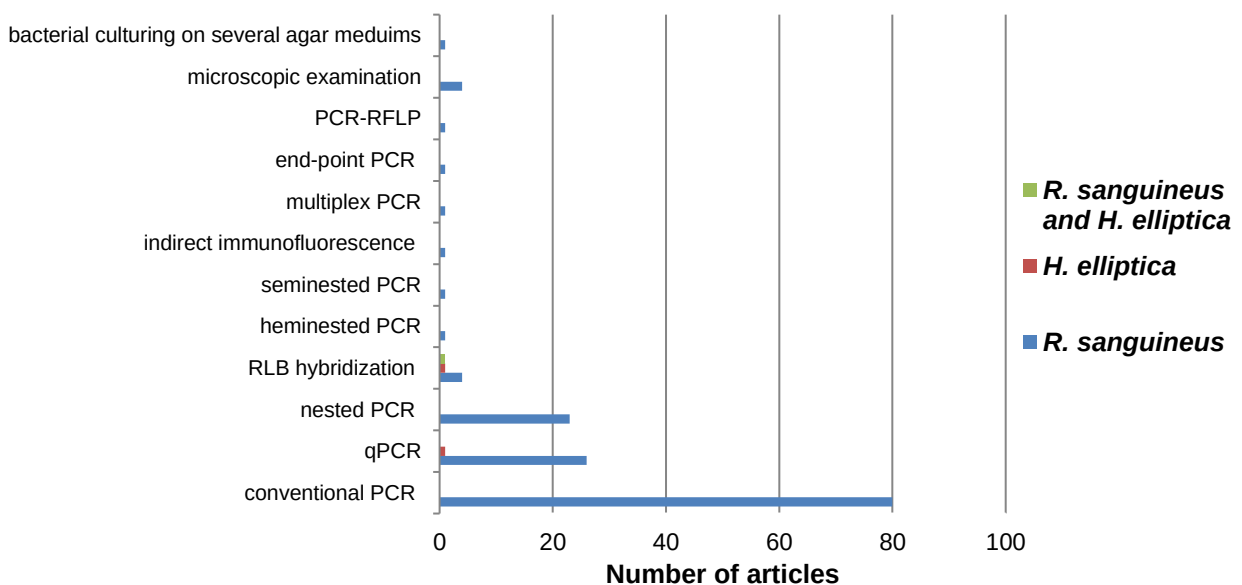


Figure 3-20: Frequency of detection of tick-borne pathogens harboured by *R. sanguineus* and/or *H. elliptica* ticks

Table 3-1: Summary containing the list, as well as study characteristics of articles included in the systematic review, reporting on the global prevalence of *R. sanguineus* and *H. elliptica*

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Ramos <i>et al.</i> (2014b)	Italy	Morphological identification	Dogs (n=34)	267	267	nd
Horak <i>et al.</i> (2009)	South Africa	Morphological identification	Dogs (n=200) and vegetation	13667	25 [from dogs (n=25)]	201 [from dogs (n=193) and vegetation (n=8)]
Horak <i>et al.</i> (2009)	Mozambique	Morphological identification	Dogs (n=132) and vegetation	15192	153 [from dogs (n=153)]	449 [from dogs (n=447) and vegetation (n=2)]
Chao <i>et al.</i> (2017)	Taiwan	Morphological identification	Dogs (n=236)	1195	1195	nd
Dall'Agnol <i>et al.</i> (2017)	Brazil	Morphological identification	Dogs (n=14)	63	63	nd
A. B. Silva <i>et al.</i> (2017)	Brazil	Morphological identification	Dogs (n=300)	369	369	nd
Chisu <i>et al.</i> (2019)	Italy	Morphological identification	Mouflon (n=5), Corsican red deer (n=7), European pine marten (n=5), dogs (n=83), cats (n=1), goats (n=15), and vegetation	116	97 [from mouflon (n=3), Corsican red deer (n=1), European pine marten (n=5), dogs (n=83), cats (n=1), goats (n=2), and vegetation (n=2)]	nd
Manoj <i>et al.</i> (2020)	India	Morphological identification	Dogs (n=230)	1294	1272; 98.3%	nd
Dzul-Rosado <i>et al.</i> (2021)	Mexico	Morphological identification	Dogs (n=50)	260	239	nd
Carvalho <i>et al.</i> (2017)	Uruguay	Morphological identification	Dogs (n=191)	135	135	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Santos <i>et al.</i> (2017)	Brazil	Morphological identification	Dogs (n=83)	1814	1814	nd
Vila <i>et al.</i> (2019)	Spain	Morphological identification	Dogs (n=660)	1628	53%	nd
Mediannikov <i>et al.</i> (2012)	Democratic Republic of the Congo	Morphological identification	Dogs (n=19)	125	111	nd
Trotta <i>et al.</i> (2012)	Italy	Morphological identification, conventional PCR	Dogs (n=21)	91	78	nd
Ybañez <i>et al.</i> (2012)	Philippines	Morphological identification	Dogs (n=36)	164	164	nd
Ramos <i>et al.</i> (2014a)	Italy	Morphological identification	Vegetation	1091	1091	nd
Beck <i>et al.</i> (2014)	Germany	Morphological identification	Dogs (n=441)	914	1	nd
Maia <i>et al.</i> (2014)	Portugal	Morphological identification	Dogs (n=116), cats (n=14), and vegetation	925	882 [from dogs (n=616), cats (n=36), and vegetation (n=230)]	nd
Chisu <i>et al.</i> (2014)	Italy	Morphological identification	Dogs (n=3)	254	17	nd
Cicuttin <i>et al.</i> (2014)	Argentina	Morphological identification	Dogs (n=52)	207	207	nd
Iwakami <i>et al.</i> (2014)	Japan	Morphological identification	Dogs (n=1162) and cats (n=136)	4535	72 [from dogs (n=70) and cats (n=2)]	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Barbieri <i>et al.</i> (2014)	Brazil	Morphological identification	Dogs (n=53)	153	6	nd
Cicuttin <i>et al.</i> (2015)	Argentina	Morphological identification, conventional PCR	Dogs (n=101)	181	181	nd
Ionita <i>et al.</i> (2016)	Romania	Morphological identification	Dogs (n=38)	384	225	nd
Almazán <i>et al.</i> (2016)	Mexico	Morphological identification	Dogs (n=100)	519	519	nd
Witter <i>et al.</i> (2016)	Brazil	Morphological identification	Mammals (n=87)	4467	2 [from Crab-eating fox (n=1) and Black-tailed marmoset (n=1)]	nd
Cetinkaya <i>et al.</i> (2016)	Turkey	Morphological identification	Dogs (n=400)	912	850	nd
Azmi <i>et al.</i> (2016)	Israel	Morphological identification	Dogs (n=117), sheep (n=66), and goats (n=6)	516	305 [from dogs (n=250), sheep (n=42), and goats (13)]	nd
Golezardy <i>et al.</i> (2016)	South Africa	Morphological identification	Cheetahs (n=191) and vegetation	19699	nd	16474 [from cheetahs (n=3175) and vegetation (n=13299)]
da Silva <i>et al.</i> (2016)	Cuba	Morphological identification	Dogs (n=100)	431	431	nd
Scarpulla <i>et al.</i> (2016)	Italy	Morphological identification	Dog (n=1) and vegetation	113	25 [from dog (n=16) and vegetation (n=9)]	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Campos-Calderón <i>et al.</i> (2016)	Costa Rica	Morphological identification	Dogs (n=165)	165	160	nd
Rogovskyy <i>et al.</i> (2017)	Ukraine	Morphological identification	Vegetation	971	1	nd
Licari <i>et al.</i> (2017)	Malta	Morphological identification, conventional PCR	Dogs (n=99)	105	105	nd
de Sousa <i>et al.</i> (2018)	Brazil	Morphological identification	Thrichomys fosteri (n=77)	1582	1	nd
Springer <i>et al.</i> (2018)	Costa Rica and Nicaragua	Morphological identification	Dogs (n=680)	1023	956	nd
Hornok <i>et al.</i> (2018)	Malta	Morphological identification, conventional PCR	Dogs (n=41), cats (n=31), hedgehogs (n=2), goat (n=1), and vegetation	540	540 [from dogs (n=363), cats (n=133), hedgehogs (n=8), goat (n=1), and vegetation (n=35)]	nd
Chao <i>et al.</i> (2019a)	Taiwan	Morphological identification	Dogs (n=138)	1082	1082	nd
G. M. B. de Oliveira <i>et al.</i> (2020)	Brazil	Morphological identification	Dogs (n=147)	222	119	nd
Chandra <i>et al.</i> (2020)	Australia	Morphological identification, conventional PCR	Dogs (n=48)	294	294	nd
Penzhorn <i>et al.</i> (2020)	South Africa	Morphological identification	Black-backed jackals (n=9)	52	nd	25

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Barradas <i>et al.</i> (2020)	Portugal	Morphological identification, conventional PCR	Dogs (n=200)	221	221	nd
Forlano <i>et al.</i> (2005)	Brazil	Morphological identification	Dogs (n=9)	566	345	nd
Rojas <i>et al.</i> (2014)	Costa Rica	Morphological identification	Dogs (n=146)	425	42	nd
Thomas <i>et al.</i> (2016)	United States	Morphological identification	Cats (n=673)	373	4	nd
Mediannikov <i>et al.</i> (2011)	New Caledonia	Morphological identification	Dogs (n=64)	258	235	nd
Hsu <i>et al.</i> (2011)	Taiwan	Morphological identification	Dogs (n=62)	386	151	nd
Bessas <i>et al.</i> (2016)	Algeria	Morphological identification	Dogs (n=117) and cats (n=107),	532	532 [from dogs (n=520) and cats (n=12)]	nd
Sipin <i>et al.</i> (2020)	Malaysia	Morphological identification, conventional PCR	Dogs (n=220)	140	140	nd
González <i>et al.</i> (2004)	Argentina	Morphological identification	Dogs (n=116)	3791	3791	nd
Zabó <i>et al.</i> (2007)	Brazil	Morphological identification	Dogs (n=2)	2509	16	nd
M'ghirbi & Bouattour (2008)	Tunisia	Morphological identification	Dogs (n=233)	780	780	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Silveira <i>et al.</i> (2009)	Brazil	Morphological identification	Dogs (n=12)	7318	7318	nd
Brianti <i>et al.</i> (2010)	Italy	Morphological identification	Dogs (n=24)	7816	7816	nd
Bermúdez <i>et al.</i> (2011)	Panama	Morphological identification	Dogs (n=20)	157	57	nd
René <i>et al.</i> (2012)	France	Morphological identification, conventional PCR	Dogs (n=12)	83	83	nd
Loftis <i>et al.</i> (2013)	Saint Kitts and Nevis	Morphological identification	Dogs (n=165)	268	268	nd
Giannelli <i>et al.</i> (2013)	Italy	Morphological identification	Dogs (n=1)	400	400	nd
Giannelli <i>et al.</i> (2013)	Italy	Morphological identification	Vegetation	35	35	nd
Aktas (2014)	Turkey	Morphological identification	Vegetation	2333	46	nd
Sándor <i>et al.</i> (2014)	Romania	Morphological identification, conventional PCR	Dogs (n=84)	1068	33	nd
Cao <i>et al.</i> (2015)	China	Morphological identification	Dogs (n=1170)	1776	1158	nd
Low <i>et al.</i> (2017)	Malaysia	Morphological identification	Dogs (n=29)	120	120	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
de Toledo Vieira <i>et al.</i> (2018)	Brazil	Morphological identification	Dogs (n=378)	941	938	nd
Little <i>et al.</i> (2018)	United States	Morphological identification	Cats (n=332)	796	1	nd
Oskam <i>et al.</i> (2017)	Australia	Morphological identification	Dogs (n=74)	199	199	nd
Monje <i>et al.</i> (2016)	Argentina	Morphological identification, conventional PCR	Dogs (n=42)	138	138	nd
Alieva <i>et al.</i> (2020)	Ukraine	Morphological identification	Dogs (n=7) and cat (n=1)	84	84 [from dogs (n=80) and cat (n=4)]	nd
Ghafar & Amer (2012)	Egypt	Morphological identification	Dogs (n=72)	413	413	nd
Ebani <i>et al.</i> (2015)	Italy	Morphological identification	Dogs (n=117)	119	36	nd
Sarani <i>et al.</i> (2014)	Iran	Morphological identification	Sheep (n= 44), goats (n=63), cattle (n=99), and camels (n=13)	225	170 [from sheep (n=56), goats (n=85), cattle (n=20), and camels (n=9)]	nd
Hayati <i>et al.</i> (2020)	Sudan	Morphological identification	Cattle (n=200)	1089	59	nd
Kumsa <i>et al.</i> (2019)	Ethiopia	Morphological identification	Dogs (n=200) and cats (n=137)	515	277 [from dogs (n=251) and cats (n=26)]	167 [from dogs (n=167)]
De Matos <i>et al.</i> (2008)	Mozambique	Morphological identification	Dogs (n=132)	1 768	196	592

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Aydin <i>et al.</i> (2020)	Turkey	Morphological identification	Vegetation	6186	3698	nd
Billeter <i>et al.</i> (2012)	Thailand	Morphological identification	Dogs (n=164)	92	92	nd
Jacobs <i>et al.</i> (2001)	South Africa	Morphological identification	Dogs (n=36)	21636	20150	1290
Inokuma <i>et al.</i> (2001)	Japan	Morphological identification	Dogs (n=22)	121	121	nd
Andoh <i>et al.</i> (2013)	Japan	Morphological identification	Dogs (n=48)	261	74	nd
Wallach <i>et al.</i> (2008)	Israel	Morphological identification	Deer (n=4)	74	1	nd
Sosa-Gutiérrez <i>et al.</i> (2016)	Mexico	Morphological identification	Deer (n=139)	139	128	nd
Watanabe <i>et al.</i> (2015)	Malaysia	Morphological identification, conventional PCR	Dogs (n=23)	53	53	nd
Bogunović <i>et al.</i> (2018)	Serbia	Morphological identification	Dogs (n=53)	316	288	nd
Santos <i>et al.</i> (2013)	Brazil	Morphological identification	Dogs (n=398)	235	194	nd
Adamu <i>et al.</i> (2014)	Nigeria	Morphological identification	Dogs (n=100)	146	107	28

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Rautenbach <i>et al.</i> (1991)	South Africa	Morphological identification	Dogs (n=220)	7398	7337	nd
J. C. P. de Oliveira <i>et al.</i> (2020)	Brazil	Morphological identification	Dogs (n=22)	344	48	nd
Ferreira <i>et al.</i> (2020)	Brazil	Morphological identification	Dogs (n=33)	88	88	nd
Costa <i>et al.</i> (2019)	Brazil	Morphological identification	Dogs (n=20) and vegetation	299	299 [from dogs (n=124) and vegetation (n=175)]	nd
Sthitmatee <i>et al.</i> (2016)	Thailand	Morphological identification	Dogs (n=402)	376	376	nd
Potkonjak <i>et al.</i> (2013)	Serbia	Morphological identification	Dogs (n=100)	62	15	nd
Díaz-Medina <i>et al.</i> (2016)	Mexico	Morphological identification	Dogs (n=200)	1 116	1 116	nd
Cordeiro <i>et al.</i> (2015)	Brazil	Morphological identification	Dogs (n=283)	773	610	nd
Sparagano <i>et al.</i> (2003)	Italy	Morphological identification	Dogs (n=3)	292	292	nd
Yuasa <i>et al.</i> (2017)	Taiwan	Morphological identification	Dogs (n=175)	315	306	nd
Horak (1995)	South Africa	Morphological identification	Dogs (n=395)	3554	1084	2152

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Jacques <i>et al.</i> (2015)	Benin	Morphological identification	Dogs (n=40)	773	673	92
Aktas <i>et al.</i> (2009)	Turkey	Morphological identification	Sheep (n=4) and human (n=1)	220	5 [from sheep (n=4) and human (n=1)]	nd
Aktas <i>et al.</i> (2013)	Turkey	Morphological identification	Dogs (n=63)	328	328	nd
de Almeida <i>et al.</i> (2013)	Brazil	Morphological identification	Dogs (n=148)	2015	1992	nd
De Waal <i>et al.</i> (2020)	Ireland	Morphological identification	Dogs (n=148)	120	1	nd
Khamassi Khbou <i>et al.</i> (2021)	Tunisia	Morphological identification	Sheep (n=439)	707	646	nd
Augustine <i>et al.</i> (2017)	India	Morphological identification	Dogs (n=80)	50	29	nd
Balinandi <i>et al.</i> (2020)	Uganda	Morphological identification, conventional PCR	Cattle (n=100)	4362	3	nd
Tsai <i>et al.</i> (2011)	Taiwan	Morphological identification	Dogs (n=120) and cats (n=103)	386	154 [from dogs (n=151) and cats (n=3)]	nd
Burroughs <i>et al.</i> (2016)	United States	Morphological identification	Dogs (n=121) and cats (n=15)	1337	1243 [from dogs (n=1242) and cats (n=1)]	nd
Chao <i>et al.</i> (2016)	Taiwan	Morphological identification	Dogs (n=30)	578	578	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Chao <i>et al.</i> (2019a)	Taiwan	Morphological identification	Dogs (n=2025)	9467	8759	nd
Konto <i>et al.</i> (2014)	Nigeria	Morphological identification	Dogs (n=400)	4216	456	nd
Ayan <i>et al.</i> (2019)	Turkey	Morphological identification	Dogs (n=55)	220	220	nd
da Costa <i>et al.</i> (2021)	Brazil	Morphological identification	Dogs (n=190)	1962	49	nd
Dantas-Torres <i>et al.</i> (2010)	Brazil and Italy	Morphological identification	Dogs (n=26)	95	95	nd
Costa <i>et al.</i> (2015)	Brazil	Morphological identification	Dogs (n=322)	929	866	nd
Araujo <i>et al.</i> (2015)	Brazil	Morphological identification	Dogs (n=404)	1511	1511	nd
Arroyave <i>et al.</i> (2020)	Colombia	Morphological identification	Dogs (n=300)	193	178	nd
Dantas-Torres <i>et al.</i> (2009)	Brazil	Morphological identification	Dogs (n=41)	159	107	nd
Oliveira <i>et al.</i> (2021)	Brazil	Morphological identification	Dogs (n=22)	344	48	nd
Rozental <i>et al.</i> (2002)	Brazil	Morphological identification	Dogs (n=129)	578	209	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Cordeiro <i>et al.</i> (2020)	Cuba	Morphological identification	Dogs (n=176)	158	158	nd
Campos <i>et al.</i> (2020b)	Brazil	Morphological identification	Dogs (n=130)	353	337	nd
Campos <i>et al.</i> (2020a)	Brazil	Morphological identification	Dogs (n=115)	135	47	nd
de Farias Rotondano <i>et al.</i> (2015)	Brazil	Morphological identification	Dogs (n=100)	1151	1151	nd
Dantas-Torres (2009)	Brazil	Morphological identification	Dogs (n=34)	1405	515	nd
Nascimento <i>et al.</i> (2016)	Brazil	Morphological identification	Dogs (n=83)	100	45	nd
Dumitrache <i>et al.</i> (2014)	Romania	Morphological identification	Dogs (n=8)	893	3	nd
Petney <i>et al.</i> (2004)	South Africa	Morphological identification	Striped mice (n=169)	16599	nd	49
Neves <i>et al.</i> (2004)	Mozambique	Morphological identification	Dogs (n=89)	1898	1484	257
Bryson <i>et al.</i> (2000)	South Africa	Morphological identification	Dogs (n=344)	14724	14226	420
Kumsa <i>et al.</i> (2012)	Ethiopia	Morphological identification	Sheep (n=1325)	600	12	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Roshan <i>et al.</i> (2021)	Pakistan	Morphological identification	Dogs (n=69)	542	248	nd
Ferrell <i>et al.</i> (2017)	Panama	Morphological identification	Dogs (n=355)	2074	2021	nd
Fonseca <i>et al.</i> (2020)	Brazil	Morphological identification	Several mammals (n=16)	238	1 [from guinea pig (n=1)]	nd
Galaviz-Silva <i>et al.</i> (2013)	Mexico	Morphological identification	Dogs (n=338)	2543	2408	nd
Greay <i>et al.</i> (2016)	Australia	Morphological identification	Dogs (n=685) and cats (n=153)	4765	3070 [from dogs (n=3069) and cats (n=1)]	nd
Soares <i>et al.</i> (2020)	Brazil	Morphological identification	Dogs (n=73)	432	432	nd
Hazihan <i>et al.</i> (2019)	China	Morphological identification, conventional PCR	Dogs (n=32)	178	178	nd
Gillingham <i>et al.</i> (2020)	Croatia and Italy	Morphological identification	Humans (n=59)	68	2 [from Croatia (n=1) and Italy (n=1)]	nd
Shimada <i>et al.</i> (2003)	Japan	Morphological identification	Dogs (n=651)	4122	276	nd
Papazahariadou <i>et al.</i> (2003)	Greece	Morphological identification	Dogs (n=249)	2812	2511	nd
Pacheco <i>et al.</i> (2011)	Brazil	Morphological identification	Dogs (n=250)	122	122	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Toledo <i>et al.</i> (2011)	Brazil	Morphological identification	Dogs (n=73)	182	58	nd
Fritz <i>et al.</i> , (2012)	United States	Morphological identification	Dogs (n=116)	200	200	nd
Szabó <i>et al.</i> (2010)	Brazil	Morphological identification	Dogs (n=413)	835	674	nd
Motaghipisheh <i>et al.</i> (2016)	Iran	Morphological identification	Dogs (n=100)	408	408	nd
Banafshi <i>et al.</i> (2018)	Iraq	Morphological identification	Cows (n=62), sheep (n=506), goats (n=73), and lambs (n=23)	1209	726 [from cows (n=17), sheep (n=572), goats (n=65) and lambs (n=72)]	nd
Rahmani-Varmale <i>et al.</i> (2019)	Iran	Morphological identification	Goats (n=400)	420	95	nd
Goldberg <i>et al.</i> (2002)	Georgia	Morphological identification	Dogs (n=324)	917	275	nd
Sabatini <i>et al.</i> (2010)	Brazil	Morphological identification	Dogs (n=146)	2439	59	nd
Costa <i>et al.</i> (2013)	Brazil	Morphological identification	Dogs (n=622)	1582	1235	nd
Özübek & Aktas (2018)	Turkey	Morphological identification	Dogs (n=282) and vegetation	5403	5403 [from dogs (n=418) and vegetation (n=4985)]	nd
Persichetti <i>et al.</i> (2016)	Italy	Morphological identification	Cats (n=42)	73	25	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Szabó <i>et al.</i> (2001)	Brazil	Morphological identification	Dogs (n=140)	179	159	nd
Kamani <i>et al.</i> (2018)	Nigeria	Morphological identification	Dogs (n=31)	66	66	nd
Kamani <i>et al.</i> (2019)	Nigeria	Morphological identification	Dogs (n=608)	1196	1066	42
Klimpel <i>et al.</i> (2010)	Brazil	Morphological identification	Dogs (n=46)	208	208	nd
Kolo <i>et al.</i> (2016)	South Africa	Morphological identification	Dogs (n=64)	103	27	30
Köseoğlu <i>et al.</i> (2021)	Turkey	Morphological identification, conventional PCR	Dogs (n=46)	110	27	nd
Laatamna <i>et al.</i> (2020, 2021)	Algeria	Morphological identification, conventional PCR	Dogs (n=174)	1043	1043	nd
Latrofa <i>et al.</i> (2017)	Greece	Morphological identification	Dogs (n=310)	757	531	nd
Satta <i>et al.</i> (2011)	Italy	Morphological identification	Dogs (n=80), sheep (n=36), goats (n=41), cattle (n=13), and hedgehogs (n=2)	1485	1045 [from dogs (n=965), sheep (n=45), goats (n=20), cattle (n=10), and hedgehogs (n=5)]	nd
Lee <i>et al.</i> (2015)	Mauritius	Morphological identification	Dogs (n=52)	178	175	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Leulmi <i>et al.</i> (2016)	Algeria	Morphological identification	Dogs (n=50), cattle (n=123), goats (n=125), sheep (n=250), boars (n=2), jackals (n=2), mongoose (n=1), and hedgehogs (n=4)	1773	1241 [from dogs (n=323), cattle (n=316), goats (n=104), sheep (n=454), boars (n=9), jackals (n=23), mongoose (n=2), and hedgehogs (n=10)]	nd
Lineberry <i>et al.</i> (2020)	United States	Morphological identification	Dogs (n=250)	112	77	nd
Marquez <i>et al.</i> (2008)	Spain	Morphological identification	Dogs (n=500)	2229	2229	nd
Ramezani <i>et al.</i> (2014)	Iran	Morphological identification	sheep (n=450), dogs (n=9), and goats (n=70)	1208	9 [from sheep (n=1), dogs (n=6), and goats (n=2)]	nd
Kolo <i>et al.</i> (2020)	South Africa	Morphological identification	Dogs (n=56)	160	160	nd
Mirzaei <i>et al.</i> (2016)	Iran	Morphological identification	Dogs (n=102)	101	101	nd
Cervantes S. <i>et al.</i> (2020)	Peru	Morphological identification, conventional PCR	Dogs (n=74)	297	297	nd
Rodriguez <i>et al.</i> (2015)	Cuba	Morphological identification	Dogs (n=55)	152	152	nd
Pat-Nah <i>et al.</i> (2015)	Mexico	Morphological identification	Dogs (n=50)	200	200	nd
Dantas-Torres <i>et al.</i> (2012)	Italy	Morphological identification	Dogs (n=34) and vegetation	567	567 [from dogs (n=276) and vegetation (n=300)]	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Mumcuoglu <i>et al.</i> (1993)	Israel	Morphological identification	Dogs (n=16)	960	960	nd
Melo <i>et al.</i> (2016)	Brazil	Morphological identification, conventional PCR	Dogs (n=320)	930	708	nd
Aktas & Özübek (2017a)	Turkey	Morphological identification	Dogs (n=282) and vegetation	2389	2389 [from dogs (n=204) and vegetation (n=2185)]	nd
Aktas & Özübek (2018)	Turkey	Morphological identification	Dogs (n=282) and vegetation	5389	5389 [from dogs (n=404) and vegetation (n=4985)]	nd
Cevidanes <i>et al.</i> (2021)	Chile	Morphological identification, conventional PCR	Dogs (n=111) and Andean foxes (n=79)	574	511 [from dogs (n=505) and Andean foxes (n=6)]	nd
Ndip <i>et al.</i> (2010)	Cameroon	Morphological identification	Dogs (n=5)	63	63	nd
V. L. Nguyen <i>et al.</i> (2019)	Vietnam	Morphological identification, conventional PCR	Dogs (n=359)	466	466	nd
V. L. Nguyen <i>et al.</i> (2020)	China, Taiwan, Indonesia, Malaysia, Singapore, Thailand, Philippines, and Vietnam	Morphological identification, conventional PCR	Dogs (n=1229), cats (n=1152)	392	377 [from dogs (n=341), cats (n=41), and unspecified hosts (n=10)]	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Nijhof <i>et al.</i> (2007)	Netherlands	Morphological identification	Dogs (n=1281)	4298	12	nd
Hassissen <i>et al.</i> (2019)	Algeria	Morphological identification	Dogs (n=631)	339	174	nd
de Oliveira <i>et al.</i> (2019)	Brazil	Morphological identification	Dogs (n=356)	1344	1270	nd
Oyarzún-Ruiz <i>et al.</i> (2021)	Chile	Morphological identification, conventional PCR	Dogs (n=47)	119	119	nd
Davari <i>et al.</i> (2017)	Iran	Morphological identification	Cattle (n=3044), sheep (n=15365), and goats (n=8678)	549	239 [from cattle (n=42), sheep (n=113), and goats (n=84)]	nd
Zhang <i>et al.</i> (2017)	China	Morphological identification	Dogs (n=562)	1550	1058	nd
Cicculi <i>et al.</i> (2019)	France	Morphological identification, conventional PCR	Dogs (n= 13)	1117	24	nd
Díaz-Sánchez <i>et al.</i> (2020)	Cuba	Morphological identification	Dogs (n=100)	248	248	nd
Sharifdini <i>et al.</i> (2021)	Iran	Morphological identification	Feral raccoons (n=30)	255	167	nd
Gehrke <i>et al.</i> (2009)	Brazil	Morphological identification	Dogs (n=7)	191	191	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Márquez & Millán (2009)	Spain	Morphological identification	Iberian lynx (n=32) and dogs (n=47)	430	263 [from Iberian lynx (n=1) and dogs (n=262)]	nd
Kamani <i>et al.</i> (2013)	Nigeria	Morphological identification	Rodents (n=177)	85	79	6
Ereqat <i>et al.</i> (2016)	Palestine	Morphological identification	Dogs (n=234), sheep (n=68), goats (n=5), horses (n=1), wolves (n=1)	1123	694 [from dogs (n=634), sheep (n=41), goats (n=16), horses (n=2), and wolves (n=1)]	nd
Diarra <i>et al.</i> (2017)	Mali	Morphological identification, conventional PCR	Dogs (n=9)	1333	5	nd
Quintero V <i>et al.</i> (2017)	Colombia	Morphological identification	Humans (n=597)	458	3	nd
Costa <i>et al.</i> (2017)	Brazil	Morphological identification	Dogs (n=1560)	959	652	nd
Agu <i>et al.</i> (2020)	Nigeria	Morphological identification	Dogs (n=420)	171	158, 37.6%	4, 1%
Proboste <i>et al.</i> (2015)	Uganda	Morphological identification	Dogs (n=251)	430	4	324
Qablan <i>et al.</i> (2012)	Jordan	Morphological identification	Dogs (n=45)	268	255	nd
Ramos <i>et al.</i> (2013)	Italy	Morphological identification	Dogs (n=1)	184	10	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
René-Martellet <i>et al.</i> (2015)	France	Morphological identification, conventional PCR	Dogs (n=155) and vegetation	667	605 [from dogs (n=574) and vegetation (n=31)]	nd
Medeiros-Silva <i>et al.</i> (2015)	Brazil	Morphological identification	Dogs (n=48)	130	130	nd
Li <i>et al.</i> (2015)	China	Morphological identification, conventional PCR	Camels (n=279) and vegetation	382	84 [from camels (n=63) and vegetation (n=21)]	nd
Xu <i>et al.</i> (2015)	China	Morphological identification	Dogs (n=1114)	146	146	nd
Pennisi <i>et al.</i> (2015)	Italy	Morphological identification	Cats (n=308)	132	28	nd
Andersson <i>et al.</i> (2018)	Romania	Morphological identification, conventional PCR	Dogs (n=545)	824	314	nd
Greay <i>et al.</i> (2018)	Australia	Morphological identification	Dogs (n=498)	711	184	nd
Maurelli <i>et al.</i> (2018)	Italy	Morphological identification	Dogs (n=3026)	2439	1822	nd
Livanova <i>et al.</i> (2018)	Russia	Morphological identification	Dogs (n=636)	1010	112	nd
Galay <i>et al.</i> (2018)	Philippines	Morphological identification	Dogs (n=248)	157	157	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Regier <i>et al.</i> (2019)	Germany	Morphological identification	Dogs (n=54) and cats (n=1)	189	2 [from dogs (n=1) and cats (n=1)]	nd
Checa <i>et al.</i> (2019)	Spain	Morphological identification	Dogs (n=756)	130	64	nd
Ortega-Morales <i>et al.</i> (2019)	Mexico	Morphological identification	Dogs (n=300)	1238	1238	nd
Saleh <i>et al.</i> (2019)	United States	Morphological identification	Dogs (n=1494) and cats (n=336)	10087	6266 [from dogs (n=6252) and cats (n=14)]	nd
Choe <i>et al.</i> (2011)	South Korea	Morphological identification	Dogs (n=2500)	411	2	nd
Santos <i>et al.</i> (2018)	Brazil	Morphological identification	Dogs (n=75)	758	758	nd
Minervino <i>et al.</i> (2015)	Brazil	Morphological identification	Dogs (n=327)	182	33	nd
Solano-Gallego <i>et al.</i> (2012)	Italy	Morphological identification, conventional PCR	Dogs (n=41)	118	118	nd
Tarasevich <i>et al.</i> (1991)	Russia	Morphological identification	Dogs (n=480)	104	104	nd
López-Pérez <i>et al.</i> (2022)	United States	Morphological identification	Wild carnivores (n=23)	447	405 [from coyotes (n=8) and wolves (n=397)]	nd
Temoche <i>et al.</i> (2018)	Peru	Morphological identification	Dogs (n=216)	984	977	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Tsai <i>et al.</i> (2012)	Taiwan	Morphological identification	Dogs (n=92), goats (n=9), and vegetation	594	292 [from dogs (n=285), goats (n=3), and vegetation (n=4)]	nd
Medrano-Bugarini <i>et al.</i> (2019)	Mexico	Morphological identification, conventional PCR	horses (n=144)	98	8	nd
Merino <i>et al.</i> (2005)	Spain	Morphological identification	Humans (n=179)	185	3	nd
Moerbeck <i>et al.</i> (2016)	Brazil	Morphological identification	Dogs (n=188), cattle (n=28), donkeys (n=18), rodents (n=9), humans (n=4), and vegetation	1575	514 [from dogs (n=490), cattle (n=16), donkeys (n=2), rodents (n=3), humans (n=1) and vegetation (n=2)]	nd
Poo-Muñoz <i>et al.</i> (2016)	Chile	Morphological identification	Dogs (n=107)	139	139	nd
Durden <i>et al.</i> (2016)	United States	Morphological identification	Dogs (n=79) and humans (n=13)	160	24 [from dogs (n=23) and humans (n=1)]	nd
Aktas & Özübek (2017b)	Turkey	Morphological identification	Dogs (n=16) and vegetation	4103	4103 [from dogs (n=418) and vegetation (n=3685)]	nd
Low <i>et al.</i> (2018); Prakash <i>et al.</i> (2018a); Prakash <i>et al.</i> (2018b)	Malaysia	Morphological identification	Dogs (n=240)	140	140	nd
Oliveira <i>et al.</i> (2019)	Brazil	Morphological identification	Dogs (n=72)	720	720	nd
Beeler <i>et al.</i> (2011)	United States	Morphological identification, conventional PCR	Dogs (n=4) and vegetation	332	332 [from dogs (n=272) and vegetation (n=50)]	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Zhang <i>et al.</i> (2012)	China	Morphological identification	Dogs (n=219) and vegetation	1580	76 [from dogs (n=40) and vegetation (n=36)]	nd
Horak <i>et al.</i> (2010)	South Africa	Morphological identification	Domestic cats (n=130), cheetahs (n=23), caracals (n=21), lions (n=9), and leopards (n=8)	2654	nd	2239 [from domestic cats (n=1978), cheetahs (n=119), caracals (n=31), lions (n=53), and leopards (n=58)]
Henning <i>et al.</i> (2014)	United States	Morphological identification	Vegetation	2396	60	nd
Araes-Santos <i>et al.</i> (2015)	Brazil	Morphological identification	Dogs (n=504)	285	285	nd
Shoorijeh <i>et al.</i> (2008)	Iran	Morphological identification	Dogs (n=160)	142	142	nd
Mengfan <i>et al.</i> (2020)	China	Morphological identification	Dogs (n=297)	709	709	nd
Kiouani <i>et al.</i> (2020)	Algeria	Morphological identification	Dogs (n=189)	242	242	nd
Vieira <i>et al.</i> (2018)	Brazil	Morphological identification	Dogs (n=1483)	665	665	nd
Wells <i>et al.</i> (2012)	Malaysia	Morphological identification	Dogs (n=212)	245	170	nd
Lucero-Velasco <i>et al.</i> (2018)	Mexico	Morphological identification	Dogs (n=18)	70	70	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Ramos <i>et al.</i> (2020)	Brazil	Morphological identification	Dogs (n=145)	2435	354	nd
B. R. D. Silva <i>et al.</i> (2017)	Brazil	Morphological identification	Dogs (n=56)	253	176	nd
Zanet <i>et al.</i> (2020)	Italy	Morphological identification	Dogs (n=1454)	2681	1930	nd
Zheng <i>et al.</i> (2019)	China	Morphological identification	Dogs (n=171), goats (n=85), cattle (n=48), Chinese hare (n=21), Ring-necked Pheasants (n=7), sheep (n=17), and vegetation	2193	420 [from dogs (n=266), goats (n=1), cattle (n=13), Chinese hare (n=4), Ring-necked Pheasants (n=14), sheep (n=76), and vegetation (n=46)]	nd
Khrouf <i>et al.</i> (2014)	Tunisia	Morphological identification	Dogs (n=28) and sheep (n=11)	663	663 [from dogs (n=657) and sheep (n=6)]	nd
Yaglom <i>et al.</i> (2018)	United States	Morphological identification	Dogs (n=217)	57	57	nd
Pieracci <i>et al.</i> (2019)	United States	Morphological identification	Dogs (n=1136)	942	942	nd
H. M. Nguyen <i>et al.</i> (2020)	Laos	Morphological identification	Dogs (n=112)	3151	3151	nd
Diyes & Rajakaruna. (2015)	Sri Lanka	Morphological identification	Goats (n=740)	2628	55	nd
Miranda & Mattar (2015)	Colombia	Morphological identification	Dogs (n=87)	1105	353	nd

Table 3-1: (continue)

Reference	Country	Tick identification method	Hosts ^a	Total number of ticks collected ^b	<i>Rhipicephalus sanguineus</i> prevalence	<i>Heamaphysalis elliptica</i> prevalence
Fryxell et al. (2012)	United States	Morphological identification	Canines (n=173) and white-tailed deer (n=250)	2123	341 [from canines (n=317) and white-tailed deer (n=24)]	nd

^aOnly hosts infected with *R. sanguineus* or *H. elliptica* are reported.

^bRefers to total of all tick species collected during a study, not just *R. sanguineus* and *H. elliptica* ticks.

nd: not detected.

Table 3-2: Summary containing the list, as well as study characteristics of articles included in the systematic review, reporting on the global prevalence of tick-borne pathogens harboured by *R. sanguineus* and *H. elliptica*

Reference	Geographical location	Tick-borne pathogens detected in <i>R. sanguineus</i> ticks	Tick-borne pathogens detected in <i>H. elliptica</i> ticks	Tick-borne pathogens detection method
Ramos et al. (2014b)	Italy	<i>A. platys</i> (n=129/267 ticks)	nd	qPCR and conventional PCR
Chao et al. (2017)	Taiwan	<i>B. vogeli</i> and <i>B. gibsoni</i> [1.59% (n=19/1195 ticks)]	nd	nested PCR
Dall'Agnol et al. (2017)	Brazil	<i>Candidatus Rickettsia asemboensis</i> (n=1/63 ticks)	nd	PCR
Dzul-Rosado et al. (2021)	Mexico	Spotted Fever Group Rickettsia (n=89/239 ticks) Typhus Group Rickettsia (n=86/239 ticks)	nd	nested PCR

Table 3-2: (continue)

Reference	Geographical location	Tick-borne pathogens detected in <i>R. sanguineus</i> ticks	Tick-borne pathogens detected in <i>H. elliptica</i> ticks	Tick-borne pathogens detection method
Vila <i>et al.</i> (2019)	Spain	<i>Coxiella</i> sp. (<i>R. sanguineus</i> symbiont) [100% (315/315 tick pools)] <i>R. massiliae</i> [37% (116/315 tick pools)] <i>Rickettsia sibirica</i> subsp. <i>Mongolitimona</i> [2.2% (7/315 tick pools)] <i>Rickettsia monacensis</i> [0.6% (2/315 tick pools)] <i>Rickettsia raoultii</i> [0.3% (1/315 tick pools)]	nd	qPCR and conventional PCR
Trotta <i>et al.</i> (2012)	Italy	<i>Ri. conorii</i> [1% (n=1/73 ticks)] <i>Ri. massiliae</i> [4% (n=3/73 ticks)] <i>L. infantum</i> [3% (n=2/73 ticks)]	nd	qPCR and conventional PCR
Ybañez <i>et al.</i> (2012)	Philippines	<i>E. canis</i> [% (n=7/164 ticks)] <i>A. platys</i> [% (n=1/164 ticks)]	nd	screening PCR and semi-nested PCR
Ramos <i>et al.</i> (2014a)	Italy	<i>H. canis</i> [13.47% (n=147/1091 ticks)]	nd	morphologically identified
Maia <i>et al.</i> (2014)	Portugal	<i>Ri. massiliae</i> [4.0% (n=35/882 ticks)] <i>Ri. conorii</i> [0.5% (n=4/882 ticks)] <i>H. canis</i> [0.2% (n=2/882 ticks)] <i>A. phagocytophylum</i> [0.1% (n=1/882 ticks)] <i>A. platys</i> [0.3% (n=3/882 ticks)] <i>Ba. vogeli</i> [0.1% (n=1/882 ticks)] <i>Bo. burgdorferi</i> s.l. [0.1% (n=1/882 ticks)]	nd	conventional PCR
Chisu <i>et al.</i> (2014)	Italy	<i>Ri. conorii israelensis</i> [17.6% (n=3/17 ticks)] <i>Ri. massiliae</i> [11.8% (n=2/17 ticks)]	nd	conventional PCR
Cicuttin <i>et al.</i> (2014)	Argentina	<i>Rickettsia</i> spp. [3.4% (n=7/207 ticks)]	nd	conventional PCR
Barbieri <i>et al.</i> (2014)	Brazil	<i>Rickettsia</i> sp. (n=5/6 ticks)	nd	conventional PCR
Cicuttin <i>et al.</i> (2015)	Argentina	<i>Ehrlichia</i> spp. (n=3/181 ticks) <i>Anaplasma</i> spp. (n=2/181 ticks)	nd	PCR assay

Table 3-2: (continue)

Reference	Geographical location	Tick-borne pathogens detected in <i>R. sanguineus</i> ticks	Tick-borne pathogens detected in <i>H. elliptica</i> ticks	Tick-borne pathogens detection method
Ionita et al. (2016)	Romania	<i>Ri. conorii</i> [0.8% (n=1/120 ticks)] <i>E. canis</i> [15.8% (n=19/120 ticks)]	nd	conventional PCR; q-PCR
Almazán et al. (2016)	Mexico	<i>Anaplasma</i> sp. (n=3/500 ticks)	nd	conventional PCR
Cetinkaya et al. (2016)	Turkey	<i>E. canis</i> [(n=284/850 ticks)] <i>A. platys</i> (n=8/850 ticks)	nd	nested PCR
Azmi et al. (2016)	Israel	<i>Theileria ovis</i> (n=7/305 ticks) <i>Babesia ovis</i> (n=1/305 ticks) <i>H. canis</i> (n=21/305 ticks) <i>B. vogeli</i> (n=2/305 ticks)	nd	PCR-RFLP
da Silva et al. (2016)	Cuba	<i>A. platys</i> [10.20% (n=5/49 tick pools)]	nd	nPCR and conventional PCR
Scarpulla et al. (2016)	Italy	<i>R. massiliae</i> [4% (n=1/25 ticks)] <i>R. conorii</i> [4% (n=1/25 ticks)] <i>Rickettsia</i> sp. [4% (n=1/25 ticks)]	nd	conventional PCR
Campos-Calderón et al. (2016)	Costa Rica	<i>E. canis</i> [26% (n=43/160 ticks)] <i>A. phagocytophylum</i> [1.3% (n=2/160 ticks)] <i>A. platys</i> [3% (n=5/160 ticks)] <i>Rickettsia</i> sp. [15.6% (n=25/160 ticks)]	nd	conventional PCR
Springer et al. (2018)	Costa Rica and Nicaragua	<i>Rickettsia</i> sp. [0.7% (n=7/281 tick pools)]	nd	qPCR
Chao et al. (2019b)	Taiwan	<i>H. canis</i> [23.3% (n=252/1082 ticks)]	nd	nested-PCR
G. M. B. de Oliveira et al. (2020)	Brazil	<i>Ehrlichia</i> spp. (n=2/33 tick pools)	nd	conventional PCR
Barradas et al. (2020)	Portugal	<i>Rickettsia</i> spp. [4.5% (n=10/221 ticks)]	nd	nested-PCR

Table 3-2: (continue)

Reference	Geographical location	Tick-borne pathogens detected in <i>R. sanguineus</i> ticks	Tick-borne pathogens detected in <i>H. elliptica</i> ticks	Tick-borne pathogens detection method
Hsu <i>et al.</i> (2011)	Taiwan	<i>Rickettsia</i> spp. [1.9% (n=3/151 ticks)]	nd	PCR
Bessas <i>et al.</i> (2016)	Algeria	<i>R. massiliae</i> [24.35% (n=28/115 randomly selected ticks)] <i>R. conorii</i> [0.87% (n=1/115 randomly selected ticks)] <i>E. canis</i> [6.95% (n=8/115 randomly selected ticks)]	nd	qPCR
Sipin <i>et al.</i> (2020)	Malaysia	<i>A. platys</i> [1.43% (n=2/140 ticks)] <i>E. canis</i> [0.71% (n=1/140 ticks)] <i>B. vogeli</i> [0.71% (n=1/140 ticks)]	nd	conventional PCR
M'ghirbi & Bouattour (2008)	Tunisia	<i>B. vogeli</i> [0.6% (n=1/160 selected ticks)]	nd	conventional PCR and Reverse line blot hybridization
Bermúdez <i>et al.</i> (2011)	Panama	<i>R. amblyommii</i> [12.3% (n=7/44 tick pools)]	nd	conventional PCR
René <i>et al.</i> (2012)	France	<i>B. vogeli</i> [22.2% (n=8/36 selected ticks)]	nd	conventional PCR
Giannelli <i>et al.</i> (2013)	Italy	<i>H. canis</i> (n=31/120 selected ticks)	nd	Morphological identification (Giemsa-stained blood smears)
		<i>H. canis</i> [95.8% (n=23/24 tick pools)]	nd	PCR
Giannelli <i>et al.</i> (2013)	Italy	<i>H. canis</i> [88.6% (n=31/35 ticks)]	nd	conventional PCR
Aktas (2014)	Turkey	<i>E. canis</i> [2.35% (n=1/5 tick pools)]	nd	Reverse line blot hybridization
		<i>H. canis</i> [7.10% (n=3/5 tick pools)]	nd	conventional PCR
Low <i>et al.</i> (2017)	Malaysia	<i>R. asembonensis</i> [1.7% (n=2/120 ticks)]	nd	Conventional PCR
Oskam <i>et al.</i> (2017)	Australia	<i>Coxiella</i> sp. [100% (n=199/199 ticks)]	nd	qPCR

Table 3-2: (continue)

Reference	Geographical location	Tick-borne pathogens detected in <i>R. sanguineus</i> ticks	Tick-borne pathogens detected in <i>H. elliptica</i> ticks	Tick-borne pathogens detection method
Monje <i>et al.</i> (2016)	Argentina	<i>R. massiliae</i> [5.1% (n=7/138 ticks)]	nd	qPCR
Alieva <i>et al.</i> (2020)	Ukraine	<i>Rickettsia</i> sp. [19.1% (n=16/84 ticks)]	nd	qPCR
Ghafar & Amer (2012)	Egypt	<i>A. phagocytophylum</i> [1.7% (n=55/104 ticks)]	nd	conventional PCR
Billeter <i>et al.</i> (2012)	Thailand	<i>B. rochalimae</i> (n=1/76 adult ticks)	nd	conventional PCR
Sosa-Gutiérrez <i>et al.</i> (2016)	Mexico	<i>Ehrlichia</i> sp. (n=28/128 ticks); <i>E. canis</i> (n=27/128 ticks)	nd	conventional PCR
Watanabe <i>et al.</i> (2015)	Malaysia	<i>C. burnetii</i> [59% (n=26/44 selected ticks)]	nd	nested PCR
Bogunović <i>et al.</i> (2018)	Serbia	<i>C. burnetii</i> [10.53% (n=24/288 ticks)]	nd	conventional PCR
Santos <i>et al.</i> (2013)	Brazil	<i>A. phagocytophylum</i> [7.76% (n=5/194 ticks)]	nd	qPCR
J. C. P. de Oliveira <i>et al.</i> (2020)	Brazil	<i>Rickettsia felis</i> (n=1/31 tick pools)	nd	conventional PCR
Costa <i>et al.</i> (2019)	Brazil	<i>E. canis</i> (n=11/299 ticks)	nd	qPCR
Yuasa <i>et al.</i> (2017)	Taiwan	<i>A. platys</i> [23.2% (n=71/306 ticks)] <i>E. canis</i> [1% (n=3/306 ticks)]	nd	nested PCR
Aktas <i>et al.</i> (2009)	Turkey	<i>A. ovis</i> (n=2/5 ticks)	nd	conventional PCR
Aktas <i>et al.</i> (2013)	Turkey	<i>Hepatozoon</i> spp. [20.58% (n=14/68 ticks pools)]	nd	conventional PCR
Tsai <i>et al.</i> (2011)	Taiwan	<i>Bartonella</i> (n=11/68 ticks from dogs)	nd	conventional PCR
Chao <i>et al.</i> (2016)	Taiwan	<i>B. vogeli</i> [1.04% (n=6/578 ticks)]	nd	nested PCR
Ayan <i>et al.</i> (2019)	Turkey	<i>E. canis</i> (n=50/220 ticks) <i>E. ewingii</i> (n=3/220 ticks)	nd	nested PCR

Table 3-2: (continue)

Reference	Geographical location	Tick-borne pathogens detected in <i>R. sanguineus</i> ticks	Tick-borne pathogens detected in <i>H. elliptica</i> ticks	Tick-borne pathogens detection method
Dantas-Torres <i>et al.</i> , (2010)	Brazil and Italy	<i>L. infantum</i> (n=2/4 salivary gland pools of ticks)	nd	conventional PCR
		<i>L. infantum</i> (n=9/73 ticks)	nd	qPCR
Araujo <i>et al.</i> (2015)	Brazil	<i>B. vogeli</i> [6% (n=3/50 adult ticks) and 8,7% (n=7/80 nymphal tick pools)]	nd	conventional PCR
Arroyave <i>et al.</i> (2020)	Colombia	<i>E. canis</i> [11.8% (n=21/178 ticks)] <i>A. platys</i> [3.4% (n=6/178 ticks)]	nd	conventional PCR
Campos <i>et al.</i> (2020b)	Brazil	<i>Rickettsia</i> spp. [0.89% (n=3/337 ticks)]	nd	conventional PCR
Campos <i>et al.</i> (2020a)	Brazil	<i>Rickettsia</i> spp. (n=2/47 ticks)	nd	conventional PCR
Ferrell <i>et al.</i> (2017)	Panama	<i>R. amblyommii</i> (n=1/244 selected ticks from different species)	nd	conventional PCR
Soares <i>et al.</i> (2020)	Brazil	<i>E. canis</i> [24.65% (n=18/73 tick pools)] <i>A. platys</i> [19.17% (n=14/73 tick pools)]	nd	nested PCR
		<i>H. canis</i> [5.47% (n=4/73 tick pools)]	nd	conventional PCR
Hazihan <i>et al.</i> (2019)	China	<i>Candidatus R. barbariae</i> [61.9% (n=13/178 ticks)] <i>R. massiliae</i> [23.8% (n=5/178 ticks)] <i>R. conorii</i> [14.3% (n=3/178 ticks)]	nd	conventional PCR
Pacheco <i>et al.</i> (2011)	Brazil	<i>R. rickettsii</i> [25% (n=5/20 male ticks) and 9% (n=9/100 tick eggs)]	nd	conventional PCR
Motaghipisheh <i>et al.</i> (2016)	Iran	<i>Ehrlichia</i> spp. [6% (n=6/100 tick pools)]	nd	conventional PCR
Sabatini <i>et al.</i> (2010)	Brazil	<i>Rickettsia</i> sp. [1.9% (n=1/54 ticks)]	nd	conventional PCR
Kamani <i>et al.</i> (2018)	Nigeria	<i>B. rossi</i> [12,1% (n=8/66 ticks)]	nd	conventional PCR

Table 3-2: (continue)

Reference	Geographical location	Tick-borne pathogens detected in <i>R. sanguineus</i> ticks	Tick-borne pathogens detected in <i>H. elliptica</i> ticks	Tick-borne pathogens detection method
Kolo <i>et al.</i> (2016)	South Africa	<i>Ehrlichia</i> / <i>Anaplasma</i> , <i>Neoehrlichia</i> genus-specific probe (n=1/2 tick pools)	<i>Ehrlichia</i> / <i>Anaplasma</i> genus-specific probe (n=1/3 tick pools)	RLB
		<i>Rickettsia</i> sp. (n=1/2 tick pools)	<i>R. africae</i> (n=1/3 tick pools)	qPCR
Köseoğlu <i>et al.</i> (2021)	Turkey	<i>Hepatozoon</i> spp. (n=1/27 ticks) <i>Hepatozoon canis</i> (n=6/27 ticks)	nd	conventional PCR
Satta <i>et al.</i> (2011)	Italy	<i>Rickettsia</i> (SFG) (n=9/209 tick pools) <i>E. canis</i> (n=1/209 tick pools) <i>C. burnetii</i> (n=5/209 tick pools) <i>Bartonella</i> spp. (n=1/209 Rh tick pools)	nd	conventional PCR
Leulmi <i>et al.</i> (2016)	Algeria	<i>R. massiliae</i> (n=38/94 ticks)	nd	qPCR
Marquez <i>et al.</i> (2008)	Spain	<i>R. massiliae</i> [18% (n=90/500 tick pools)]	nd	conventional PCR
Kolo <i>et al.</i> (2020)	South Africa	<i>Anaplasma</i> sp. [85% (n=17/20 tick pools)]	nd	qPCR
Rodriguez <i>et al.</i> (2015)	Cuba	<i>Anaplasma</i> spp. (n=12/55 tick pools) <i>Babesia</i> spp. (n=4/55 tick pools) Co-infection with <i>Anaplasma</i> spp. and <i>Babesia</i> spp. (n=1/55 tick pools)	nd	Multiplex PCR
Melo <i>et al.</i> (2016)	Brazil	<i>A. platys</i> [0.26% (n=1/392 tick pools)]	nd	Conventional and nested PCR
		<i>B. vogeli</i> 1.28% [(n=5/392 tick pools)] <i>E. canis</i> [0.26% (n=1/392 tick pools)]	nd	Conventional PCR
Aktas & Özübek (2018)	Turkey	<i>A. platys</i> (n=21/138 tick pools)	nd	Conventional PCR

Table 3-2: (continue)

Reference	Geographical location	Tick-borne pathogens detected in <i>R. sanguineus</i> ticks	Tick-borne pathogens detected in <i>H. elliptica</i> ticks	Tick-borne pathogens detection method
Ndip <i>et al.</i> (2010)	Cameroon	<i>E. chaffeensis</i> [56% (n=33/63 ticks)] <i>E. canis</i> [6% (n=4/63 ticks)] Co-infection with <i>E. chaffeensis</i> and <i>E. canis</i> (n=1/63 ticks)	nd	qPCR
V. L. Nguyen <i>et al.</i> (2019)	Vietnam	<i>H. canis</i> (n=3/302 tick pools) <i>E. canis</i> (n=1/302 tick pools) <i>B. vogeli</i> (n=1/302 tick pools)	nd	Conventional PCR
V. L. Nguyen <i>et al.</i> (2020)	China; Taiwan; Indonesia; Malaysia; Singapore; Thailand; Philippines; and Vietnam	<i>H. canis</i> (n=21/367 ticks) <i>B. vogeli</i> (n=4/367 ticks) <i>Rickettsia asembonensis</i> (n=1/367 ticks) <i>Rickettsia felis</i> (n=1/367 ticks) <i>E. canis</i> (n=7/367 ticks) <i>A. platys</i> (n=3/367 ticks)	nd	Conventional PCR
Zhang <i>et al.</i> (2017)	China	<i>E. canis</i> [11.03% (n=50/453 ticks)] <i>A. platys</i> [2.65% (n=12/453 ticks)]	nd	Nested PCR
		<i>B. gibsoni</i> [1.77% (n=8/453 ticks)]	nd	conventional PCR
		<i>Borrelia burgdorferi</i> [4.4% (n=2/453 ticks)]	nd	Nested PCR
		Co-infection with <i>B. gibsoni</i> and <i>E. canis</i> [0.22% (n=1/453 ticks)] Co-infection with <i>B. gibsoni</i> and <i>A. platys</i> [0.22% (n=1/453 ticks)]; Co-infection with <i>E. canis</i> and <i>A. platys</i> [1.10% (n=5/453 ticks)] Co-infection with <i>E. canis</i> , <i>A. platys</i> and <i>B. burgdorferi</i> [0.22% (n=1/453 ticks)]	nd	Nested PCR and conventional PCR
Cicculi <i>et al.</i> (2019)	France	<i>Ri. massiliae</i> (n=1/1 tick pools)	nd	qPCR; conventional PCR
Gehrke <i>et al.</i> (2009)	Brazil	<i>R. rickettsii</i> [3.4%]	nd	conventional PCR

Table 3-2: (continue)

Reference	Geographical location	Tick-borne pathogens detected in <i>R. sanguineus</i> ticks	Tick-borne pathogens detected in <i>H. elliptica</i> ticks	Tick-borne pathogens detection method
Márquez & Millán (2009)	Spain	<i>Ri. massiliae</i> (n=28/81 tick pools)	nd	conventional PCR
Kamani <i>et al.</i> (2013)	Nigeria	<i>Bartonella</i> spp. (n=1/10 tick pools)	nd	conventional PCR
Ereqat <i>et al.</i> (2016)	Palestine	<i>R. massiliae</i> (n=11/61 PCR <i>Rickettsia</i> positive ticks) <i>C. R. barbariae</i> (n=3/61 PCR <i>Rickettsia</i> positive ticks) <i>C. R. goldwasesrii</i> (n=7/61 PCR <i>Rickettsia</i> positive ticks) <i>Rickettsia</i> spp. (n=4/61 PCR <i>Rickettsia</i> positive ticks)	nd	conventional PCR
Proboste <i>et al.</i> (2015)	Uganda	nd	<i>Ehrlichia</i> sp. [25% (n=10/40 tick pools)] <i>Rickettsia</i> sp. [25% (n=10/40 tick pools)] <i>B. rossi</i> [2.5% (n=1/40 tick pools)]	qPCR
René-Martellet <i>et al.</i> (2015)	France	<i>B. canis</i> [1.6% (n=4/248 selected ticks)]	nd	nested PCR
		<i>B. vogeli</i> [10.5 % (n=26/248 selected ticks)]	nd	conventional PCR
Medeiros-Silva <i>et al.</i> (2015)	Brazil	<i>Leishmania infantum</i> (n=5/44 tick pools)	nd	conventional PCR
Li <i>et al.</i> (2015)	China	<i>A. platys</i> (n=9/84 ticks)	nd	conventional PCR
Xu <i>et al.</i> (2015)	China	<i>E. canis</i> [4.1% (n=6/146 ticks)]	nd	qPCR
		<i>B. vogeli</i> [3.4% (n=5/146 ticks)]		
Pennisi <i>et al.</i> (2015)	Italy	<i>Bartonella clarridgeiae</i> [3.5% (n=1/28 ticks)] <i>Rickettsia</i> spp. [7.1% (n=2/28 ticks)] <i>Rickettsia monacensis</i> [7.1% (n=2/28 ticks)] <i>B. vogeli</i> [3.5% (n=1/28 ticks)] <i>Leishmania infantum</i> [10.7% (n=3/28 ticks)]	nd	qPCR

Table 3-2: (continue)

Reference	Geographical location	Tick-borne pathogens detected in <i>R. sanguineus</i> ticks	Tick-borne pathogens detected in <i>H. elliptica</i> ticks	Tick-borne pathogens detection method
Andersson <i>et al.</i> (2018)	Romania	<i>Ehrlichia</i> sp. HF (n=1/314 ticks) <i>Rickettsia helvetica</i> (n=1/314 ticks) <i>Rickettsia monacensis</i> (n=1/314 ticks) <i>Rickettsia raoultii</i> (n=1/314 ticks) <i>Rickettsia slovaca</i> (n=2/314 ticks)	nd	qPCR
Greay <i>et al.</i> (2018)	Australia	<i>B. vogeli</i> (n=2/184 ticks)	nd	conventional PCR
Livanova <i>et al.</i> (2018)	Russia	<i>E. canis</i> [0.9% (n=1/112 ticks)] <i>B. vogeli</i> [0.9% (n=1/112 ticks)]	nd	qPCR
Galay <i>et al.</i> (2018)	Philippines	<i>E. canis</i> [3.2% (n=5/157 ticks)] <i>A. platys</i> [0.6% (n=1/157 ticks)] <i>B. vogeli</i> [0.6% (n=1/157 ticks)] <i>H. canis</i> [0.6% (n=1/157 ticks)]	nd	nested PCR, followed by end-point PCR for positive samples
Ortega-Morales <i>et al.</i> (2019)	Mexico	<i>R. rhipicephali</i> (n=2/30 tick pools) <i>R. rickettsii</i> (n=2/30 tick pools) Co-infections with <i>R. rickettsii</i> and <i>R. rhipicephali</i> (n=2/30 tick pools)	nd	conventional PCR
Santos <i>et al.</i> (2018)	Brazil	<i>C. bairdii</i> [0.93% (n=7/758 ticks)] <i>H. canis</i> [0.53% (n=4/758 ticks)]	nd	Microscopy and conventional PCR
Solano-Gallego <i>et al.</i> (2012)	Italy	<i>Leishmania infantum</i> [10.1% (n=13/118 ticks)]	nd	qPCR
Tarasevich <i>et al.</i> (1991)	Russia	<i>SFG rickettsiae</i> [7.6% (n=8/104 ticks)]	nd	Indirect immunofluorescence technique
López-Pérez <i>et al.</i> (2022)	United States	<i>Rickettsia</i> spp. (n=11/405 ticks)	nd	Conventional PCR
Merino <i>et al.</i> (2005)	Spain	<i>Rickettsia</i> spp. (n=1/3 ticks)	nd	conventional PCR

Table 3-2: (continue)

Reference	Geographical location	Tick-borne pathogens detected in <i>R. sanguineus</i> ticks	Tick-borne pathogens detected in <i>H. elliptica</i> ticks	Tick-borne pathogens detection method
Moerbeck <i>et al.</i> (2016)	Brazil	<i>Rickettsia</i> sp. (n=1/294 selected ticks)	nd	conventional PCR
Aktas & Özübek (2017b)	Turkey	<i>H. canis</i> (n=77/117 pooled ticks)	nd	conventional PCR
		<i>H. canis</i> (n=25/25 dissected ticks hemolymph)		
		<i>H. canis</i> (n=8/25 dissected ticks hemolymph)	nd	Microscopy
Low <i>et al.</i> (2018); Prakash <i>et al.</i> (2018a); Prakash <i>et al.</i> (2018b)	Malaysia	<i>B. vogeli</i> [0.71% (n=1/140 ticks)]	nd	conventional PCR
		<i>B. gibsoni</i> [0.71% (n=1/140 ticks)]		
		<i>H. canis</i> [0.71% (n=1/140 ticks)]		
		<i>A. platys</i> [2.9% (n=4/140 ticks)] by nested PCR <i>E. canis</i> [0.7% (n=1/140 ticks)]	nd	nested PCR
		Co-infection with <i>E. canis</i> and <i>B. gibsoni</i> [0.7% (n=1/140 ticks)]	nd	conventional PCR and nested PCR
Oliveira <i>et al.</i> (2019)	Brazil	<i>E. canis</i> [31.94% (n=23/72 pooled ticks)]	nd	qPCR
Beeler <i>et al.</i> (2011)	United States	<i>Rickettsia massiliae</i> (n=149/332 ticks)	nd	conventional PCR
Zhang <i>et al.</i> (2012)	China	<i>A. phagocytophylum</i> (n=5/76 ticks)	nd	Nested PCR
Henning <i>et al.</i> (2014)	United States	<i>R. parkeri</i> [3.33% (n=2/60 ticks)]	nd	nested PCR
Araes-Santos <i>et al.</i> (2015)	Brazil	<i>Rickettsia</i> spp. [1.1% (n=3/285 ticks)]	nd	conventional PCR
		<i>Ehrlichia</i> spp. [4.9% (n=14/285 ticks)]	nd	heminested PCR
Mengfan <i>et al.</i> (2020)	China	<i>E. canis</i> [15.23% (n=108/709 ticks)]	nd	conventional PCR

Table 3-2: (continue)

Reference	Geographical location	Tick-borne pathogens detected in <i>R. sanguineus</i> ticks	Tick-borne pathogens detected in <i>H. elliptica</i> ticks	Tick-borne pathogens detection method
Lucero-Velasco et al. (2018)	Mexico	<i>Staphylococcus epidermidis</i> (n=4/14 tick pools) <i>Staphylococcus sacc(capitis)</i> (n=1/14 tick pools) <i>Bacillus clausii</i> (n=2/14 tick pools) <i>Staphylococcus cohnii</i> (n=1/14 tick pools) <i>Bacillus megaterium</i> (n=1/14 Rh tick pools) <i>Acinetobacter lwoffii</i> (n=1/14 Rh tick pools)	nd	morphologically identified (streak plates performed on blood agar, trypticase soy agar, Baird-Parker agar, and Mannitol salt agar); conventional PCR
Zanet et al. (2020)	Italy	<i>B. canis</i> (n=6/949 tick pools) <i>B. capreoli</i> (n=12/949 tick pools) <i>B. capreoli/divergens</i> (n=1/949 tick pools) <i>B. microti</i> (n=22/949 tick pools) <i>B. microti-Munich type</i> (n=4/949 tick pools) <i>Babesia</i> spp. (n=51/949 tick pools) <i>B. venatorum</i> (n=61/949 tick pools) <i>B. vogeli</i> (n=10/949 tick pools) <i>B. vulpes n. p.</i> (n=2/949 tick pools) <i>T. buffeli/ sergenti/ orientalis</i> (n=36/949 tick pools) <i>T. cervi</i> (n=2/949 tick pools) <i>T. equi</i> (n=2/949 tick pools) <i>T. ovis</i> (n=5/949 tick pools) <i>Theileria</i> spp. (n=2/949 tick pools) <i>A. ovis</i> (n=3/949 tick pools) <i>A. phagocytophylum</i> (n=17/949 tick pools) <i>A. platys</i> (n=6/949 tick pools) <i>Anaplasma</i> spp. (n=12/949 tick pools) <i>E. canis</i> (n=2/949 tick pools) <i>Ehrlichia</i> spp. (n=2/949 tick pools) <i>B. burgdorferi s.l.</i> (n=5/949 tick pools)	nd	conventional PCR
Khrouf et al. (2014)	Tunisia	<i>R. conorii</i> (n=69/198 selected ticks) Spotted fever group (n=5/198 selected ticks)	nd	RLB
		<i>Rickettsia massiliae</i> (n=2/5 randomly selected ticks) <i>R. conorii</i> (n=5/7 randomly selected ticks)	nd	conventional PCR
Yaglom et al. (2018)	United States	<i>Rickettsia</i> spp. [7% (n=4/57 ticks)]	nd	qPCR

Table 3-2: (continue)

Reference	Geographical location	Tick-borne pathogens detected in <i>R. sanguineus</i> ticks	Tick-borne pathogens detected in <i>H. elliptica</i> ticks	Tick-borne pathogens detection method
Pieracci <i>et al.</i> (2019)	United States	<i>Rickettsia</i> spp. [25% (n=38/150 selected ticks)] <i>R. rickettsii</i> [0.67% (n=1/150 selected ticks)]	nd	qPCR
H. M. Nguyen <i>et al.</i> (2020)	Laos	<i>Rickettsia</i> spp. [6.7% (n=7/105 tick pools)] <i>Anaplasmataceae</i> [14.3% (n=15/105 tick pools)] <i>A. platys</i> [100% (n=2/2 selected tick pools positive for <i>Anaplasma</i> spp.)] <i>Brucella</i> spp. [12.4% (n=13/105 tick pools)]	nd	qPCR
Miranda & Mattar (2015)	Colombia	<i>Ehrlichia</i> spp. [3.4% (n=3/87 tick pools)]	nd	conventional PCR
Fryxell <i>et al.</i> (2012)	United States	<i>Borrelia</i> spp. [25,8% (n=88/341 ticks)]	nd	conventional PCR
Dantas-Torres <i>et al.</i> (2012)	Italy	<i>H. canis</i> [2.8% (n=12/567 ticks)]	nd	conventional PCR
Pat-Nah <i>et al.</i> (2015)	Mexico	<i>E. canis</i> [18.5% (n=37/200 ticks)]	nd	nested PCR
Özübek & Aktas (2018)	Turkey	<i>Babesia</i> spp. (n=5/140 pooled ticks) <i>Babesia ovis</i> (n=2/140 pooled ticks)	nd	nested PCR

nd: not detected.

3.3.3 Quality assessment (risk of bias) of studies

The quality assessment was done on each eligible individual study using the Joanna Briggs Institute (JBI) critical appraisal tool for prevalence studies (Munn *et al.*, 2015) to determine if the study was a low (quality score of 5 or more) or high (quality score of less than 5) risk of bias (Appendix 3B). All studies included in this systematic review had a quality score ranging from 5 to 8 and were considered a low risk of bias.

3.4 Discussion

The results of the systematic review demonstrated that *R. sanguineus* was more prevalent than *H. elliptica* in terms of countries where the ticks were documented, as well as the prevalence of the ticks collected. There were 64 countries that reported the presence of *R. sanguineus*, whereas *H. elliptica* was only reported in 6 countries. It is widely considered that *R. sanguineus* has a global prevalence mainly in regions that are tropical or subtropical (Wall, 2016). The global prevalence of *R. sanguineus* is largely associated with their hosts and adaptation ability (Gray *et al.*, 2013; Wall, 2016). This tick is mainly associated with Canidae hosts (mainly dogs) but is also documented to infest a wide selection of hosts including, several mammalian, reptilian, and aves hosts. It is largely speculated that *R. sanguineus* initially infected burrowing carnivores, but adjusted as dogs were domesticated, adapting to the habitat of their preferred host and with time adjusting to human residences (Gray *et al.*, 2013). The adaptive ability of these ticks is so good that it is most often located inside urban areas, usually inside cracks and crevices, or vegetation used by the hosts. In addition, the close proximity between *R. sanguineus* and travelling hosts (such as humans and domestic dogs) or migratory hosts (such as birds), is one of the contributors to the global prevalence of this tick (Dantas-Torres, 2008; Gray *et al.*, 2013). Additionally, Buczek & Buczek (2020) suggested that *R. sanguineus* are the most generally imported tick species.

On the other hand, *H. elliptica* is mainly documented to be located in South Africa (Wall, 2016). The majority of articles, included in the systematic review, reporting the presence of *H. elliptica* were from South Africa, with some articles reporting the presence of this tick in countries with close proximity to South Africa. Buczek & Buczek (2020) stated that ticks may be transported to other areas within the same continent or to other continents by several means. This includes owners travelling with infected companion animals, the importation of exotic infected wild animals, or eggs and ticks unattached from the host incidentally imported with transport containers containing animal fur (Keirans & Durden, 2001; Sparagano *et al.*, 2015). Claerebout *et al.* (2013), Claerebout *et al.* (2016), Dutto & Selmi (2009), Hansford *et al.* (2014), and Jameson *et al.* (2010)

reported on ticks transported from one country to another by means of travelling companion animals which included *Amblyomma americanum*, *Dermacentor variabilis*, *Dermacentor albipictus*, *H. elliptica*, *Ixodes hexagonus*, *Ixodes ricinus*, *Ixodes pacificus*, *R. sanguineus*, and *Hyalomma lusitanicum*.

The most tick-borne pathogens by far were detected within *R. sanguineus*, which were reported in 38 countries, whereas haemoparasite infections within *H. elliptica* were only reported in 2 countries. In addition, haemoparasite infections within both tick species were detected in 1 country. Since it is considered that *R. sanguineus* has a global prevalence, the same may be considered for the pathogens that they harbour, thus explaining the widespread distribution of *R. sanguineus* tick-borne pathogens detected in the articles of the systematic review (Jongejan & Uilenberg, 2004; Wall, 2016). Within the systematic review, the tick-borne pathogens detected within *H. elliptica* were only detected in South Africa and Uganda. Since *H. elliptica* is mainly documented to be located in South Africa (Bryson *et al.*, 2000; Golezardy *et al.*, 2016; Horak, 1995; Horak *et al.*, 2009, 2010; Jacobs *et al.*, 2001; Kolo *et al.*, 2016; Penzhorn *et al.*, 2020; Petney *et al.*, 2004) and several surrounding countries (Adamu *et al.*, 2014; Agu *et al.*, 2020; De Matos *et al.*, 2008; Horak *et al.*, 2009; Jacques *et al.*, 2015; Kamani *et al.*, 2013, 2019; Kumsa *et al.*, 2019; Neves *et al.*, 2004; Proboste *et al.*, 2015), the same prevalence may be expected for the tick-borne pathogens that *H. elliptica* ticks harbour (Jongejan & Uilenberg, 2004; Wall, 2016). As ticks have the ability to travel from and to new geographical areas and inhabit those areas, the same is true for their associated tick-borne pathogens. Tick-borne pathogens may be introduced to new niches when travelling with infected ticks or infected tick hosts. One of the common transmission routes of tick-borne pathogens is the transmission of infected ticks attached to companion hosts travelling with humans. With the increase in economic migration and tourism rates, the transmission risk of tick-borne tick-borne pathogens also increases. Generally, when ticks are transported to new geographical areas by means of travelling companion animals, these ticks are often in the parasitic stage (Buczek & Buczek, 2020). In turn, this means that uninfected travelling companion animals and their human owners or indigenous dogs have a greater risk of infection from potentially infected ticks with substantial vector competence (Buczek & Buczek, 2020; Parola & Paddock, 2019). Another major means of transmission of ticks potentially infected with tick-borne pathogens is by the travelling of migrating tick-infected animals, such as migrating birds travelling between continents. They can effortlessly cross barriers and thus may spread ticks faster than other migrating animals. Migratory birds are primarily responsible for the transmission of immature ticks, in the larval and nymphal developmental stages meaning the ticks may detach from the bird host, molt to the next developmental stage, and attach to a new native host, thus beginning a tick infestation and possible transmission of tick-borne pathogens in that area

(Sparagano *et al.*, 2015). Socolovschi *et al.* (2012) reported the migration of *R. sanguineus* and *Haemaphysalis* spp. infected with *Rickettsia* spp. to Southern Europe, whereas Wallménus *et al.* (2022) reported the movement of *Haemaphysalis* spp. infected with *Rickettsia* spp. to Western Europe by means of migrating birds.

Several tick species, including *R. sanguineus* and *H. elliptica*, which have the potential to adapt to novel environments with favourable environmental conditions (such as adequate vegetation coverage, temperature, moisture, photoperiod, and suitable hosts), may inhabit those areas (Dantas-Torres, 2015). This happens when ticks in the juvenile stages molt and enter the adult stage of development or engorged females lay eggs which then hatch the new habitat (Buczek & Buczek, 2020). In addition, if the ticks are infected with pathogens, the pathogens may infect tick hosts or uninfected ticks and increase the prevalence of infections of those pathogens or create a new niche of infection (Buczek & Buczek, 2020; Dantas-Torres, 2015; Parola *et al.*, 2008; Parola & Paddock, 2019).

One of the main drivers for tick adaptation may be changes in temperature caused by climate change, especially when considering questing ticks. Changes in the climate with adequate moisture and photoperiod may expand the climate niche of certain tick species that were formerly unsuitable. While climate change in terms of massive temperature increases, in zones that are already tropical, may result in unsuitable habitats and be disastrous for certain tick species, compelling these ticks to leave and search for newly suitable areas (Dantas-Torres, 2015). These ticks may also include ticks, such as *R. sanguineus*, that are highly physiologically adaptable to dry areas with low humidity (Dantas-Torres, 2015; Parola *et al.*, 2008). Spring and autumn seasons that are increasingly becoming longer along with the winter season becoming warmer may contribute to the increasing distribution of several tick species to higher altitudes and towards northern latitudes (Dantas-Torres, 2015). Climate may also result in shorter tick developmental stages or hastened lifecycles, increases in tick populations in already-infected areas, as well as longer seasonal activity of several tick species (Bouchard *et al.*, 2019; Randolph, 2010). As tick activity prolongs due to the changing seasons, the prevalence of tick-borne pathogens will increase and become more widespread. Generally, Lyme disease, babesiosis, anaplasmosis, *Borellia miyamotoi* disease, and Powassan virus are likely to emerge and escalate (Bouchard *et al.*, 2019). Several tick species rely on wild rodents as hosts during the immature stages, including *H. elliptica* ticks, whereas other tick species require wildlife as hosts during the adult stages to lay eggs. In addition, the main reservoir hosts for tick-borne diseases are also wildlife and rodents. Increasing temperatures not only affect the distribution of ticks but also the distribution of wildlife and rodents that act as hosts for ticks and reservoir hosts of tick-borne diseases. This in turn affects the population of ticks and tick-borne pathogens in a given area (Bouchard *et al.*, 2019;

Dantas-Torres, 2015; Parola *et al.*, 2008). Climate change also increases the contact of humans with ticks as people spend longer time outdoors and in areas as potential habitats of ticks, which prolongs exposure of humans to ticks (Bouchard *et al.*, 2019; Randolph, 2010).

Articles included in this systematic review reported the presence of *R. sanguineus* and *H. elliptica* on several different hosts. Most articles reported the collection of both *R. sanguineus* and *H. elliptica* from companion animals, followed by collections from vegetation, several collections from livestock and humans, as well as the least collections from several wildlife species. Both *R. sanguineus* and *H. elliptica* are three-host ticks, that require a new host with each blood meal to molt to the new developmental stage (Dantas-Torres, 2008; Gray *et al.*, 2013). *Rhipicephalus sanguineus* mainly prefer dogs as their hosts. As previously mentioned, due to the evolutionary relationship between *R. sanguineus* and dogs, these ticks have adapted to the environment of their hosts, where humans are also frequently parasitized by *R. sanguineus* (Dantas-Torres, 2008). Baneth (2014) suggests that dogs do not have effective immune responses to protect themselves from natural infections of *R. sanguineus*, which may contribute to dogs being preferred *R. sanguineus* hosts. In certain instances, these ticks display opportunistic host selection where the larval and nymphal developmental stages feed on small mammals, like rodents, and the adult developmental stages feed on large animals and humans (Dantas-Torres, 2008). Gray *et al.* (2013) suggest that *R. sanguineus* has a defined rhythm of host detachment, where the larval ticks generally detach from the host during the day, to aid with maximum distribution, and the nymphal and adult ticks detach at night, to aid with contact with favoured hosts. In addition, opportunistic host selection of *R. sanguineus* may be due to massive increases in tick populations, resulting in increases in environmental infestation and tick exposure of hosts other than dogs (Dantas-Torres, 2008). The larval and nymphal developmental stages of *H. elliptica* prefer to infest rodents, whereas the adult developmental stage prefers companion animals, including dogs and cats, as well as wild felines and some wild canine species as hosts. These ticks are more prevalent on farms or large domestic plots, mainly because of the presence of rodents and companion animals that serve as hosts for all developmental stages of *H. elliptica* (Fourie *et al.*, 2010; Penzhorn *et al.*, 2020). Although *H. elliptica* ticks infesting humans are uncommon, it is not impossible as humans may be in close contact with companion animals or sometimes wildlife, that may act as hosts of *H. elliptica* (Fourie *et al.*, 2010). Several of the articles included in the systematic review reported the collection of *R. sanguineus* and/or *H. elliptica* from vegetation, this is because three-host tick species spend the majority of their lifecycle (approximately 94 to 97%) off of hosts (Dantas-Torres, 2008).

Several articles included in this systematic review reported the presence of tick-borne pathogens harboured by *R. sanguineus* and/or *H. elliptica* ticks. *Theileria* spp. are amongst detected tick-

borne pathogens from *R. sanguineus* ticks and this is not surprising as this is a common tick-borne pathogen harboured by ixodid ticks that spread to livestock and several wildlife species when the ticks take a blood meal (Bishop *et al.*, 2004). *Theileria ovis* (responsible for ovine theileriosis in sheep and goats), *T. equi* (responsible for equine piroplasmiasis in horses and donkeys), *T. cervi* (responsible for theileriosis in deer), as well as the *T. buffeli/sergenti/orientalis* group (responsible for bovine theilerioses in buffaloes and cattle) were amongst reported species by articles in this review (Zaeemi *et al.*, 2011; Kappmeyer *et al.*, 2012; Pavón-Rocha *et al.*, 2020; Chisu *et al.*, 2021).

The *Staphylococcus* genus detected within *R. sanguineus* was also reported in articles of this systematic review including *S. cohnii* (opportunistic pathogen causing meningitis, endocarditis, and bloodstream infections in humans), *S. epidermidis* (emerging pathogen responsible for nosocomial infections in humans), and *S. saccharitidis* (occasionally responsible for prosthetic valve infectious endocarditis, device-related joint and bone infections, as well as catheter-related bloodstream infections in humans) (Olayide & Bamidele, 2008; Mendoza-Olazarán *et al.*, 2017; Brooks *et al.*, 2019; Ahmad *et al.*, 2022).

Several species of the *Rickettsia* genus were detected within *R. sanguineus* and *H. elliptica* ticks. The *Rickettsia* genus occurs globally and has a wide range of hosts, including arthropods (Behravesh & Massung, 2016). It is widely considered that most species of *Rickettsia* are endosymbionts that are not pathogenic to their hosts. Mammals and humans are considered to be accidental hosts of *Rickettsia*, due to pathogen transmission of infected ticks, mites, or fleas during a blood meal (Ahantari & Trinachartvanit, 2013). In this systematic review *R. africae* (responsible for African tick-bite fever in humans) was detected from *H. elliptica* ticks. On the other hand, *R. sibirica* subsp. *mongolitimonae*, *R. rickettsia*, *R. rhipicephali*, *R. raoultii* and *R. slovaca*, *R. parkeri*, *R. monacensis* and *R. massiliae*, *R. helvetica*, *R. felis* and *R. conorii israelensis* were detected from *R. sanguineus*. Of the aforementioned pathogens, *R. sibirica* subsp. *Mongolitimonae* is responsible for North Asian tick typhus in humans. The *R. rickettsi* is responsible for Rocky Mountain spotted fever in humans. The *R. rhipicephali* may cause moderately severe illness in meadow voles. The *R. raoultii* and *R. slovaca* is responsible for scalp eschar and neck lymphadenopathy after a tick bite, or SENLAT, syndrome in humans. The *R. parkeri* causes mild, eschar associated rickettsiosis or *R. parkeri* rickettsiosis. Both *R. monacensis* and *R. massiliae* causes spotted fever in humans. The *R. helvetica* causes an unnamed rickettsiosis in humans. The *R. felis* is responsible for flea-borne spotted fever or cat-flea typhus in humans. The *R. conorii israelensis* is responsible for Israeli tick typhus or Israeli tick bite fever, Mediterranean spotted fever or Boutonneuse fever (Parola *et al.*, 2013). Other less commonly known detected *Rickettsia* included *R. amblyommii*, *C. R. goldwasesarii*,

C. R. barbariae and *R. asembonensis* which have unknown pathogenic significance (Ereqat et al., 2016; López-Pérez et al., 2022; Moonga et al., 2021; Pornwiroon et al., 2016; Zhao et al., 2016), as well as *C. R. asemboensis* that may be pathogenic (Dall'Agnol et al., 2017).

Several articles included in this systematic review also reported the presence of *L. infantum* (responsible for canine visceral leishmaniasis, and less commonly cutaneous leishmaniasis in humans) within *R. sanguineus* ticks. Domestic dogs and several wild canids act as natural reservoirs of this pathogen and it is transmitted by the *Lutzomyia longipalpis* sandfly. Although *L. longipalpis* sandflies are considered the only recognized vector, transmission by means of other arthropods, including ticks was previously considered a possibility, but the association between *L. infantum* and *R. sanguineus* is not yet fully understood (Solano-Gallego et al., 2012).

Other articles in this systematic review reported the detection of *Hepatozoon* spp. from the *R. sanguineus* ticks. *Hepatozoon* is a well-documented and recognized pathogen of veterinary importance, especially among companion animals. There are two species of veterinary importance, namely *H. canis* and *H. americanum* and have been reported to be transmitted by *R. sanguineus* and *Amblyomma maculatum* ticks (Baneth et al., 2003; Allen et al., 2011). The *H. canis* is associated with canine hepatozoonosis in dogs and possibly other members of canines and cats (Behravesh & Massung, 2016).

Several *Ehrlichia* spp. were detected in *R. sanguineus* and *H. elliptica* ticks in the articles used in the current systematic review. The species of *Ehrlichia* transmitted by ticks are well-documented and are of veterinary importance, especially *E. canis*, and some are considered zoonotic pathogens, such as *E. ewingii* and *E. chaffeensis* (Lin et al., 2021). Of the articles included in the systematic review, the *Ehrlichia* species infecting *R. sanguineus* were identified as *Ehrlichia* sp. HF, also known as *Ixodes ovatus Ehrlichia* (IOE) agent, *E. chaffeensis*, and *E. canis*. Of the aforementioned pathogens, *Ehrlichia* sp. HF, also known as *Ixodes ovatus Ehrlichia* (IOE) agent are responsible for laboratory mice acute fatal infection, sharing similarities with acute fatal human monocytic ehrlichiosis from *E. chaffeensis* (Lin et al., 2021). The *E. ewingii* are responsible for human infections. The *E. chaffeensis* are responsible for human monocytic ehrlichiosis, or HME, in humans and potentially mammals (Lin et al., 2021). The *E. canis* are responsible for canine monocytic ehrlichiosis, or CME, in dogs, and animals classified under the family Canidae (Harrus et al., 2016).

Articles used in this systematic review reported that *Coxiella* spp. were detected in *R. sanguineus* ticks. Several articles identified *Coxiella* spp. as belonging to *C. burnetii* (responsible for Q fever in humans and subclinical infections in dogs and cats) (Angelakis & Raoult, 2010; Rezaei et al.,

2016). Even though the *Coxiella* genus only has one species, namely *C. burnetii*, there were previous reports of *Coxiella*-like endosymbionts, harboured by ixodid ticks. Even though the functional role of *Coxiella*-like endosymbionts is not fully known, they are described as obligate tick symbionts and are highly prevalent (Gottlieb *et al.*, 2015). The *C. burnetii* can spread with aerosol particles contaminated with spores but are also transmitted by arthropods, especially ticks, which is vital for wildlife reservoir cycles (Pfeffer *et al.*, 2016).

Brucella spp. was detected in *R. sanguineus* ticks as reported by some articles used in this systematic review. This pathogen is responsible for brucellosis in humans and may also infect other animals. Transmission mainly occurs by the consumption of contaminated food or water, but tick transmission is also possible. Once *Brucella* is obtained by ticks it occurs in all of the developmental stages of ticks, from eggs to adults, and is transmitted to other hosts during a blood meal (Jia *et al.*, 2022).

Other articles used in this review have reported that *Borrelia* spp. were detected in *R. sanguineus* ticks. Several members of the *Borrelia* genus are well-documented pathogens that are zoological and of veterinary importance, whereas others are non-pathogenic. The *B. burgdorferi*, *B. garinii*, *B. bavariensis*, *B. persica* and *B. afzelii* are known for their infections in animals, specifically companion animals, as well as humans. *B. recurrentis*, and *B. hermsii* are responsible for human infections, mainly associated with relapsing fever. Several species are also emerging pathogens, such as *B. miyamotoi* and *B. theileri*. The presence of multiple *Borrelia* spp. in a vector, mainly ticks and lice, is possible. The aforementioned may be problematic when multiple *Borrelia* spp. are transmitted to the host resulting in co-infections that may be difficult to diagnose (Straubinger, 2016). Several of the articles in the systematic review identified *B. burgdorferi* (associated with lyme disease in humans and animals). In addition, several birds and wild mammals serve as reservoirs of several subspecies of *B. burgdorferi*, that maintain the pathogen in wildlife reservoir cycles (Radolf *et al.*, 2021).

Bartonella spp. were detected in *R. sanguineus* ticks, in several articles of this systematic review. The *Bartonella* genus consists of a wide range of species that are responsible for the infection of humans and animals. The majority of *Bartonella* spp. have natural cycles that require infection and maintenance in a mammalian host, generally, an asymptomatic and chronic infection, followed by infection of hematophagous arthropods, responsible for the transmission of *Bartonella* spp. between the aforementioned hosts. The effect of *Bartonella* infections on maintenance mammalian hosts or accidental hosts depends on the *Bartonella* spp. and host type (Birtles, 2016). Articles have reported *B. rochalimae* and *B. clarridgeiae* from ticks. These species are associated with companion animals as *B. rochalimae* is a newly recognized emerging

pathogen of dogs (Ernst *et al.*, 2020) and the maintenance host of *B. clarridgeiae* are domestic cats and possibly other felines (Birtles, 2016).

The *Bacillus* genus consists of several bacterial species that inhabit a wide variety of natural environments. Several *Bacillus* spp. are not pathogenic while others may cause infection in humans and animals, where pathogenicity may be mild (like *B. cereus* causing food poisoning) to severe (like *B. anthracis* causing anthrax) (Ehling-Schulz *et al.*, 2019). In this systematic review, articles have reported detection of *B. megaterium* (considered to be non-pathogenic) and *B. clausii* (commercially used as a probiotic for acute diarrhea and adjunctive therapy of infection caused by *Helicobacter pylori*) from ticks. Although both species are not considered pathogenic, *B. megaterium* was responsible for a soft tissue infection in a human (Bocchi *et al.*, 2020) and prolonged use of *B. clausii* in an immunocompetent child resulted in bacteremia (Khatri *et al.*, 2021).

Some of the articles of this systematic review detected the presence of *Babesia* spp. in *R. sanguineus* and *H. elliptica* ticks. *Babesia* spp. mainly this pathogen infects domestic dogs and cats and wild canines and felids, but babesiosis is also an emerging zoonotic disease (Irwin, 2016). *Babesia rossi* was identified in both *R. sanguineus* and *H. elliptica* ticks. Furthermore, *B. vulpes*, *B. vogeli*, *B. venatorum*, *B. ovis*, *B. microti*, *B. gibsoni*, *B. capreoli/divergens*, *B. capreoli*, and *B. canis* were identified in *R. sanguineus* ticks from several articles in this systematic review. Even though several of the abovementioned *Babesia* spp. are uncomplicated (or cause mild forms of infection), several of the infections are severe, like *B. rossi* which causes the most severe form of babesiosis in South African dogs (Leisewitz *et al.*, 2019). The rate of reported *Babesia* spp. infections of canines in non-endemic areas have also increased alarmingly, especially in areas where humans and their companion animals return from traveling. Several *Babesia* spp. causing infections in humans through wildlife are also of concern, such as *B. microti* that infects rodents and may be arthropod transmitted to humans (Irwin, 2016).

The occurrence of *Anaplasma* spp. from *R. sanguineus* ticks has been reported by several articles in this systematic review. *Anaplasma* spp. are of veterinary importance, whereas several species infect humans or have zoonotic potential, such as *A. platys* (Arraga-Alvarado *et al.*, 2014; Harrus *et al.*, 2016). *Anaplasma platys* (responsible for infectious canine cyclic thrombocytopenia mainly in dogs), *A. phagocytophylum* (responsible for granulocytotropic anaplasmosis in humans and animals), and *A. ovis* (responsible for ovine anaplasmosis mainly in sheep and goats) have been detected from *R. sanguineus* ticks (Harrus *et al.*, 2016).

Acinetobacter lwoffii which is considered a bacterium that normally forms part of the human skin microbiota has also been detected in *R. sanguineus* ticks from articles used in this systematic

review. It was also previously reported in nature, water, food products, and hospitals due to its ability to survive extremely low temperatures, low pH levels, dry conditions, irradiation, desiccation, and disinfectants (Malta *et al.*, 2020). Although it is part of the skin microbiota, human infections were previously reported, specifically gastroenteritis and catheter-associated infections of hospitalized immunocompromised patients (Regalado *et al.*, 2009).

Several articles in the systematic review reported co-infections of *R. sanguineus* ticks by *B. gibsoni* and *E. canis*, *Anaplasma* spp. and *Babesia* spp., *B. gibsoni* and *A. platys*, *E. canis* and *A. platys*, *E. chaffeensis* and *E. canis*, *R. rickettsii* and *R. rhipicephali*, as well as *E. canis*, *A. platys* and *B. burgdorferi*. Certain arthropods may maintain certain pathogens through several generations by acting both as a reservoir and a vector. An example of the aforementioned is the *R. sanguineus* tick acting both as a vector and a reservoir for *E. canis* and *R. conorii* (Dantas-Torres, 2008). In addition, several wildlife species may also act as reservoir hosts for certain pathogens. An example of the aforementioned is wild rodents that may be simultaneously infected with several *Bartonella* spp., as well as *A. phagocytophylum*, *B. microti*, and *B. burgdorferi* (Bown, 2016). The pathological effect of co-infections depends on the species of host or vector and the species of pathogens present (Moutailler *et al.*, 2016; Beasley *et al.*, 2021). An example of the aforementioned is *Microtus agrestis*, commonly known as field voles, infected with *Bartonella* spp. which reduces the chances of infection with *B. microti*, and contrariwise. On the other hand, *B. microti* infections increase the chances of *A. phagocytophylum* infections in *M. agrestis* host (Taylor *et al.*, 2018). In situations where hosts are not considered reservoirs, they may still be important in infection epidemiology. An example of this is several deer species infected with *B. burgdorferi*. The deer host has borreliacidal activity making them poor hosts of *B. burgdorferi*. However, they are still important for *B. burgdorferi* epidemiology as they are hosts of several tick species (Roome *et al.*, 2017).

Most articles in this systematic review identified *R. sanguineus* and/or *H. elliptica* using morphological methods, including published keys and microscopy, as well as a combination of morphological identification and conventional PCR. The presence of tick-borne pathogens occurring in *R. sanguineus* and/or *H. elliptica* was determined by using molecular identification methods, including microscopic examination and bacterial culturing on several agar mediums, as well as molecular methods including conventional PCR, qPCR, nested PCR, RLB hybridization, heminested PCR, seminested PCR, indirect immunofluorescence, multiplex PCR, end-point PCR, nPCR, PCR-RFLP. In addition, the use of DNA-based assays has increased in the last decade, since DNA has a stable and unique structure it serves as an exceptional template to use in diagnostic assays (Ahmadi *et al.*, 2015; Bailet *et al.*, 2019). During the last two decades the increased use of DNA-based identification techniques, along with the numerous amounts of

sequence data generated has provided new insights of pathogen epidemiology (Perkins *et al.*, 2011).

There are several limitations of this systematic review, like multiple articles often use the pooling of samples, especially when screening for the presence of pathogens, which may result in heterogeneity. Another one of the limitations of this systematic review includes the low number of samples from some studies. The scarcity of data in some countries, especially concerning the studies focusing on *H. elliptica*, as well as the unequal distribution of the countries is also worrisome. Lastly, publication bias is also of concern.

This is the first systematic review to focus on the distribution of *R. sanguineus* and *H. elliptica* ticks, as well as tick-borne pathogens they harbour. All of the studies included in this systematic review were cross-sectional studies and to further increase our knowledge of the prevalence of the aforementioned ticks and the pathogens they carry, such as case controls also should be included in future research. In addition, this will assist to gain further insights on other associated subjects, such as the expansion of ticks and their tick-borne pathogens to non-endemic areas, the effects of co-infections, and tick identification and parasite detection methods.

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CHAPTER 4

IDENTIFICATION OF TICKS INFESTING DOGS FROM THE POTCHEFSTROOM ANIMAL WELFARE SOCIETY AND THE PATHOGENS THEY HARBOUR

4.1 Introduction

Certain Ixodid ticks, including *R. sanguineus* and *H. elliptica* follow a three-host life cycle and have four different developmental stages (namely eggs, larvae, nymphs, and adults). Ticks that follow a three-host life cycle require a new host to feed on when they enter a new developmental stage or when mating occurs. Respectively, this means that ticks may acquire or transmit tick-borne pathogens from or to their hosts during a blood meal, often resulting in illnesses of the hosts (Edlow & McGillicuddy, 2008; Magnarelli, 2009). Additionally, tick infestations may result in paralysis, toxicosis, allergic reactions, and irritation of their hosts, hide damage, as well as livestock production decreases (Uilenberg & Jongejan, 2004; Dantas-Torres *et al.*, 2012; Manjunathachar *et al.*, 2014).

Several of the tick-borne pathogens that *R. sanguineus* and *H. elliptica* transmit include but are not limited to *A. phagocytophylum*, *E. canis*, *C. burnetii*, and *Rickettsia* spp. The *A. phagocytophylum* (associated with granulocytic anaplasmosis) was previously naturally recorded in humans and several domestic and wild animals (Dumler *et al.*, 2005; Stuen *et al.*, 2013; van Nunen, 2018). There were lethal cases reported in dogs, humans, sheep, cattle, horses, and rodents (Woldehiwet, 2010; Stuen *et al.*, 2013; Taylor *et al.*, 2015). This tick-borne pathogen may be present in ticks for the majority of their lifecycle and is transstadially transmitted (Taylor *et al.*, 2015). *Ehrlichia canis* (associated with Canine Monocytic Ehrlichiosis or CME) was previously documented as only a veterinary pathogen but is currently recognized as a tick-borne pathogen which also infects humans (Parola & Raoult, 2001). This tick-borne pathogen is transmitted transstadially (Moraes-Filho *et al.*, 2015). The *C. burnetii* (associated with Q fever) is harboured by humans, as well as several free-living animals, birds, and arthropods. This tick-borne pathogen is transmitted by transovarial and transstadial transmission as well as inhalation of air particles contaminated with *C. burnetii* spores (Woldehiwet, 2010). Ticks, mites, and fleas are often associated as vectors of *Rickettsia* spp. (associated with boutonneuse fever or Mediterranean spotted fever) (Alberdi *et al.*, 2012). This pathogen is transmitted by ticks by means of transstadial and transovarial transmission (Parola & Raoult, 2001). There is limited information

available on ticks, their associated tick-borne pathogens in South Africa, particularly in Potchefstroom. These results obtained from this study will aid to fill the gap.

4.2 Materials and methods

4.2.1 Study area

4.2.1.1 North-West Province

South Africa is made up of nine provinces, including the North-West province, Limpopo, Mpumalanga, Gauteng, Free State, Kwa-Zulu Natal, Northern Cape, Western Cape, and the Eastern Cape, as illustrated in Figure 4.1. The North-West province makes up 9.5% of South Africa, which is an area of about 104 822 km² making it the sixth largest province. On the Northern side of the North-West province lies the Republic of Botswana; the Free-state province is located on the south-eastern side, with the Northern Cape located on the western side, whereas Gauteng and Limpopo are located on the northeast (Bik *et al.*, 2012). Generally, the climate of the North-West province is characterized by distinct seasons where the summers are warm and the winters are sunny and windy. Rain season generally commences in October and finishes in March. The climate and rainfall may differ depending on the ecological zones (North West Department of Rural, Environmental and Agricultural Development, 2013).

The population of the North-West province consists of around 3.7 million people. Of the population around 65% lives in rural areas (Main, 2017). In this province, Brits, Rustenburg, and Ga-Rankuwa are the populated industrial regions. Mahikeng is regarded as the provincial capital, whereas Klerksdorp, Potchefstroom, Ventersdorp, Lichenberg, and Vryburg are also major towns. The North-West province is made up of 4 district municipalities that are further divided into 18 local municipalities, as illustrated in Figure 4.2. This study was executed in the Dr Kenneth Kaunda District Municipality, located on the Gauteng province border about 65 km southwest of Johannesburg, which occupies 16% of the North-West province. This municipality is further divided into 3 local municipalities, namely JB Marks, Maquassi Hills, and the City of Matlosana, as illustrated in Figure 4.3. Additionally, the JB Marks Local Municipality is the biggest of the above-mentioned municipalities covering an area of about 6 398 km². It originated in 2016 with the merger of the Ventersdorp and Tlokwe Local Municipalities and includes Ventersdorp and Potchefstroom, as illustrated in Figure 4.4. The suburbs of the JB Marks Local Municipality are classified as an urban and rural environment, with several industrial areas and the entirety of the municipality is surrounded by vegetation and agricultural areas, as illustrated in Figure 4.5.



Figure 4-1: South Africa map, with the provinces. Modified from Main (2017)

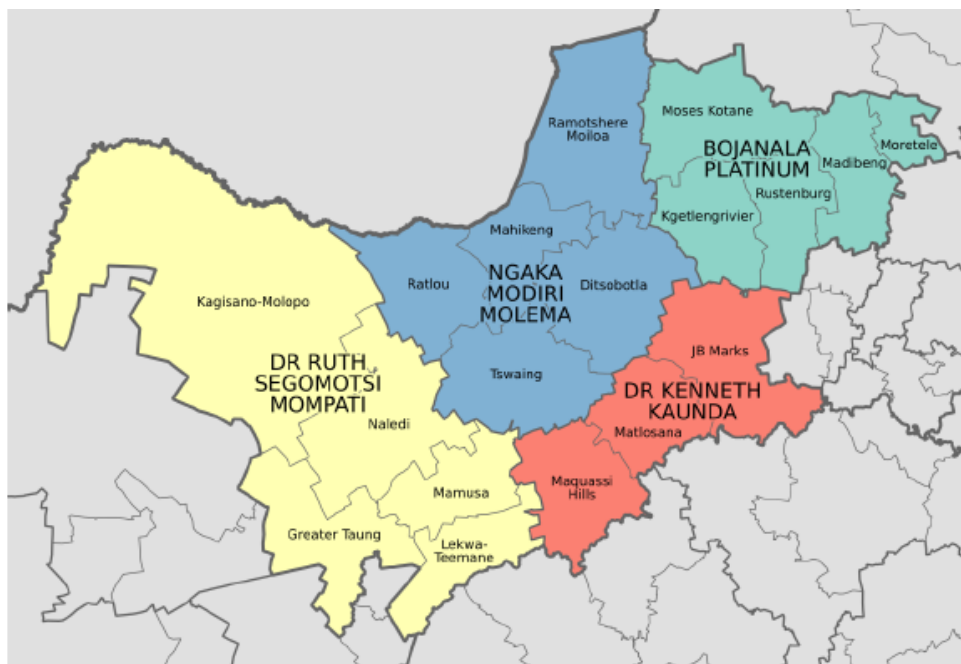


Figure 4-2: North-West Province map, with the district and local municipalities. Modified from Main (2017)



Figure 4-3: Dr Kaunda District Municipality map. Modified from Main (2017)



Figure 4-4: JB Marks Local Municipality map. Modified from Main (2017)

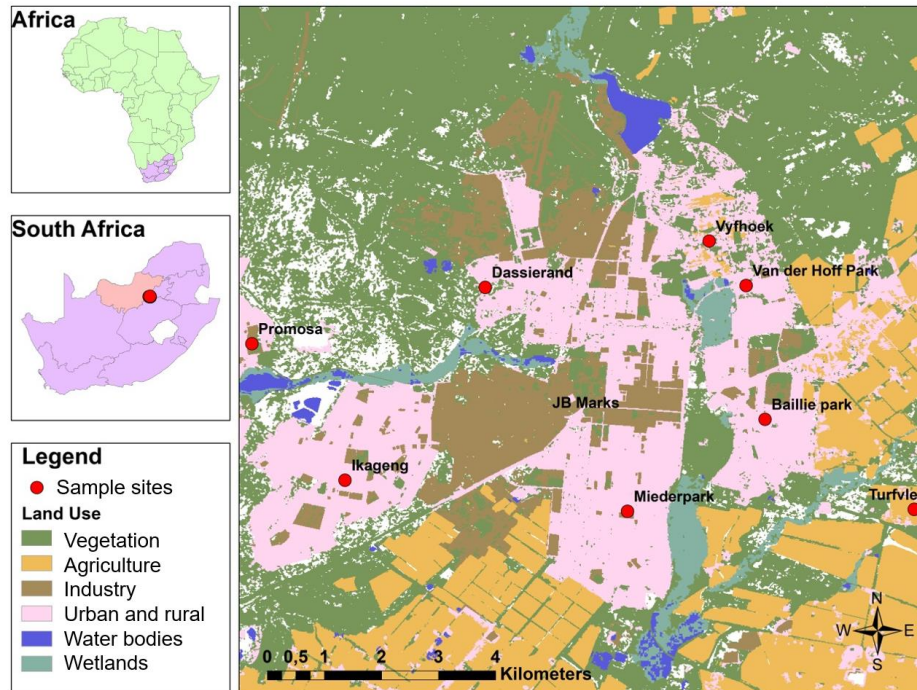


Figure 4-5: Land use map of the JB Marks Local municipality showing areas of origin of the sampled dogs (Map made using ArcGIS).

4.2.1.2 Origin of dogs within the study area

All of the tick specimens and blood samples were collected by the Potchefstroom Animal Welfare Society (PAWS), from rescued stray dogs from several suburbs within the JB Marks Local Municipality or from dogs residing in the PAWS kennels. All of the tick specimens and dog blood samples were collected by the PAWS veterinarian, Dr Stallone. Stray dogs that were rescued from PAWS originated from the following suburbs; Dassierand (26° 41' 43.08" S, 27° 4' 19.559" E), Ikageng (26° 43' 32.52" S, 27° 3' 0" E), Miederpark (26° 43' 50.16" S, 27° 5' 40.2" E), Baillie park (26° 42' 57.96" S, 27° 6' 58.319" E), Promosa (26° 42' 15.12" S, 27° 2' 7.079" E), Turfvlei (26° 43' 49.08", 27° 8' 22.92" E), Van der Hoff Park (26° 41' 42" S, 27° 6' 47.519" E), and Vyfhoek (26° 41' 16.8" S, 27° 6' 26.639" E), as illustrated by Figure 4.6.

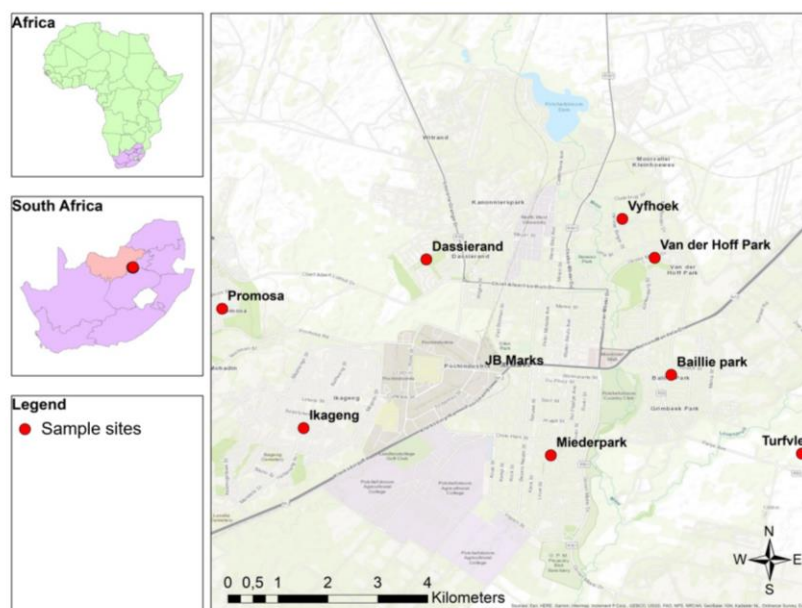


Figure 4-6: Map of Potchefstroom-JB Marks Local Municipality showing exact locations of origin of the stray dogs before admission at PAWS (Map made using ArcGIS).

4.2.2 Sample collection

For this study 300 tick specimens were collected from 50 dogs. Additionally, blood samples were collected from the dogs. Tick specimens ($n=300$) and blood samples ($n=50$) from their associated dog hosts were collected weekly from PAWS from August 2019 to February 2022. Ticks and dog blood were collected from stray dogs rescued by and transported to PAWS or from dogs residing in the PAWS kennels. Upon collection, ticks were transferred to collection vials and stored in 70% ethanol, whereas the blood from the infected dog host was collected, with 4 drops of blood from each dog placed on Whatman Chromatography FTA™ (Flinders Technology Associate) cards (Sigma-Aldrich, USA) (Odeniran *et al.*, 2019) and transported to the North-West University for further analysis.

4.2.3 Morphological identification of ticks

Using morphological features, ticks were identified to species level microscopically using tick identification keys that were published by Walker (2003) and Barker & Walker (2014). Table 4.1 gives a summary of the morphological features of ticks that were used in this study. In addition to the identification of tick species, the ticks' life stage and sex, as well as the location of ticks and host origin were noted. After ticks were morphologically identified, the ticks were stored in vials with 70% ethanol until further analysis. In the presence of eggs, the eggs were also collected and stored in vials with 70% ethanol until further analysis. To confirm the morphological identification,

selected tick specimens were sent to the Gertrud Theiler Tick Museum, at the Onderstepoort Veterinary Institute (OVI) where voucher specimens were issued.

Table 4-1: Summary of morphological features used to identify tick species infecting dogs in South Africa, adapted from Walker (2003) and Barker & Walker (2014)

Tick species	Morphological features
<i>Rhipicephalus</i> genus	Festoons are present. Eyes are present. Basis capituli generally has a hexagonal shape. Mouth parts have a short to medium size. Males may have adanal and accessory adanal plates.
<i>R. sanguineus</i>	Spiracle plate is half the size of the festoons. Convex eyes. The setiferous punctuations are impossible to differentiate. Interstitial punctuation size ranges from small to medium. In the females: Genital aperture posterior lips have a broad V or U shape. Cervical fields texture contain wrinkled areas. Scapular grooves are steep. Scutum colours are generally pale. Scutum posterior end margin are somewhat sinuous. Cervical fields are straight and big. Palp pedicles are tiny. Porose areas are widely separated. Basis capituli lateral angles are sharp. In males: Evident, smooth textured lateral grooves. Deep, wide, and wrinkled posterior grooves. Caudal appendage appears to be broad after males took a blood meal. Narrow and trapezoid shaped adanal plates. Pale conscutum. Ambiguous cervical fields indent. Dorsally unnoticeable coxae 1 anterior spurs.
<i>Heamaphysalis</i> genus	No adanal plates in males. Festoons are present. Eyes are absent. Inornate scutum. Rectangular shaped basis capituli. Mouthparts appear to be short and broad.
<i>H. elliptica</i>	Dense distributed punctuation. Has eleven festoons. Large, conical shaped palp articles 2 lateral extensions. In females: Lateral groove enclose every third festoon. Medium length coxae 1 to 3 spurs. Palp articles 3 have a ventral spur. Palp articles 2 have a dorsal spur. In males: Cornua is lengthy. Lateral groove encloses every second festoon. Medium length of coxae 4 spurs. Palp articles 2 has a dorsal and ventral spur.

4.2.4 Molecular identification of ticks

4.2.4.1 DNA extractions

Prior to DNA extractions, tick specimens were washed with 10% Tween 20 for 1 hour. Afterward, the ticks were respectively rinsed twice and thrice with 70% Ethanol and double distilled water. Each time the ticks were rinsed for a period of 1 hour. For the molecular identification of tick species, 15 selected individual ticks from each species, that were previously morphologically identified, were used for DNA extractions. Tick pools (n=81) for the molecular detection of tick-borne pathogens were made if the ticks were from the same host, location, species, and life stage for DNA extractions. The number of ticks in each pool varied, where some pools consisted of individual ticks, while the maximum number of ticks in a pool was 5. Egg batches (n=6) were made if the eggs were from the same host, location of origin, and tick species. Each egg batch consisted of 50 eggs. DNA was extracted from whole ticks (n=30), tick pools (n=81), egg batches (n=6), as well as blood (n=50) from the associated dog hosts by using a modified salting out method (Gaaib *et al.*, 2011). For DNA extractions, the tick specimens and eggs were crushed in 1.5 ml Eppendorf tubes using a sterilized metal pistil, whereas a drop of blood from each sample was transferred in a 1.5 ml Eppendorf tube. Subsequently, 480 µl of DNA extraction buffer was added to each tube. DNA extraction buffer consisted of 10 mM Tris-HCl (pH 8.0), 10 mM EDTA, and 1% sodium dodecyl sulphate (also known as SDS). Additionally, 20 µl Proteinase K was added to each tube. The tubes were then placed into the TissueLyser LT (Qiagen, DE), and the contents were mixed for 10 minutes. The tubes were placed into a heat block and incubated at 56°C overnight. After incubation, the tubes were centrifuged at 12 000 rpm for 5 minutes. Subsequently, the supernatant was transferred to a new 1.5 ml Eppendorf tube and 180 µl of 5 M NaCl was added to each tube. The tubes were vortexed for 30 seconds to mix the contents, centrifuged for 5 minutes at 13 000 rpm and the supernatant was transferred to a new 1.5 ml Eppendorf tube. Subsequently, 420 µl of cold isopropanol was added to each tube. The tubes were inverted 50 times and centrifuged at 4°C for 15 minutes at 13 500 rpm to allow DNA precipitation. Afterward, the supernatant was discarded. The DNA pellet was washed by adding 250 µl of 70% Ethanol to each tube. The tubes were briefly vortexed and centrifuged at 13 500 rpm for 5 minutes. The DNA pellet was washed again by discarding the supernatant, adding 250 µl of 70% Ethanol, briefly vortexing, and then centrifuging the tubes at 13 000 rpm for 5 minutes. Subsequently, the supernatant was discarded and the samples were air-dried for 1 hour at room temperature to allow the evaporation of the 70% Ethanol. The DNA pellet was resuspended in 50 µl of double distilled water. To confirm the presence and concentration of DNA the NanoDrop One/OneC Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, ZA) was used. The concentrations of the extracted DNA of the selected tick specimens that was used for molecular

identification of tick species ranged from 0.947 to 3.786 ng/μl. Concentrations of the extracted DNA of tick pools, dog blood samples and tick eggs that were used for the screening of tick-borne pathogens ranged from 2.693 to 4.971 ng/μl. Extracted DNA was stored at -20°C until further analysis.

4.2.4.2 Polymerase chain reaction

Polymerase chain reaction was performed for the molecular identification of tick species targeting the *cytochrome oxidase subunit 1 (CO1)* and *18 small subunit ribosomal RNA (18S rRNA)* genes. For the detection of tick-borne pathogens in dog blood, as well as tick pools, and egg batches, PCR was respectively performed for *A. phagocytophylum*, *Rickettsia* spp., *C. burnetii*, and *E. canis* targeting the *16 small subunit ribosomal RNA (16S rRNA)*, *citrate synthase (gltA)*, *superoxide dismutase 1 (SOD1)*, and *16S rRNA* genes. The primers used during PCR are shown in Table 4.2. For the identification of tick species, conventional PCR was performed where each PCR reaction mixture was made up to a total volume of 25 μl and consisted of 12.5 μl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 8.5 μl of double distilled water, 1 μl of 10 μM of the forward primer, 1 μl of 10 μM of the reverse primer, and 2 μl of template DNA. Double distilled water was used as the PCR negative control and *R. sanguineus* DNA was used as a positive control. PCR consisted of the following conditions: initial denaturation at 95°C for 10 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 47°C (for the *CO1* gene) and 52°C (for the *18S rRNA* gene) for 30 seconds and extension at 72°C for 60 seconds; this was followed by a final extension at 72°C for 7 minutes and infinite final hold at 4°C by using the ProFlex PCR System (Thermo Fisher Scientific, US). For the detection of tick-borne pathogens, including *E. canis*, *A. phagocytophylum*, and *Rickettsia* spp., conventional PCR was performed where each PCR reaction mixture was made up to a total volume of 25 μl and consisted of 12.5 μl of OneTaq 2x Master Mix with standard Buffer (New England Biolabs, US), 9.5 μl of double distilled water, 0.5 μl of 10 μM of the forward primer, 0.5 μl of 10 μM of the reverse primer, and 2 μl of the template DNA. Double distilled water was used as the PCR negative control. For the positive controls *A. phagocytophylum* (for the detection of *A. phagocytophylum*), *E. canis* (for the detection of *E. canis*), and *R. africae* (for the detection of *Rickettsia* spp.) DNA were used. PCR consisted of the following conditions: initial denaturation at 94°C for 30 seconds, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 59°C (for *A. phagocytophylum*), 52°C (for *Rickettsia* spp.), and 55°C (for *E. canis*) for 60 seconds and extension at 68°C for 15 seconds (for *A. phagocytophylum*), 23 seconds (for *Rickettsia* spp.), and 23 seconds (for *E. canis*); this was followed by a final extension at 68°C for 5 minutes and infinite final hold at 4°C by using the ProFlex PCR System (Thermo Fisher Scientific, US). For the detection of *C. burnetii*, touchdown PCR was performed (Ogawa *et al.*, 2004) where each PCR reaction mixture was

made up to a total volume of 25 µl and consisted of 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, US), 8.5 µl of double distilled water, 1 µl of 10 µM of the forward primer, 1 µl of 10 µM of the reverse primer, and 2 µl of template DNA. Double distilled water was used as the PCR negative control and *C. burnetii* DNA was used as a positive control. PCR consisted of the following conditions: initial denaturation at 54°C for 10 minutes, followed by the touchdown step for 10 cycles, starting at 60°C and ending at 50°C (where the temperature was lowered by 1°C during every cycle). The touchdown step was followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 20 seconds and extension at 72°C for 1 minute, followed by final extension at 72°C for 5 minutes and infinite final hold at 4°C by using the ProFlex PCR System (Thermo Fisher Scientific, US). Visualization of the PCR amplicons was done by using a 1% (w/v) agarose electrophoresis gel, stained with Ethidium Bromide. The electrophoresis conditions were 30 minutes at 100 V. The gel was visualized using the ENDURO GDS Gel Documentation System (Labnet International, Inc., US) under UV light. Subsequently, the DNA amplicons were sent for Sanger sequencing at Inqaba Biotechnical Industries (Pty) Ltd. (Pretoria, South Africa).

Table 4-2: Oligonucleotide primers used for molecular identification of ticks and detection of tick-borne pathogens

Tick species or name of tick-borne pathogen	Target gene	Primer sequence (5'-3')	Fragment size (bp)	Annealing temperature (°C)	Reference
<i>R. sanguineus</i> or <i>H. elliptica</i>	<i>CO1</i>	LCO1490: 5'- GGT CAA CAA ATC ATA AAG ATA TTG G -3' HCO2198: 5'- TAA ACT TCA GGG TGA CCA AAA AAT CA -3'	710	47	Licari <i>et al.</i> (2017)
<i>R. sanguineus</i> or <i>H. elliptica</i>	<i>18S rRNA</i>	18S-F: 5'- CAT TAA ATC AGT TAT GGT TCC -3' 18S-R: 5'- CGC CGC AAT ACG AAT GC -3'	780	52	Lv <i>et al.</i> (2014)
<i>A. phagocytophylum</i>	<i>16S rRNA</i>	EHR-521: 5'- TGT AGG CGG TTC GGT AAG TTA AAG -3' EHR-747: 5'- GCA CTC ATC GTT TAC AGG GTG -3'	250	59	Welc-Fałęciak <i>et al.</i> (2009)
<i>Rickettsia</i> spp.	<i>gltA</i>	Rp877p: 5'- GGG GAC CTG CTC ACG GCG G -3' Rp1258n: 5'- ATT GCA AAA AGT ACA GTG AAC A -3'	381	52	Parola <i>et al.</i> (2003)
<i>C. burnetti</i>	<i>SOD1</i>	CB1: 5'- ACT CAA CGC ACT GGA ACC GC -3' CB2: 5'- TAG CTG AAG CCA ATT CGC C -3'	257	50	Ogawa <i>et al.</i> (2004)
<i>E. canis</i>	<i>16S rRNA</i>	ECAN5: 5'- CAA TAA TTT ATA GCC TCT GGC TAT AGG A -3' HE3: 5'- TAT AGG TAC CGT CAT TAT CTT CCC TAT -3'	387	55	Lakshmanan <i>et al.</i> (2007)

4.2.5 Statistical analysis

To determine the prevalence of ticks, confidence interval (CI) with an average of 95% was used. Tick distribution was determined using Pearson's chi-square test on R (R Core Team, 2013). Sequences that were obtained from Inqaba Biotechnical Industries (Pty) Ltd. after Sanger Sequencing were viewed/visualized and edited using Molecular Evolutionary Genetics Analysis 11 (MEGA11) version 11.0.11 (Tamura *et al.*, 2021). Degenerate nucleotides (B, D, H, K, W, Y, S, M, and R) were replaced with a correct nucleotide (A, T, G, and C) determined by the colour peaks given by the chromatogram (Ma *et al.*, 2015). To confirm the identification of ticks and to identify the detected tick-borne pathogens, the DNA sequences generated in this study were compared to DNA sequences available in the National Center for Biotechnology Information (NCBI) database by using the Basic Local Alignment Search Tool (BLAST) for nucleotides. Additionally, the DNA sequences from this study were submitted to the NCBI database to obtain accession numbers. Homologous sequences of other related tick species from the NCBI database were acquired. For the construction of each phylogenetic tree of ticks, all of the sequences obtained from the study as well as those obtained from the NCBI database (reference sequences and outgroups) were added to an alignment explorer using MEGA11. Subsequently, the sequences were aligned by ClustalW (Wallace *et al.*, 2005). The construction of phylogenetic trees was done by means of the maximum likelihood (ML) statistical method (Jin *et al.*, 2006) using 10 000 bootstrap replications. During phylogenetic analysis, the lowest Bayesian Information Criterion (BIC) score was used to determine which nucleotide substitution models were used (Nylander *et al.*, 2004). Furthermore, gaps or missing data were eradicated and the rates among sites were handled as uniform rates. Geographical maps generated in this study were made by using ArcGIS Desktop 10.5.

4.3 Results

4.3.1 Collected tick specimens

During this study, 300 ticks were collected from 50 dogs admitted to PAWS located in Potchefstroom, in the JB Marks Local Municipality. The ticks were originally from Baillie park (2%), Dassierand (10.3%), Ikageng (2.3%), Miederpark (81.3%), Promosa (0.3%), Turfvlei (1%), Van der Hoff Park (2.3%), and Vyfhoek (0.3%) (Table 4.3). There was no significant statistical difference for either *R. sanguineus* ($X^2 = 659.6$, $df = 4$, $p\text{-value} < 2.2^{-16}$) or *H. elliptica* ($X^2 = 161.43$, $df = 5$, $p\text{-value} < 2.2^{-16}$) occurrences based on geographical localities of collections.

Table 4-3: Ticks from sampled dogs at PAWS and their location of collection

Location of collection	Tick species		Total number of ticks from each location	Overall occurrence of ticks from each location (%)
	<i>R. sanguineus</i>	<i>H. elliptica</i>		
Baillie park	3	3	6	2
Dassierand	2	29	31	10.3
Ikageng	7	0	7	2.3
Miederpark	193	51	244	81.3
Promosa	0	1	1	0.3
Turfvlei	0	3	3	1
Van der Hoff Park	7	0	7	2.3
Vyfhoek	0	1	1	0.3
Total ticks collected	212	88	300	

4.3.2 Ticks morphological identification

Two tick species from naturally-infected dogs were identified. These ticks were morphologically identified as *R. sanguineus* (Plate 4.1) and *H. elliptica* (Plate 4.2), using distinguishing morphological features published by Walker (2003) and Barker & Walker (2014) (Plate 4.3 and Plate 4.4). For the verification of the morphological identification of tick species, several tick specimens were submitted to the tick museum, located at the ARC-Onderstepoort Veterinary Research, where voucher specimens were provided for *R. sanguineus* (GTTM voucher accession number: OP5078) and *H. elliptica* (GTTM voucher accession number: OP5113).

**Plate 4-1:** *Rhipicephalus sanguineus***Plate 4-2:** *Haemaphysalis elliptica*

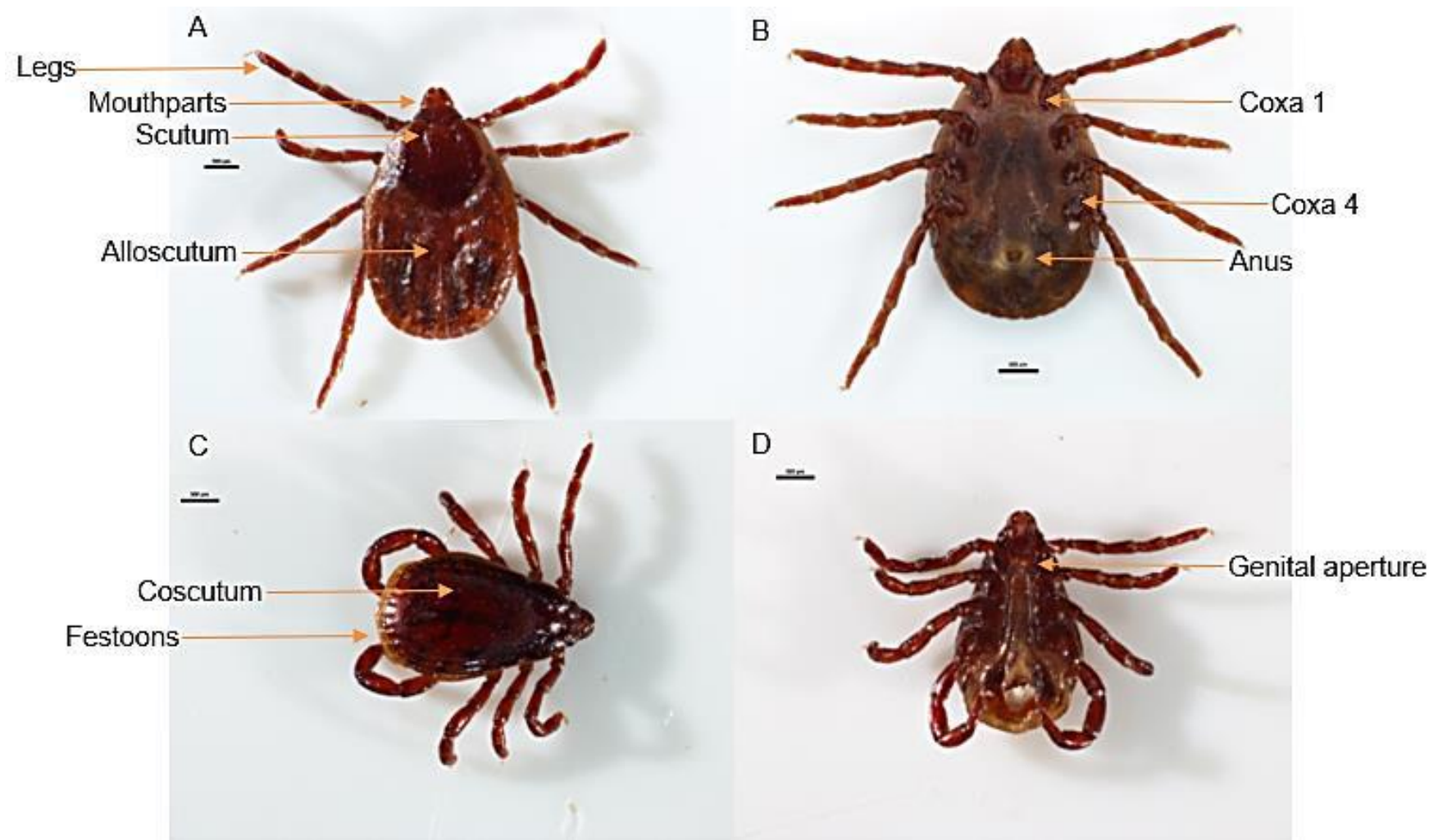


Plate 4-3: Morphological features of *Rhipicephalus sanguineus*. Dorsal view (A) and ventral view (B) of females. Dorsal view (C) and ventral view (D) of males

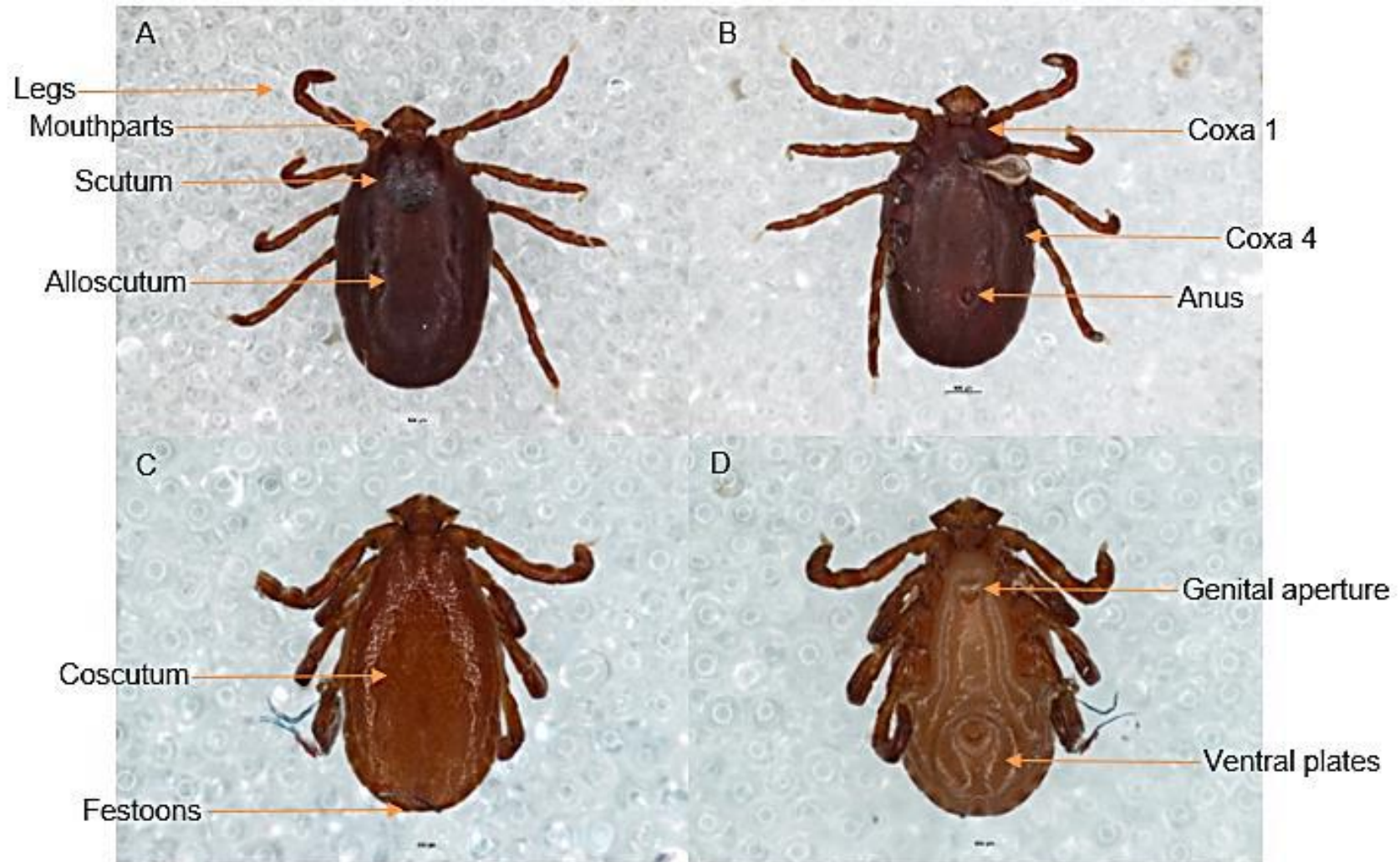


Plate 4-4: Morphological features of *Haemaphysalis elliptica*. Dorsal view (A) and ventral view (B) of females. Dorsal view (C) and ventral view (D) of males

During this study *R. sanguineus* was more abundant than *H. elliptica*, where *R. sanguineus* had an overall abundance of 70.7% (212/300) (143 nymphs and 69 female adults) and *H. elliptica* had an overall abundance of 29.3% (88/300) (32 nymphs and 56 female adults), as illustrated in Figure 4.7. The *R. sanguineus* was the most abundant in Miederpark [64.3% (193/300)], followed by Van der Hoff Park [2.3% (7/300)], Ikageng [2.3% (7/300)], Baillie park [1% (3/300)], and Dassierand [0.7% (2/300)], as illustrated in Figure 4.8. The *H. elliptica* was the most abundant in Miederpark [17% (51/300)] and Dassierand [9.7% (29/300)], followed by Baillie park [1% (3/300)], Turfvlei [1% (3/300)], Promosa [0.3% (1/300)], and Vyfhoek [0.3% (1/300)], as illustrated in Figure 4.8.

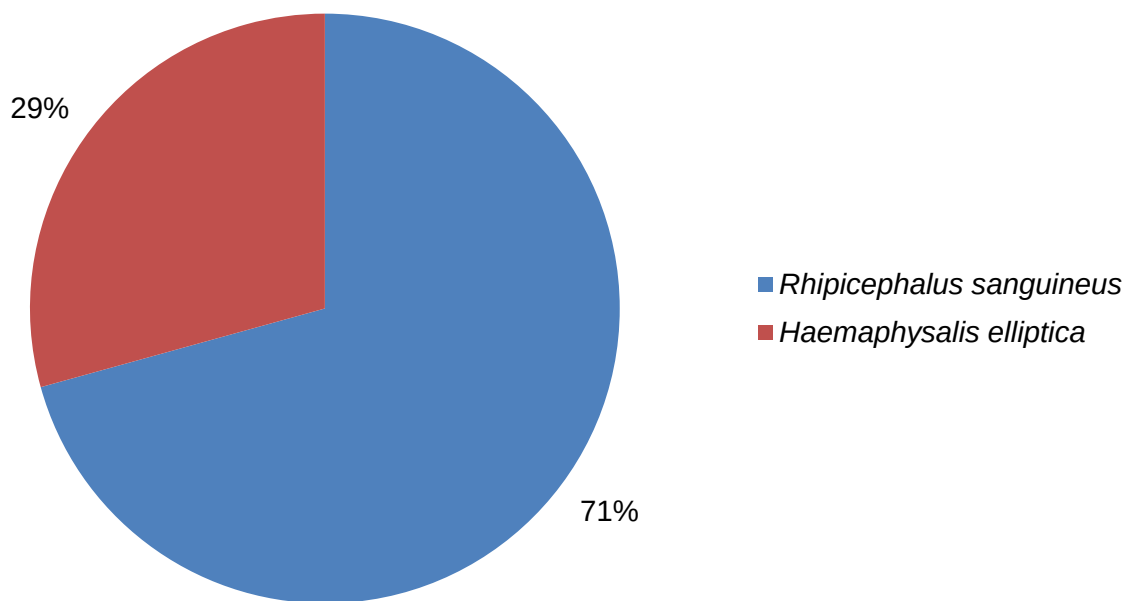


Figure 4-7: Overall occurrence of ticks naturally infesting dogs, collected at PAWS

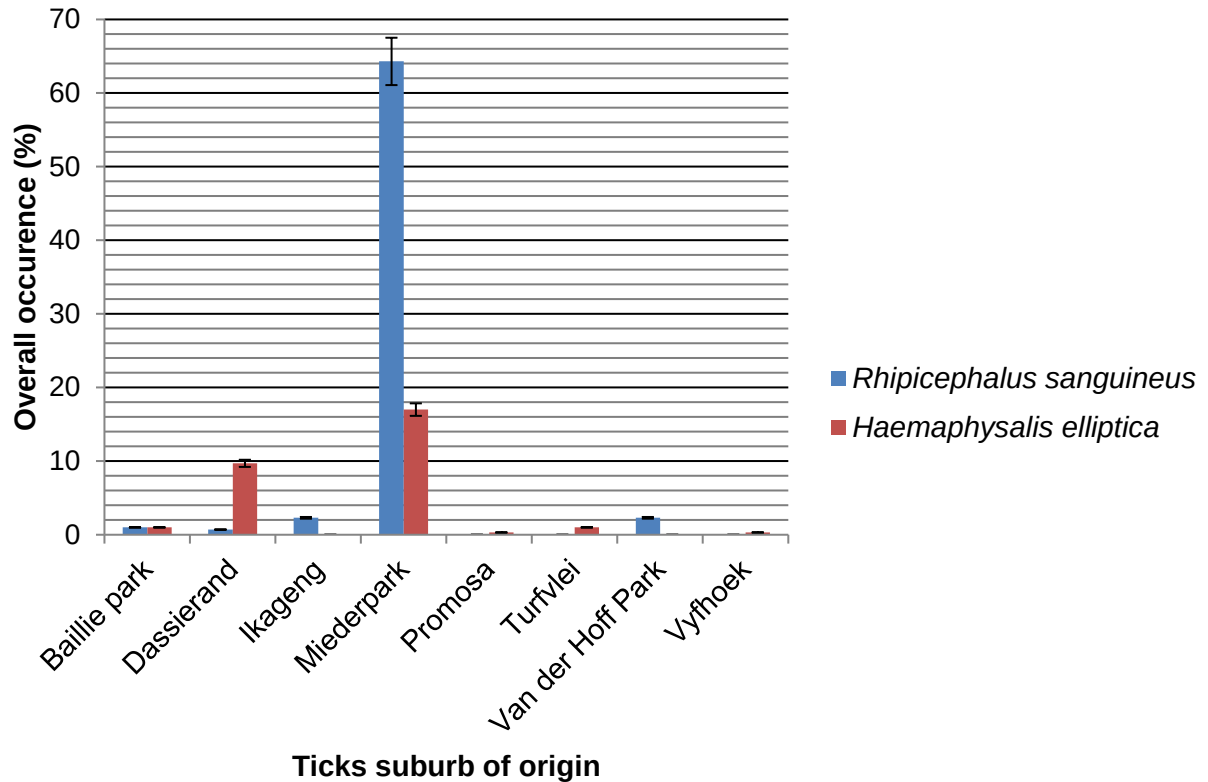


Figure 4-8: Overall occurrence of ticks naturally infesting dogs, admitted to PAWS, originating from several suburbs

4.3.3 Tick genetic identification

To supplement the tick's morphological identification, molecular identification was done to determine the tick species. Molecular identification was done by extracting DNA from selected whole tick species, followed by PCR assays to amplify both the *CO1* and *18S rRNA* genes.

4.3.3.1 The *CO1* gene

The DNA from tick specimens was amplified by conventional PCR targeting the *CO1* gene. The PCR amplification yielded fragments with an expected size of 710 bp (Figure 4.9). After Sanger sequencing, the lengths of the sequences ranged between 681 to 693 bp (Table 4.4). The nucleotide sequences generated in this study were submitted to GenBank under the following accession numbers: OQ319944, OQ318402, OQ319945, OQ324896, OQ318446, OQ324889, OQ324922, OQ329972, OQ329973, OQ306570, OQ329974, OQ318487, OQ324763, OQ318511, OQ320811, OQ323460, and OQ319993. The BLASTn search results of sequences from tick specimens used for molecular identification are summarized in Table 4.4. All of the tick specimens morphologically identified as *H. elliptica* (Sample IDs: H1.1, H1.2, H1.3, H1.4, H1.5,

H1.6, H1.7, H1.8, H1.9, and H1.10) were 96.89 to 97.74% similar to *H. elliptica* sequences on the NCBI database (GenBank accession number MZ351133.1) with maximum and total scores ranging between 1096 to 1131, query coverages ranging between 92 to 99% and E-values of 0. The tick species morphologically identified as *R. sanguineus* (Sample IDs: R1.1, R1.3, R1.4, R1.6, R17, R1.8, and R1.9) were 97.20 to 99.84% similar to *R. sanguineus* sequences on the NCBI database (GenBank accession numbers: JQ737084.1, KT906182.1, KT906183.1, OM368327.1, OM368323.1, OM368322.1, KT906185.1, KT906184.1, JX416325.1, HM193874.1, KF200112.1, KF200096.1, KF437543.1, KF200113.1, KF200084.1, KX360367.1, MZ401443.1, MZ401441.1, MZ401440.1, MG969507.1, ON134479.1, ON134475.1, ON134473.1, ON134471.1, ON134470.1, ON134469.1, ON134468.1, ON134467.1, ON134466.1, ON134465.1, ON134463.1, ON134462.1, ON134460.1, ON134459.1, ON134458.1, ON134455.1, ON134454.1, ON134452.1, ON134451.1, ON134450.1, ON134447.1, ON134446.1, ON134443.1, ON134442.1, ON134440.1, ON134439.1, ON134438.1, ON134437.1, ON134435.1, ON134434.1, ON134433.1, ON134432.1, ON134431.1, ON134430.1, ON134428.1, ON134427.1, ON134426.1, ON134424.1, ON134421.1, ON134420.1, ON134419.1, ON134417.1, ON134415.1, ON134414.1, ON134413.1, ON134412.1, ON134411.1, ON134409.1, ON134408.1, ON134407.1, ON134405.1, ON134404.1, ON134402.1, ON134400.1, ON134399.1, ON134398.1, ON134397.1, ON134396.1, ON134394.1, ON134393.1, ON134392.1, ON134391.1, KT906186.1). Additionally, the BLASTn search revealed that the aforementioned sequences had maximum and total scores ranging between 1092 to 1210, query coverages ranging between 88 to 99%, and E-values of 0.

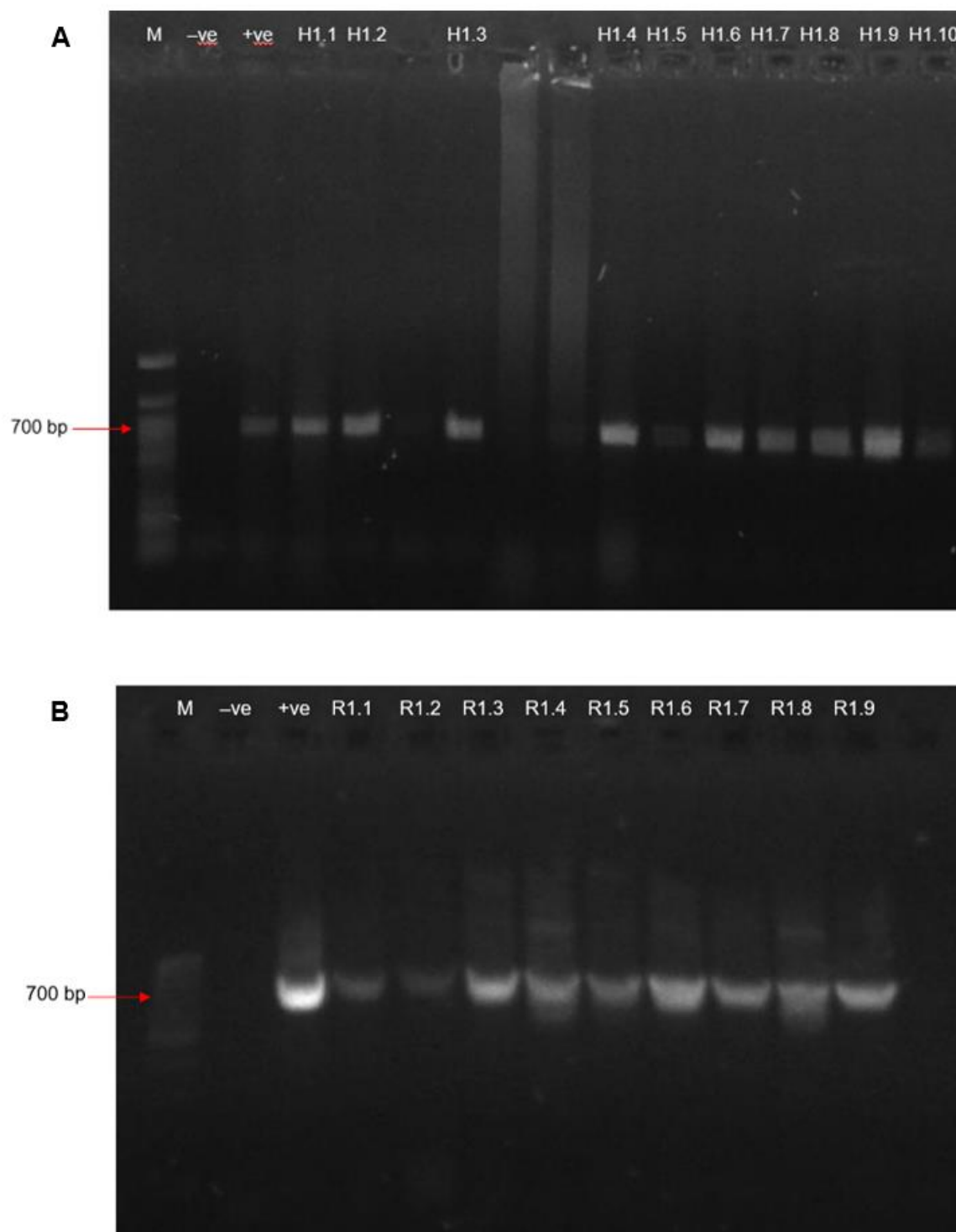


Figure 4-9: Agarose gel electrophoresis (1% w/v) of amplified DNA products of ticks, obtained by conventional PCR targeting the *CO1* gene, with an estimated 710 bp product size. Electrophoresis gel 1 (marked as **A**) represents the amplified DNA products of *H. elliptica* tick specimens. In this gel, lane 1 (marked as **M**) contained the 50 bp molecular DNA marker. Lane 2 (marked as **-ve**) contained the no template control. Lane 3 (marked as **+ve**) contained the positive

control. The remainder of the labelled lanes (marked as **H1.1** to **H1.10**) contained the amplified DNA products, whereas the unlabelled lanes contain the samples that did not amplify. Electrophoresis gel 2 (marked as **B**) represents the amplified DNA products of *R. sanguineus* tick specimens. In this gel, lane 1 (marked as **M**) contained the 50 bp molecular DNA marker. Lane 2 (marked as **-ve**) contained the no template control. Lane 3 (marked as **+ve**) contained the positive control. The remainder of the labelled lanes (marked as **R1.1** to **R1.9**) contained the amplified DNA products, whereas the unlabelled lanes contained the samples that did not amplify

Table 4-4: Ticks CO1 gene BLASTn results

Sample ID	Gene size (bp)	Morphological identification	BLASTn match	Maximum score	Total score	Query cover (%)	E-value	Identity (%)	Accession number*
H1.1	690	<i>H. elliptica</i>	<i>H. elliptica</i>	1096	1096	95	0	97.08	MZ351133.1
H1.2	688	<i>H. elliptica</i>	<i>H. elliptica</i>	1101	1101	94	0	97.24	
H1.3	690	<i>H. elliptica</i>	<i>H. elliptica</i>	1105	1105	94	0	97.38	
H1.4	685	<i>H. elliptica</i>	<i>H. elliptica</i>	1103	1103	94	0	97.24	
H1.5	684	<i>H. elliptica</i>	<i>H. elliptica</i>	1110	1110	95	0	97.25	
H1.6	691	<i>H. elliptica</i>	<i>H. elliptica</i>	1094	1094	92	0	97.65	
H1.7	685	<i>H. elliptica</i>	<i>H. elliptica</i>	1112	1112	94	0	97.54	
H1.8	685	<i>H. elliptica</i>	<i>H. elliptica</i>	1116	1116	95	0	97.74	
H1.9	685	<i>H. elliptica</i>	<i>H. elliptica</i>	1131	1131	99	0	96.90	
H1.10	682	<i>H. elliptica</i>	<i>H. elliptica</i>	1131	1131	98	0	96.89	

*Accession number of gene sequence of BLASTn searches representing all the sequences generated in this study.

Table 4-4: (continue)

Sample ID	Gene size (bp)	Morphological identification	BLASTn match	Maximum score	Total score	Query cover (%)	E-value	Identity (%)	Accession number*
R1.1	685	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1120-1194	1120-1194	89-95	0	98.63-99.68	JQ737084.1, KT906182.1, KT906183.1,
R1.3	685	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1125-1210	1125-1210	88-98	0	97.73-99.84	OM368327.1, OM368323.1, OM368322.1,
R1.4	679	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1094-1206	1094-1206	89-99	0	97.81-99.37	KT906185.1, KT906184.1, JX416325.1,
R1.6	688	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1075-1160	1075-1160	89-97	0	97.34-98.37	HM193874.1, KF200112.1, KF200096.1,
R1.7	693	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1125-1195	1125-1195	89-95	0	98.93-99.84	KF437543.1, KF200113.1, KF200084.1,
R1.8	681	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1092-1160	1092-1160	89-99	0	97.20-98.86	KX360367.1, MZ401443.1, MZ401441.1,
R1.9	690	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1123-1205	1123-1205	89-99	0	97.86-99.84	MZ401440.1, MG969507.1, ON134479.1,
									ON134475.1, ON134473.1, ON134471.1,
									ON134470.1, ON134469.1, ON134468.1,
									ON134467.1, ON134466.1, ON134465.1,
									ON134463.1, ON134462.1, ON134460.1,
									ON134459.1, ON134458.1, ON134455.1,
									ON134454.1, ON134452.1, ON134451.1,
									ON134450.1, ON134447.1, ON134446.1,
									ON134443.1, ON134442.1, ON134440.1,
									ON134439.1, ON134438.1, ON134437.1,
									ON134435.1, ON134434.1, ON134433.1,
									ON134432.1, ON134431.1, ON134430.1,
									ON134428.1, ON134427.1, ON134426.1,
									ON134424.1, ON134421.1, ON134420.1,
									ON134419.1, ON134417.1, ON134415.1,
									ON134414.1, ON134413.1, ON134412.1,
									ON134411.1, ON134409.1, ON134408.1,
									ON134407.1, ON134405.1, ON134404.1,
									ON134402.1, ON134400.1, ON134399.1,
									ON134398.1, ON134397.1, ON134396.1,
									ON134394.1, ON134393.1, ON134392.1,
									ON134391.1, KT906186.1

*Accession numbers of gene sequences of BLASTn searches representing all the sequences generated in this study.

Phylogenetic analysis was conducted using sequences of *R. sanguineus* and *H. elliptica* from this study, as well as reference sequences from *Rhipicephalus* and *Haemaphysalis* genera obtained from the NCBI database, with *Varroa destructor* used as an outgroup, as illustrated in Figure 4.10. The bootstrap support values of all the clades were greater than 50%. On the phylogenetic tree, there are two major clades. In the first major clade, the sequences of *R. sanguineus* ticks collected from dogs in this study clustered with sequences of other *R. sanguineus* and *Rhipicephalus* spp. from the NCBI database, with a bootstrap support value of 93%. In the second major clade the sequences of *H. elliptica* ticks collected from dogs in the JB Marks Local Municipality (North West Province) clustered with sequences of other *H. elliptica* and *Haemaphysalis* spp. from the NCBI database, with a bootstrap support value of 76%.

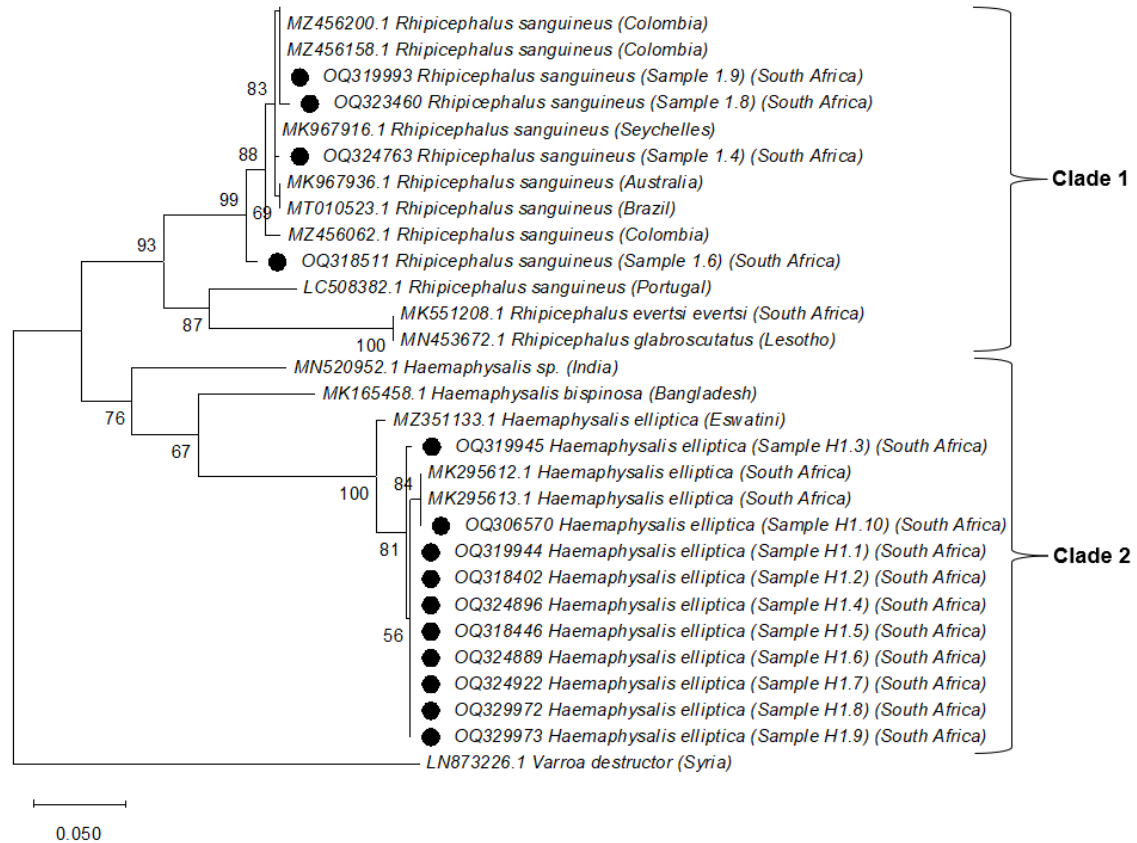


Figure 4-10: The CO1 gene phylogenetic analysis. Phylogenetic tree was obtained by using the Maximum Likelihood (ML) method and the Hasegawa-Kishino-Yano (HKY) model (Hasegawa. *et al.*, 1985). Bootstrap percentage (based on 10 000 replicates) is displayed next to the nodes of branches of associated clustered taxa. The data analysis was conducted using 33 nucleotide sequence samples. Nucleotide positions filled with missing data and gaps were removed during analysis. The sequences contain the GenBank accession number, species identification, and country of sample collection. Sequences of specimens collected during this study are indicated by a black dot and the assigned sample ID. The horizontal scale bar represents the number of base substitutions per site. Phylogenetic trees were constructed by using MEGA 11 software (Tamura *et al.*, 2021)

There were 376 nucleotide bases or positions left of each gene sequence, after sequence alignment and trimming. The aforementioned nucleotide bases or positions were used for phylogenetic analysis. Table 4.5 illustrates the base substitution numbers for each site of the sequences used for phylogenetic analysis. The sequence divergence of *H. elliptica* of this study, as well as *H. elliptica* and *Haemaphysalis* spp. sequences from GenBank ranged between 0 to 0.213. The sequence divergence of *R. sanguineus* of this study, as well as *R. sanguineus* and *Rhipicephalus* spp. sequences from GenBank ranged between 0 to 0.189. The pairwise distance (p-distance) matrix is illustrated in Table 4.6. The p-distance matrix demonstrated strong resolution power, ranging between 0 to 0.279%, between the sequences of *Rhipicephalus* spp. and *Haemaphysalis* spp. used for phylogenetic analysis. Figure 4.11 illustrates the differences among the nucleotides that were used during the phylogenetic analysis.

Table 4-5: Base substitutions number of each site using the CO1 gene

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	
[1] OQ319944_Haemaphysalis_elliptica_(Sample_H1.1)_(South_Africa)		0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.028	0.039	0.024	0.004	0.004	0.030	0.028	0.027	0.030	0.026	0.027	0.008	0.029	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.029	0.028	
[2] OQ318402_Haemaphysalis_elliptica_(Sample_H1.2)_(South_Africa)	0.000		0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.028	0.039	0.024	0.004	0.004	0.030	0.028	0.027	0.030	0.026	0.027	0.008	0.029	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.029	0.028	
[3] OQ319945_Haemaphysalis_elliptica_(Sample_H1.3)_(South_Africa)	0.005	0.005		0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.028	0.039	0.025	0.005	0.005	0.030	0.028	0.027	0.030	0.027	0.027	0.008	0.029	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.029	0.028	
[4] OQ324896_Haemaphysalis_elliptica_(Sample_H1.4)_(South_Africa)	0.000	0.000	0.005		0.000	0.000	0.000	0.000	0.000	0.004	0.028	0.039	0.024	0.004	0.004	0.030	0.028	0.027	0.030	0.026	0.027	0.008	0.029	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.029	0.028	
[5] OQ318446_Haemaphysalis_elliptica_(Sample_H1.5)_(South_Africa)	0.000	0.000	0.005	0.000		0.000	0.000	0.000	0.000	0.004	0.028	0.039	0.024	0.004	0.004	0.030	0.028	0.027	0.030	0.026	0.027	0.008	0.029	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.029	0.028	
[6] OQ324889_Haemaphysalis_elliptica_(Sample_H1.6)_(South_Africa)	0.000	0.000	0.005	0.000	0.000		0.000	0.000	0.000	0.004	0.028	0.039	0.024	0.004	0.004	0.030	0.028	0.027	0.030	0.026	0.027	0.008	0.029	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.029	0.028	
[7] OQ324922_Haemaphysalis_elliptica_(Sample_H1.7)_(South_Africa)	0.000	0.000	0.005	0.000	0.000	0.000		0.000	0.000	0.004	0.028	0.039	0.024	0.004	0.004	0.030	0.028	0.027	0.030	0.026	0.027	0.008	0.029	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.029	0.028	
[8] OQ329972_Haemaphysalis_elliptica_(Sample_H1.8)_(South_Africa)	0.000	0.000	0.005	0.000	0.000	0.000	0.000		0.000	0.004	0.028	0.039	0.024	0.004	0.004	0.030	0.028	0.027	0.030	0.026	0.027	0.008	0.029	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.029	0.028	
[9] OQ329973_Haemaphysalis_elliptica_(Sample_H1.9)_(South_Africa)	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000		0.004	0.028	0.039	0.024	0.004	0.004	0.030	0.028	0.027	0.030	0.026	0.027	0.008	0.029	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.029	0.028	
[10] OQ306570_Haemaphysalis_elliptica_(Sample_H1.10)_(South_Africa)	0.005	0.005	0.011	0.005	0.005	0.005	0.005	0.005	0.005		0.028	0.039	0.024	0.000	0.000	0.030	0.028	0.028	0.030	0.026	0.028	0.009	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	
[11] LC508382.1_Rhipicephalus_sanguineus_(Portugal)	0.227	0.227	0.227	0.227	0.227	0.227	0.227	0.227	0.227	0.227		0.037	0.028	0.028	0.028	0.022	0.020	0.020	0.022	0.027	0.020	0.028	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.019	0.020	0.021	0.020	
[12] LN873226.1_Varroa_destructor_(Syria)	0.358	0.358	0.358	0.358	0.358	0.358	0.358	0.358	0.358	0.358	0.334		0.038	0.039	0.038	0.037	0.037	0.037	0.038	0.037	0.037	0.039	0.038	0.037	0.037	0.037	0.037	0.037	0.037	0.037	0.037	0.037	0.037	
[13] MK165458.1_Haemaphysalis_bispinosa_(Bangladesh)	0.175	0.175	0.182	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.220	0.356		0.024	0.024	0.028	0.026	0.026	0.028	0.023	0.026	0.023	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.027	0.026	
[14] MK295612.1_Haemaphysalis_elliptica_(South_Africa)	0.005	0.005	0.011	0.005	0.005	0.005	0.005	0.005	0.005	0.000	0.227	0.358	0.175		0.000	0.030	0.028	0.028	0.030	0.026	0.028	0.009	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.028	0.029	0.029	
[15] MK295613.1_Haemaphysalis_elliptica_(South_Africa)	0.005	0.005	0.011	0.005	0.005	0.005	0.005	0.005	0.005	0.000	0.227	0.358	0.175	0.000		0.030	0.028	0.028	0.030	0.026	0.028	0.009	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.028	0.029	0.029	
[16] MK551208.1_Rhipicephalus_evertsi_evertsi_(South_Africa)	0.246	0.246	0.246	0.246	0.246	0.246	0.246	0.246	0.246	0.148	0.352	0.231	0.246	0.246		0.024	0.024	0.000	0.027	0.024	0.030	0.025	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.025	0.025	0.024	0.025	0.024
[17] MK967916.1_Rhipicephalus_sanguineus_(Seychelles)	0.220	0.220	0.220	0.220	0.220	0.220	0.220	0.220	0.220	0.228	0.129	0.334	0.198	0.228	0.228	0.179		0.003	0.024	0.025	0.003	0.027	0.006	0.003	0.003	0.003	0.003	0.003	0.003	0.008	0.003	0.005	0.003	
[18] MK967936.1_Rhipicephalus_sanguineus_(Australia)	0.217	0.217	0.217	0.217	0.217	0.217	0.217	0.217	0.217	0.224	0.129	0.338	0.198	0.224	0.224	0.175	0.003		0.024	0.025	0.000	0.027	0.007	0.004	0.004	0.004	0.004	0.004	0.004	0.008	0.004	0.005	0.004	
[19] MN453672.1_Rhipicephalus_glabroscutatus_(Lesotho)	0.246	0.246	0.246	0.246	0.246	0.246	0.246	0.246	0.246	0.148	0.352	0.231	0.246	0.246	0.000	0.179	0.175		0.027	0.024	0.030	0.025	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.025	0.025	0.024	0.025	0.024
[20] MN520952.1_Haemaphysalis_sp_(India)	0.198	0.198	0.206	0.198	0.198	0.198	0.198	0.198	0.198	0.206	0.343	0.158	0.198	0.198	0.219	0.191	0.188	0.219		0.025	0.027	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.025	0.026	0.026	0.026	
[21] MT010523.1_Rhipicephalus_sanguineus_(Brazil)	0.217	0.217	0.217	0.217	0.217	0.217	0.217	0.217	0.217	0.224	0.129	0.338	0.198	0.224	0.224	0.175	0.003	0.000	0.175	0.188		0.027	0.007	0.004	0.004	0.004	0.004	0.004	0.004	0.008	0.004	0.005	0.004	
[22] MZ351133.1_Haemaphysalis_elliptica_(Eswatini)	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.030	0.224	0.353	0.164	0.030	0.030	0.243	0.213	0.209	0.243	0.213	0.209		0.028	0.027	0.027	0.027	0.027	0.027	0.027	0.028	0.027	0.028	0.027	
[23] MZ456062.1_Rhipicephalus_sanguineus_(Colombia)	0.232	0.232	0.232	0.232	0.232	0.232	0.232	0.232	0.232	0.240	0.129	0.347	0.205	0.240	0.240	0.189	0.014	0.016	0.189	0.202	0.016	0.224		0.007	0.007	0.007	0.007	0.007	0.007	0.008	0.007	0.008	0.007	
[24] MZ456158.1_Rhipicephalus_sanguineus_(Colombia)	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.232	0.129	0.334	0.202	0.232	0.232	0.179	0.003	0.005	0.179	0.195	0.005	0.213	0.016		0.000	0.000	0.000	0.000	0.004	0.008	0.000	0.004	0.000	
[25] MZ456200.1_Rhipicephalus_sanguineus_(Colombia)	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.232	0.129	0.334	0.202	0.232	0.232	0.179	0.003	0.005	0.179	0.195	0.005	0.213	0.016	0.000		0.000	0.000	0.000	0.004	0.008	0.000	0.004	0.000	
[26] MZ456214.1_Rhipicephalus_sanguineus_(Colombia)	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.232	0.129	0.334	0.202	0.232	0.232	0.179	0.003	0.005	0.179	0.195	0.005	0.213	0.016	0.000	0.000		0.000	0.000	0.004	0.008	0.000	0.004	0.000	
[27] OQ329974_Rhipicephalus_sanguineus_(Sample_1.1)_(South_Africa)	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.232	0.129	0.334	0.202	0.232	0.232	0.179	0.003	0.005	0.179	0.195	0.005	0.213	0.016	0.000	0.000	0.000		0.000	0.004	0.008	0.000	0.004	0.000	
[28] OQ318487_Rhipicephalus_sanguineus_(Sample_1.3)_(South_Africa)	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.232	0.129	0.334	0.202	0.232	0.232	0.179	0.003	0.005	0.179	0.195	0.005	0.213	0.016	0.000	0.000	0.000	0.000		0.004	0.008	0.000	0.004	0.000	
[29] OQ324763_Rhipicephalus_sanguineus_(Sample_1.4)_(South_Africa)	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.232	0.125	0.338	0.202	0.232	0.232	0.182	0.003	0.005	0.182	0.195	0.005	0.216	0.016	0.005	0.005	0.005	0.005	0.005		0.008	0.004	0.005	0.004	
[30] OQ318511_Rhipicephalus_sanguineus_(Sample_1.6)_(South_Africa)	0.221	0.221	0.221	0.221	0.221	0.221	0.221	0.221	0.221	0.221	0.119	0.343	0.198	0.221	0.221	0.179	0.022	0.025	0.179	0.191	0.025	0.221	0.025	0.025	0.025	0.025	0.025	0.025	0.025		0.008	0.009	0.008	
[31] OQ320811_Rhipicephalus_sanguineus_(Sample_1.7)_(South_Africa)	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.232	0.129	0.334	0.202	0.232	0.232	0.179	0.003	0.005	0.179	0.195	0.005	0.213	0.016	0.000	0.000	0.000	0.000	0.000	0.005	0.025		0.004	0.000	
[32] OQ323460_Rhipicephalus_sanguineus_(Sample_1.8)_(South_Africa)	0.231	0.231	0.231	0.231	0.231	0.231	0.231	0.231	0.231	0.231	0.135	0.342	0.209	0.239	0.239	0.185	0.008	0.011	0.185	0.202	0.011	0.220	0.022	0.005	0.005	0.005	0.005	0.005	0.011	0.030	0.005		0.004	
[33] OQ319993_Rhipicephalus_sanguineus_(Sample_1.9)_(South_Africa)	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.232	0.129	0.334	0.202	0.232	0.232	0.179	0.003	0.005	0.179	0.195	0.005	0.213	0.016	0.000	0.000	0.000	0.000	0.000	0.005	0.025	0.000	0.005		

*Numbers in black below the diagonal line represent the base substitutions number of each site between sequences and the numbers in blue above the diagonal line

Table 4-6: Pairwise distance (p-distance) analysis of sequences using the *CO1* gene

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33		
[1] OQ319944_Haemaphysalis_elliptica_(Sample_H1.1)_(South_Africa)		0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.020	0.023	0.019	0.004	0.004	0.021	0.020	0.020	0.021	0.020	0.020	0.008	0.021	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020		
[2] OQ318402_Haemaphysalis_elliptica_(Sample_H1.2)_(South_Africa: 0.000)			0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.020	0.023	0.019	0.004	0.004	0.021	0.020	0.020	0.021	0.020	0.020	0.008	0.021	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020		
[3] OQ319945_Haemaphysalis_elliptica_(Sample_H1.3)_(South_Africa: 0.005)		0.005		0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.020	0.023	0.019	0.005	0.005	0.021	0.020	0.020	0.021	0.020	0.020	0.008	0.021	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020		
[4] OQ324896_Haemaphysalis_elliptica_(Sample_H1.4)_(South_Africa: 0.000)		0.000	0.000	0.005		0.000	0.000	0.000	0.000	0.004	0.020	0.023	0.019	0.004	0.004	0.021	0.020	0.020	0.021	0.020	0.020	0.008	0.021	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020		
[5] OQ318446_Haemaphysalis_elliptica_(Sample_H1.5)_(South_Africa: 0.000)		0.000	0.000	0.005	0.000		0.000	0.000	0.000	0.004	0.020	0.023	0.019	0.004	0.004	0.021	0.020	0.020	0.021	0.020	0.020	0.008	0.021	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020		
[6] OQ324889_Haemaphysalis_elliptica_(Sample_H1.6)_(South_Africa: 0.000)		0.000	0.000	0.005	0.000	0.000		0.000	0.000	0.004	0.020	0.023	0.019	0.004	0.004	0.021	0.020	0.020	0.021	0.020	0.020	0.008	0.021	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020		
[7] OQ324922_Haemaphysalis_elliptica_(Sample_H1.7)_(South_Africa: 0.000)		0.000	0.000	0.005	0.000	0.000	0.000		0.000	0.004	0.020	0.023	0.019	0.004	0.004	0.021	0.020	0.020	0.021	0.020	0.020	0.008	0.021	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020		
[8] OQ329972_Haemaphysalis_elliptica_(Sample_H1.8)_(South_Africa: 0.000)		0.000	0.000	0.005	0.000	0.000	0.000	0.000		0.000	0.004	0.020	0.023	0.019	0.004	0.004	0.021	0.020	0.020	0.021	0.020	0.020	0.008	0.021	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020		
[9] OQ329973_Haemaphysalis_elliptica_(Sample_H1.9)_(South_Africa: 0.000)		0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000		0.004	0.020	0.023	0.019	0.004	0.004	0.021	0.020	0.020	0.021	0.020	0.020	0.008	0.021	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020		
[10] OQ306570_Haemaphysalis_elliptica_(Sample_H1.10)_(South_Africa: 0.005)		0.005	0.005	0.011	0.005	0.005	0.005	0.005	0.005		0.020	0.023	0.019	0.000	0.000	0.021	0.020	0.020	0.021	0.020	0.020	0.009	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021		
[11] LC508382_1_Rhipicephalus_sanguineus_(Portugal)		0.194	0.194	0.194	0.194	0.194	0.194	0.194	0.194	0.194		0.023	0.020	0.020	0.020	0.018	0.017	0.017	0.018	0.020	0.017	0.020	0.017	0.017	0.017	0.017	0.017	0.017	0.016	0.016	0.017	0.017			
[12] LN873226_1_Varroa_destructor_(Syria)		0.279	0.279	0.279	0.279	0.279	0.279	0.279	0.279	0.279	0.266		0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023			
[13] MK165458_1_Haemaphysalis_bispinosa_(Bangladesh)		0.154	0.154	0.160	0.154	0.154	0.154	0.154	0.154	0.154	0.189	0.279		0.019	0.019	0.021	0.020	0.020	0.021	0.018	0.020	0.018	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020			
[14] MK295612_1_Haemaphysalis_elliptica_(South_Africa)		0.005	0.005	0.011	0.005	0.005	0.005	0.005	0.005	0.000	0.194	0.279	0.154		0.000	0.021	0.020	0.020	0.021	0.020	0.020	0.009	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021			
[15] MK295613_1_Haemaphysalis_elliptica_(South_Africa)		0.005	0.005	0.011	0.005	0.005	0.005	0.005	0.005	0.000	0.194	0.279	0.154	0.000		0.021	0.020	0.020	0.021	0.020	0.020	0.009	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021			
[16] MK551208_1_Rhipicephalus_evertsi_evertsi_(South_Africa)		0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.133	0.277	0.197	0.207	0.207		0.019	0.019	0.000	0.020	0.019	0.021	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019			
[17] MK967916_1_Rhipicephalus_sanguineus_(Seychelles)		0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.117	0.266	0.173	0.194	0.194	0.157		0.003	0.019	0.019	0.003	0.020	0.006	0.003	0.003	0.003	0.003	0.003	0.007	0.003	0.005	0.003			
[18] MK967936_1_Rhipicephalus_sanguineus_(Australia)		0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.117	0.269	0.173	0.191	0.191	0.154	0.003		0.019	0.019	0.000	0.020	0.006	0.004	0.004	0.004	0.004	0.004	0.008	0.004	0.005	0.004			
[19] MN453672_1_Rhipicephalus_glabroscutatus_(Lesotho)		0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.133	0.277	0.197	0.207	0.207	0.154	0.000	0.157	0.154		0.020	0.019	0.021	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019			
[20] MN520952_1_Haemaphysalis_sp_(India)		0.173	0.173	0.178	0.173	0.173	0.173	0.173	0.173	0.173	0.178	0.271	0.141	0.173	0.173	0.189	0.168	0.165	0.189		0.019	0.020	0.020	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.020	0.019		
[21] MT010523_1_Rhipicephalus_sanguineus_(Brazil)		0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.117	0.269	0.173	0.191	0.191	0.154	0.003	0.000	0.154	0.165		0.020	0.006	0.004	0.004	0.004	0.004	0.004	0.008	0.004	0.005	0.004			
[22] MZ351133_1_Haemaphysalis_elliptica_(Eswatini)		0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.117	0.277	0.146	0.029	0.029	0.205	0.184	0.181	0.005	0.184	0.181		0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020			
[23] MZ456062_1_Rhipicephalus_sanguineus_(Colombia)		0.197	0.197	0.197	0.197	0.197	0.197	0.197	0.197	0.197	0.117	0.274	0.178	0.202	0.202	0.165	0.013	0.016	0.165	0.178	0.016	0.191		0.006	0.006	0.006	0.006	0.006	0.006	0.008	0.006	0.007	0.006		
[24] MZ456158_1_Rhipicephalus_sanguineus_(Colombia)		0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.117	0.266	0.176	0.197	0.197	0.157	0.003	0.005	0.157	0.170	0.005	0.184	0.016		0.000	0.000	0.000	0.000	0.004	0.008	0.000	0.004	0.000		
[25] MZ456200_1_Rhipicephalus_sanguineus_(Colombia)		0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.117	0.266	0.176	0.197	0.197	0.157	0.003	0.005	0.157	0.170	0.005	0.184	0.016	0.000		0.000	0.000	0.000	0.000	0.004	0.008	0.000	0.004	0.000	
[26] MZ456214_1_Rhipicephalus_sanguineus_(Colombia)		0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.117	0.266	0.176	0.197	0.197	0.157	0.003	0.005	0.157	0.170	0.005	0.184	0.016	0.000	0.000		0.000	0.000	0.000	0.000	0.004	0.008	0.000	0.004	0.000
[27] OQ329974_Rhipicephalus_sanguineus_(Sample_1.1)_(South_Africa: 0.191)		0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.117	0.266	0.176	0.197	0.197	0.157	0.003	0.005	0.157	0.170	0.005	0.184	0.016	0.000	0.000	0.000		0.000	0.004	0.008	0.000	0.004	0.000		
[28] OQ318487_Rhipicephalus_sanguineus_(Sample_1.3)_(South_Africa: 0.191)		0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.117	0.266	0.176	0.197	0.197	0.157	0.003	0.005	0.157	0.170	0.005	0.184	0.016	0.000	0.000	0.000	0.000		0.004	0.008	0.000	0.004	0.000		
[29] OQ324763_Rhipicephalus_sanguineus_(Sample_1.4)_(South_Africa: 0.191)		0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.114	0.269	0.176	0.197	0.197	0.160	0.003	0.005	0.160	0.170	0.005	0.186	0.016	0.005	0.005	0.005	0.005		0.008	0.004	0.005	0.004	0.000		
[30] OQ318511_Rhipicephalus_sanguineus_(Sample_1.6)_(South_Africa: 0.189)		0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.109	0.271	0.173	0.189	0.189	0.157	0.021	0.024	0.157	0.168	0.024	0.189	0.024	0.024	0.024	0.024	0.024	0.024		0.008	0.009	0.008	0.000		
[31] OQ320811_Rhipicephalus_sanguineus_(Sample_1.7)_(South_Africa: 0.191)		0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.117	0.266	0.176	0.197	0.197	0.157	0.003	0.005	0.157	0.170	0.005	0.184	0.016	0.000	0.000	0.000	0.000	0.000	0.005	0.024		0.004	0.000		
[32] OQ323460_Rhipicephalus_sanguineus_(Sample_1.8)_(South_Africa: 0.197)		0.197	0.197	0.197	0.197	0.197	0.197	0.197	0.197	0.197	0.122	0.271	0.181	0.202	0.202	0.162	0.008	0.011	0.162	0.176	0.011	0.189	0.021	0.005	0.005	0.005	0.005	0.005	0.011	0.029	0.005		0.004		
[33] OQ319993_Rhipicephalus_sanguineus_(Sample_1.9)_(South_Africa: 0.191)		0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.117	0.266	0.176	0.197	0.197	0.157	0.003	0.005	0.157	0.170	0.005	0.184	0.016	0.000	0.000	0.000	0.000	0.000	0.005	0.024	0.000	0.005			

*Numbers in black below the diagonal line represent the p-distance and the numbers in blue above the diagonal line represent the standard errors.

Nucleotide position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
OQ319944 Haemaphysalis elliptica (Sample H1.1) (South Africa)	T	T	T	A	A	T	G	T	T	A	G	G	A	G	C	A	C	C	A	G
OQ318402 Haemaphysalis elliptica (Sample H1.2) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ319945 Haemaphysalis elliptica (Sample H1.3) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ314896 Haemaphysalis elliptica (Sample H1.4) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ318446 Haemaphysalis elliptica (Sample H1.5) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ314889 Haemaphysalis elliptica (Sample H1.6) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ314922 Haemaphysalis elliptica (Sample H1.7) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ319972 Haemaphysalis elliptica (Sample H1.8) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ319973 Haemaphysalis elliptica (Sample H1.9) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ386570 Haemaphysalis elliptica (Sample H1.10) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
LC588382.1 Rhipicephalus sanguineus (Portugal)	.	A	.	T	.	.	A	.	.	.	.	.	T	.	.	C	.	.	.	.
LN873226.1 Varroa destructor (Syria)	.	.	.	.	.	.	A	A	.	T	T	C	T	.	.	.	.	.	.	.
MK165458.1 Haemaphysalis bispinosa (Bangladesh)	.	C	.	.	.	.	A	.	.	.	.	.	.	.	.	T	.	.	.	.
MK295612.1 Haemaphysalis elliptica (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MK295613.1 Haemaphysalis elliptica (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MK551208.1 Rhipicephalus evertsi evertsi (South Africa)	.	A	.	T	.	.	A	.	.	.	.	.	T	.	.	T	.	.	T	.
MK967916.1 Rhipicephalus sanguineus (Seychelles)	.	A	.	T	.	.	A	C	.	.	.	.	.	.	.	T	.	.	.	.
MK967936.1 Rhipicephalus sanguineus (Australia)	.	A	.	T	.	.	A	C	.	.	.	.	.	.	.	T	.	.	.	.
MN453672.1 Rhipicephalus glabroscuratus (Lesotho)	.	A	.	T	.	.	A	C	.	.	.	T	.	.	.	T	.	.	T	.
MN520952.1 Haemaphysalis sp. (India)	.	.	.	.	.	.	.	.	6	.	.	.	.	.	.	.	.	.	.	.
MT010523.1 Rhipicephalus sanguineus (Brazil)	.	A	.	T	.	.	A	C	.	.	.	.	.	.	.	T	.	.	.	.
MZ351133.1 Haemaphysalis elliptica (Eswatini)	.	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	.	.	.	.
MZ456062.1 Rhipicephalus sanguineus (Colombia)	.	A	.	T	.	.	A	C	.	.	.	.	.	.	.	T	.	.	.	.
MZ456158.1 Rhipicephalus sanguineus (Colombia)	.	A	.	T	.	.	A	C	.	.	.	.	6	.	.	T	.	.	.	.
MZ456200.1 Rhipicephalus sanguineus (Colombia)	.	A	.	T	.	.	A	C	.	.	.	.	6	.	.	T	.	.	.	.
MZ456214.1 Rhipicephalus sanguineus (Colombia)	.	A	.	T	.	.	A	C	.	.	.	.	6	.	.	T	.	.	.	.
OQ319974 Rhipicephalus sanguineus (Sample 1.1) (South Africa)	.	A	.	T	.	.	A	C	.	.	.	.	6	.	.	T	.	.	.	.
OQ318487 Rhipicephalus sanguineus (Sample 1.3) (South Africa)	.	A	.	T	.	.	A	C	.	.	.	.	6	.	.	T	.	.	.	.
OQ314763 Rhipicephalus sanguineus (Sample 1.4) (South Africa)	.	A	.	T	.	.	A	C	.	.	.	.	.	.	.	T	.	.	.	.
OQ318511 Rhipicephalus sanguineus (Sample 1.6) (South Africa)	.	A	.	T	.	.	.	C	.	.	.	.	.	.	.	T	.	.	.	.
OQ318011 Rhipicephalus sanguineus (Sample 1.7) (South Africa)	.	A	.	T	.	.	.	C	.	.	.	.	.	.	.	T	.	.	.	.
OQ313460 Rhipicephalus sanguineus (Sample 1.8) (South Africa)	.	A	.	T	.	.	A	C	.	.	.	.	6	.	.	T	.	.	.	.
OQ319993 Rhipicephalus sanguineus (Sample 1.9) (South Africa)	.	A	.	T	.	.	A	C	.	.	.	.	6	.	.	T	.	.	.	.

Nucleotide position	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
OQ319944 Haemaphysalis elliptica (Sample H1.1) (South Africa)	A	T	A	T	A	G	C	T	T	T	T	C	C	A	C	G	A	A	T	A
OQ318402 Haemaphysalis elliptica (Sample H1.2) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ319945 Haemaphysalis elliptica (Sample H1.3) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ314896 Haemaphysalis elliptica (Sample H1.4) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ318446 Haemaphysalis elliptica (Sample H1.5) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ314889 Haemaphysalis elliptica (Sample H1.6) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ314922 Haemaphysalis elliptica (Sample H1.7) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ319972 Haemaphysalis elliptica (Sample H1.8) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ319973 Haemaphysalis elliptica (Sample H1.9) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OQ386570 Haemaphysalis elliptica (Sample H1.10) (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
LC588382.1 Rhipicephalus sanguineus (Portugal)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	T	T	.	.
LN873226.1 Varroa destructor (Syria)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
MK165458.1 Haemaphysalis bispinosa (Bangladesh)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
MK295612.1 Haemaphysalis elliptica (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MK295613.1 Haemaphysalis elliptica (South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MK551208.1 Rhipicephalus evertsi evertsi (South Africa)	.	.	.	.	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.
MK967916.1 Rhipicephalus sanguineus (Seychelles)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
MK967936.1 Rhipicephalus sanguineus (Australia)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
MN453672.1 Rhipicephalus glabroscuratus (Lesotho)	.	.	.	.	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.
MN520952.1 Haemaphysalis sp. (India)	.	.	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	.	.	.
MT010523.1 Rhipicephalus sanguineus (Brazil)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
MZ351133.1 Haemaphysalis elliptica (Eswatini)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MZ456062.1 Rhipicephalus sanguineus (Colombia)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
MZ456158.1 Rhipicephalus sanguineus (Colombia)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
MZ456200.1 Rhipicephalus sanguineus (Colombia)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
MZ456214.1 Rhipicephalus sanguineus (Colombia)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
OQ319974 Rhipicephalus sanguineus (Sample 1.1) (South Africa)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
OQ318487 Rhipicephalus sanguineus (Sample 1.3) (South Africa)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
OQ314763 Rhipicephalus sanguineus (Sample 1.4) (South Africa)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
OQ318511 Rhipicephalus sanguineus (Sample 1.6) (South Africa)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
OQ318011 Rhipicephalus sanguineus (Sample 1.7) (South Africa)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
OQ313460 Rhipicephalus sanguineus (Sample 1.8) (South Africa)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	C	.	.	.	.
OQ319993 Rhipicephalus sanguineus (Sample 1.9) (South Africa)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.

Figure 4-11: Nucleotide differences within the alignment sequences, from nucleotide position 1 to 40, used for phylogenetic analysis of the CO1 gene.

The full stops (.) represent nucleotides identical to the top nucleotide sequence

Table 4.7 illustrates the base substitution rates of nucleotide pairs used during phylogenetic analysis. With the removal of missing data and gaps, the average nucleotide frequencies were 29% for adenine, 38.7% for thymine, 19.4% for cytosine, and 12.9% for guanine.

Table 4-7: Nucleotide pairs of the *CO1* gene rates of substitution

From\To	A	T	C	G
A	-	0.078514482	0.039444997	0.075457596
T	0.058922596	-	0.115823095	0.025698023
C	0.058922596	0.23054357	-	0.025698023
G	0.173015543	0.078514482	0.039444997	-

A: adenine; T: thymine; C: cytosine; G: Guanine

4.3.3.2 The *18S rRNA* gene

The DNA of tick specimens was amplified by conventional PCR targeting the *18S rRNA* gene, resulting in PCR amplicons with an expected size of 780 bp (Figure 4.12). After Sanger sequencing, the lengths of the sequences ranged between 811 to 826 bp (Table 4.8). The sequences obtained by Sanger sequencing were submitted to GenBank on the NCBI database under the following accession numbers: OP962688, OP962689, OP962690, OP962691, OP962692, OP962693, OP962694, OP962695, OP962696, OP962697, OP962698, OP962699, OP962701, OP962702, OP962703, OP962704, OP962705, OP962709, OP962710, OP962711, and OP962712. The BLASTn search results of the tick specimens used for molecular identification are summarized in Table 4.8. All of the tick specimens morphologically identified as *H. elliptica* (Sample IDs: H2.2, H2.3, H2.4, H2.5, H2.6, H2.7, H2.8, H2.11, H2.12, H2.13, H2.14, and H2.15) were 99.26 to 99.63% similar to *H. elliptica* sequences on the NCBI database (GenBank accession number AF018647.1) with maximum and total scores ranging between 1465 to 1491, query covers of 99% and E-values of 0. All of the tick species morphologically identified as *R. sanguineus* (Sample IDs: R2.2, R2.3, R2.4, R2.5, R2.7, 2.11, R2.12, R2.13, and R2.14) were 98.53 to 99.90% similar to *R. sanguineus* sequences on the NCBI database (GenBank accession numbers: KF958435.1, KF958434.1, KF958433.1, KF958432.1, KF958431.1, KF958430.1, KF958429.1, JX987497.1, XR_005180903.1, JX987496.1, and L76342.1). Additionally, the BLASTn search revealed that the aforementioned sequences had maximum and total scores ranging between 1424 to 1489, query covers ranging between 97 to 100%, and E-values of 0.

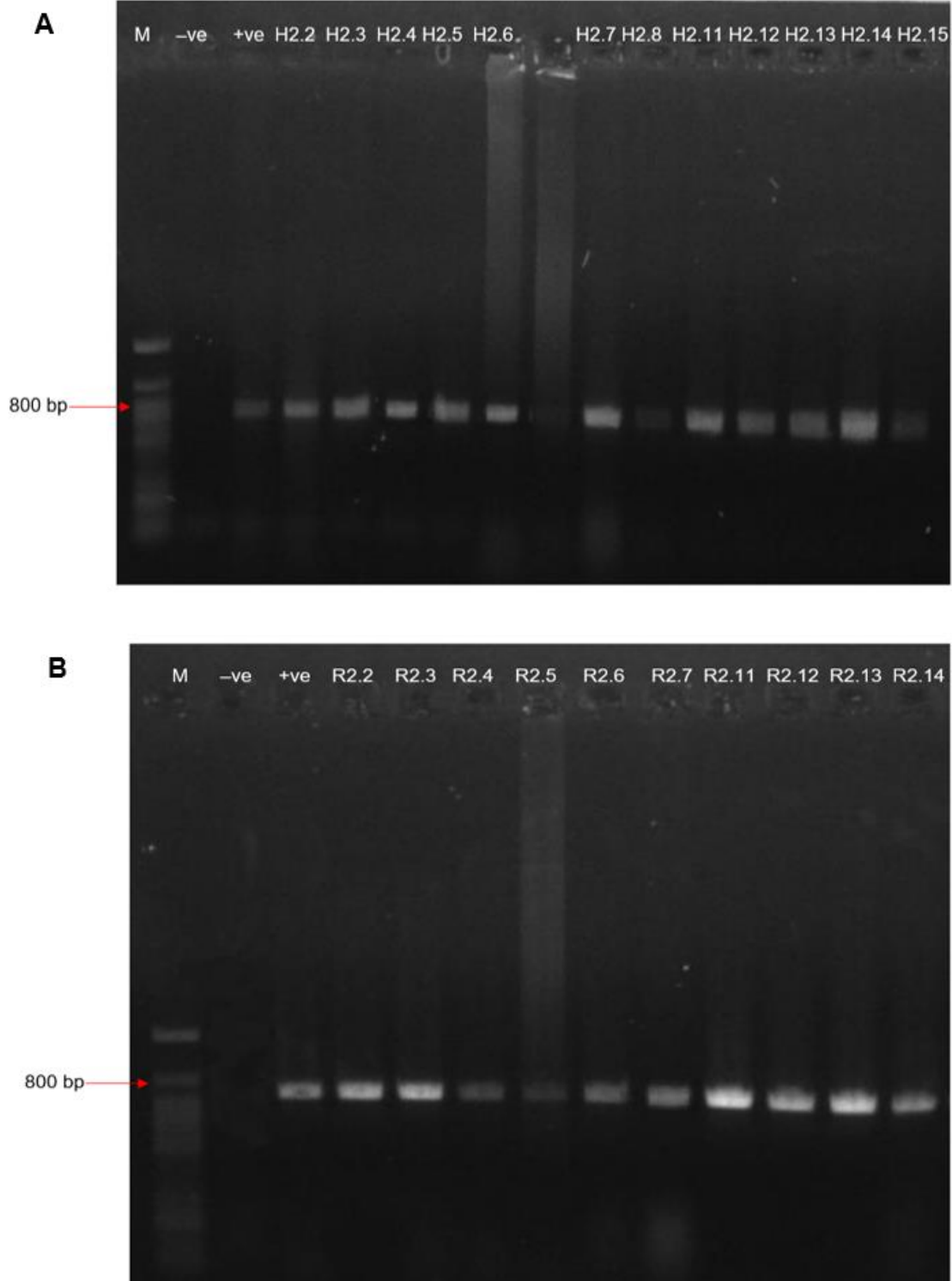


Figure 4-12: Agarose gel electrophoresis (1% w/v) of amplified DNA products of ticks, obtained by conventional PCR targeting the *18S rRNA* gene, with an estimated 780 bp product size. Electrophoresis gel 1 (marked as **A**) represents the amplified DNA products of *H. elliptica* tick specimens. In this gel, lane 1 (marked as **M**) contained the 100 bp molecular DNA marker. Lane 2 (marked as **-ve**) contained the no template control. Lane 3 (marked as **+ve**) contained the

positive control. The remainder of the labelled lanes (marked as **H2.2** to **H2.15**) contained the amplified DNA products, whereas the unlabelled lanes contained the samples that did not amplify. Electrophoresis gel 2 (marked as **B**) represents the amplified DNA products of *R. sanguineus* tick specimens. In this gel, lane 1 (marked as **M**) contained the 100 bp molecular DNA marker. Lane 2 (marked as **-ve**) contained the no template control. Lane 3 (marked as **+ve**) contained the positive control. The remainder of the lanes (marked as **R2.2** to **R2.14**) contained the amplified DNA products

Table 4-8: Ticks 18S rRNA gene BLASTn results

Sample ID	Gene size (bp)	Morphological identification	BLASTn match	Maximum score	Total score	Query cover (%)	E-value	Identity (%)	Accession number*
H2.2	818	<i>H. elliptica</i>	<i>H. elliptica</i> (formerly named <i>H. leachi</i>)	1478	1478	99	0	99.39	AF018647.1
H2.3	818	<i>H. elliptica</i>	<i>H. elliptica</i> (formerly named <i>H. leachi</i>)	1472	1472	99	0	99.38	
H2.4	815	<i>H. elliptica</i>	<i>H. elliptica</i> (formerly named <i>H. leachi</i>)	1471	1471	99	0	99.26	
H2.5	816	<i>H. elliptica</i>	<i>H. elliptica</i> (formerly named <i>H. leachi</i>)	1487	1487	99	0	99.63	
H2.6	817	<i>H. elliptica</i>	<i>H. elliptica</i> (formerly named <i>H. leachi</i>)	1478	1478	99	0	99.39	
H2.7	819	<i>H. elliptica</i>	<i>H. elliptica</i> (formerly named <i>H. leachi</i>)	1478	1478	99	0	99.39	
H2.8	819	<i>H. elliptica</i>	<i>H. elliptica</i> (formerly named <i>H. leachi</i>)	1491	1491	99	0	99.63	
H2.11	817	<i>H. elliptica</i>	<i>H. elliptica</i> (formerly named <i>H. leachi</i>)	1474	1474	99	0	99.39	
H2.12	819	<i>H. elliptica</i>	<i>H. elliptica</i> (formerly named <i>H. leachi</i>)	1465	1465	98	0	99.38	
H2.13	817	<i>H. elliptica</i>	<i>H. elliptica</i> (formerly named <i>H. leachi</i>)	1478	1478	99	0	99.39	
H2.14	819	<i>H. elliptica</i>	<i>H. elliptica</i> (formerly named <i>H. leachi</i>)	1476	1476	99	0	99.39	
H2.15	822	<i>H. elliptica</i>	<i>H. elliptica</i> (formerly named <i>H. leachi</i>)	1476	1476	99	0	99.39	

*Accession numbers of gene sequences of BLASTn searches representing all the sequences generated in this study.

Table 4-8: (continue)

Sample ID	Gene size (bp)	Morphological identification	BLASTn match	Maximum score	Total score	Query cover (%)	E-value	Identity (%)	Accession number*
R2.2	816	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1441-1465	1441-1465	98	0	99.01-99.50	KF958435.1, KF958434.1,
R2.3	816	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1447-1471	1447-1471	99	0	99.26-99.89	KF958433.1, KF958432.1,
R2.4	819	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1441-1463	1441-1463	99	0	98.65-99.14	KF958431.1, KF958430.1,
R2.5	814	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1456-1480	1456-1480	100	0	99.02-99.51	KF958429.1, JX987497.1,
R2.7	814	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1465-1489	1465-1489	99	0	99.26-99.75	XR_005180903.1,
R2.11	811	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1447-1469	1447-1469	99	0	98.89-99.38	JX987496.1, L76342.1
R2.12	815	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1441-1465	1441-1465	99	0	98.77-99.26	
R2.13	826	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1424-1447	1424-1447	97	0	98.75-99.25	
R2.14	817	<i>R. sanguineus</i>	<i>R. sanguineus</i>	1434-1458	1434-1458	99	0	98.53-98.90	

*Accession numbers of gene sequences of BLASTn searches representing all the sequences generated in this study.

Phylogenetic analysis was conducted using sequences targeting the *18S rRNA* gene of *R. sanguineus* and *H. elliptica* from this study, as well as reference sequences from *R. sanguineus* and *H. elliptica* obtained from the NCBI database, with *Gehypochthonius urticinus* used as an outgroup, as illustrated in Figure 4.13. The bootstrap support values of all the clades were greater than 50%. On the phylogenetic tree, there are two major clades. In the first major clade, the sequences of *H. elliptica* ticks collected from dogs in this study clustered with sequences of other *H. elliptica* ticks from the NCBI database, with a bootstrap support value of 82%. In the second major clade, the sequences of *R. sanguineus* ticks collected from dogs in this study clustered with sequences of other *R. sanguineus* ticks from the NCBI database, with a bootstrap support value of 52%.

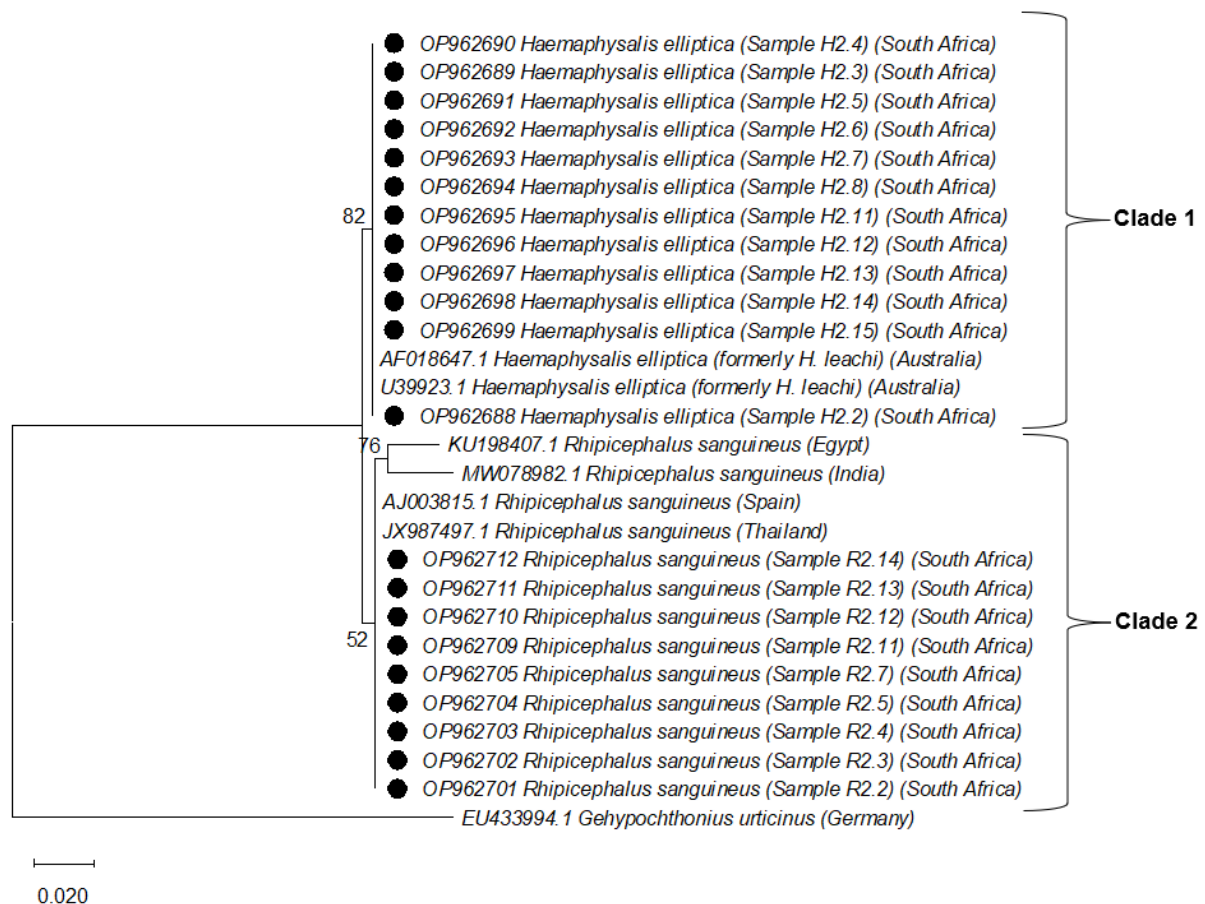


Figure 4-13: The tick 18S rRNA gene phylogenetic analysis. The phylogenetic tree was generated using the Maximum Likelihood (ML) method and the Kimura 2-parameter model (Kimura, 1980). Bootstrap percentage (based on 10000 replicates) is displayed next to the nodes branches of associated clustered taxa. The data analysis was conducted using 28 nucleotide sequence samples. Nucleotide positions filled with missing data and gaps were removed during analysis. The sequences contain the GenBank accession number, species identification, and country of sample collection. Sequences of specimens collected during this study are indicated by using a black dot and additionally the assigned sample ID. The horizontal scale bar represents the number of base substitutions per site. Phylogenetic trees were obtained by using MEGA 11 software (Tamura *et al.*, 2021)

There were 841 nucleotide bases or positions left of each gene sequence after sequence alignment and trimming. The aforementioned nucleotide bases or positions were used for phylogenetic analysis. Table 4.9 illustrates the base substitution numbers for each site of the sequences used for phylogenetic analysis. The sequence divergence of *H. elliptica* of this study, as well as sequences from GenBank ranged between 0 to 0.012. The sequence divergence of *R. sanguineus* of this study, as well as *R. sanguineus* sequences from GenBank, ranged between 0 to 0.041. The pairwise distance (p-distance) matrix is illustrated in Table 4.10. The p-distance matrix demonstrated strong resolution power, ranging between 0 to 0.227%, between the sequences of *R. sanguineus* and *H. elliptica* used for phylogenetic analysis. Figure 4.14 illustrates the differences among the nucleotides that were used during the phylogenetic analysis.

Table 4-9: Base substitutions number of each site using the *18S rRNA* gene

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
[1] AJ003815.1_Rhipicephalus_sanguineus_(Spain)		0.000	0.007	0.004	0.016	0.007	0.004	0.003	0.005	0.002	0.003	0.002	0.004	0.002	0.002	0.002	0.005	0.005	0.006	0.005	0.005	0.004	0.005	0.005	0.005	0.005	0.005	0.005
[2] JX987497.1_Rhipicephalus_sanguineus_(Thailand)	0.000		0.007	0.004	0.016	0.007	0.004	0.003	0.005	0.002	0.003	0.002	0.004	0.002	0.002	0.002	0.005	0.005	0.006	0.005	0.005	0.004	0.005	0.005	0.005	0.005	0.005	0.005
[3] KU198407.1_Rhipicephalus_sanguineus_(Egypt)	0.040	0.040		0.004	0.019	0.013	0.009	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009
[4] MW078982.1_Rhipicephalus_sanguineus_(India)	0.009	0.009	0.012		0.018	0.011	0.006	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006
[5] EU433994.1_Gehypochthonius_urticinus_(Germany)	0.166	0.166	0.217	0.176		0.040	0.016	0.017	0.017	0.016	0.016	0.016	0.017	0.016	0.016	0.016	0.016	0.017	0.017	0.017	0.017	0.016	0.016	0.016	0.016	0.017	0.016	0.016
[6] U39923.1_Haemaphysalis_elliptica_(formerly_H_leachi)_(Australia)	0.011	0.011	0.039	0.032	0.278		0.004	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
[7] AF018647.1_Haemaphysalis_elliptica_(formerly_H_leachi)_(Australia)	0.014	0.014	0.054	0.022	0.170	0.004		0.005	0.006	0.005	0.005	0.005	0.006	0.005	0.005	0.005	0.002	0.003	0.004	0.003	0.003	0.001	0.002	0.002	0.002	0.003	0.002	0.002
[8] OP962712_Rhipicephalus_sanguineus_(Sample_R2.14)_(South_Africa)	0.009	0.009	0.040	0.009	0.178	0.011	0.023		0.005	0.003	0.003	0.004	0.004	0.002	0.002	0.002	0.005	0.005	0.006	0.005	0.005	0.005	0.005	0.005	0.006	0.005	0.005	0.005
[9] OP962711_Rhipicephalus_sanguineus_(Sample_R2.13)_(South_Africa)	0.018	0.018	0.041	0.009	0.188	0.011	0.032	0.016		0.005	0.005	0.005	0.004	0.005	0.005	0.005	0.007	0.006	0.007	0.007	0.007	0.006	0.006	0.007	0.007	0.007	0.006	0.007
[10] OP962710_Rhipicephalus_sanguineus_(Sample_R2.12)_(South_Africa)	0.005	0.005	0.040	0.009	0.173	0.011	0.019	0.007	0.020		0.002	0.003	0.004	0.002	0.002	0.002	0.005	0.005	0.005	0.005	0.005	0.006	0.005	0.005	0.005	0.005	0.005	0.005
[11] OP962709_Rhipicephalus_sanguineus_(Sample_R2.11)_(South_Africa)	0.006	0.006	0.040	0.009	0.174	0.011	0.020	0.006	0.019	0.002		0.003	0.004	0.002	0.002	0.002	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
[12] OP962705_Rhipicephalus_sanguineus_(Sample_R2.7)_(South_Africa)	0.002	0.002	0.040	0.009	0.169	0.011	0.016	0.014	0.023	0.007	0.009		0.004	0.004	0.003	0.003	0.005	0.005	0.005	0.005	0.006	0.005	0.005	0.005	0.005	0.005	0.005	0.005
[13] OP962704_Rhipicephalus_sanguineus_(Sample_R2.5)_(South_Africa)	0.010	0.010	0.040	0.009	0.179	0.011	0.024	0.011	0.014	0.010	0.011	0.015		0.004	0.004	0.003	0.006	0.006	0.006	0.006	0.006	0.005	0.006	0.006	0.006	0.006	0.006	0.006
[14] OP962703_Rhipicephalus_sanguineus_(Sample_R2.4)_(South_Africa)	0.005	0.005	0.040	0.009	0.172	0.011	0.019	0.004	0.020	0.004	0.004	0.010	0.010		0.001	0.000	0.005	0.005	0.005	0.005	0.005	0.005	0.004	0.005	0.005	0.005	0.004	0.004
[15] OP962702_Rhipicephalus_sanguineus_(Sample_R2.3)_(South_Africa)	0.002	0.002	0.040	0.009	0.170	0.011	0.016	0.005	0.020	0.002	0.002	0.007	0.010	0.001		0.001	0.005	0.005	0.005	0.005	0.005	0.004	0.004	0.004	0.005	0.005	0.004	0.005
[16] OP962701_Rhipicephalus_sanguineus_(Sample_R2.2)_(South_Africa)	0.004	0.004	0.040	0.009	0.171	0.011	0.018	0.004	0.019	0.004	0.004	0.009	0.009	0.000	0.001		0.005	0.005	0.005	0.005	0.005	0.005	0.004	0.005	0.005	0.005	0.004	0.004
[17] OP962699_Haemaphysalis_elliptica_(Sample_H2.15)_(South_Africa)	0.019	0.019	0.054	0.022	0.177	0.004	0.005	0.020	0.037	0.019	0.015	0.023	0.028	0.017	0.016	0.017		0.002	0.003	0.002	0.003	0.002	0.002	0.002	0.003	0.002	0.002	0.002
[18] OP962698_Haemaphysalis_elliptica_(Sample_H2.14)_(South_Africa)	0.020	0.020	0.054	0.022	0.178	0.004	0.006	0.020	0.033	0.018	0.016	0.023	0.024	0.016	0.018	0.016	0.005		0.003	0.001	0.003	0.003	0.002	0.002	0.003	0.000	0.002	0.001
[19] OP962697_Haemaphysalis_elliptica_(Sample_H2.13)_(South_Africa)	0.024	0.024	0.054	0.022	0.183	0.004	0.010	0.024	0.035	0.023	0.021	0.024	0.026	0.021	0.023	0.021	0.007	0.006		0.003	0.002	0.004	0.003	0.003	0.004	0.002	0.003	0.002
[20] OP962696_Haemaphysalis_elliptica_(Sample_H2.12)_(South_Africa)	0.020	0.020	0.055	0.022	0.178	0.004	0.006	0.021	0.034	0.017	0.016	0.024	0.025	0.016	0.017	0.016	0.004	0.001	0.007		0.003	0.002	0.001	0.002	0.003	0.001	0.001	0.002
[21] OP962695_Haemaphysalis_elliptica_(Sample_H2.11)_(South_Africa)	0.023	0.023	0.054	0.022	0.181	0.004	0.009	0.023	0.037	0.025	0.021	0.025	0.026	0.021	0.023	0.021	0.007	0.006	0.004	0.007		0.003	0.003	0.003	0.004	0.003	0.003	0.002
[22] OP962694_Haemaphysalis_elliptica_(Sample_H2.8)_(South_Africa)	0.015	0.015	0.054	0.022	0.172	0.004	0.001	0.021	0.033	0.018	0.019	0.019	0.023	0.017	0.015	0.016	0.004	0.006	0.010	0.005	0.009		0.002	0.002	0.002	0.003	0.002	0.002
[23] OP962693_Haemaphysalis_elliptica_(Sample_H2.7)_(South_Africa)	0.019	0.019	0.054	0.022	0.176	0.004	0.005	0.018	0.033	0.018	0.018	0.023	0.024	0.014	0.015	0.014	0.002	0.002	0.006	0.001	0.006	0.004		0.002	0.003	0.002	0.000	0.001
[24] OP962692_Haemaphysalis_elliptica_(Sample_H2.6)_(South_Africa)	0.019	0.019	0.054	0.022	0.176	0.004	0.005	0.020	0.035	0.018	0.018	0.023	0.026	0.016	0.015	0.016	0.002	0.005	0.009	0.004	0.009	0.004	0.002		0.003	0.002	0.002	0.002
[25] OP962691_Haemaphysalis_elliptica_(Sample_H2.5)_(South_Africa)	0.016	0.016	0.054	0.022	0.173	0.004	0.002	0.026	0.034	0.023	0.023	0.019	0.028	0.023	0.020	0.021	0.007	0.009	0.012	0.009	0.011	0.004	0.007	0.007		0.003	0.003	0.003
[26] OP962690_Haemaphysalis_elliptica_(Sample_H2.4)_(South_Africa)	0.020	0.020	0.054	0.022	0.179	0.004	0.006	0.021	0.034	0.018	0.016	0.021	0.025	0.018	0.019	0.018	0.005	0.000	0.005	0.001	0.006	0.006	0.002	0.005	0.009		0.002	0.001
[27] OP962689_Haemaphysalis_elliptica_(Sample_H2.3)_(South_Africa)	0.019	0.019	0.054	0.022	0.176	0.004	0.005	0.018	0.033	0.018	0.018	0.023	0.024	0.014	0.015	0.014	0.002	0.002	0.006	0.001	0.006	0.004	0.000	0.002	0.007	0.002		0.001
[28] OP962688_Haemaphysalis_elliptica_(Sample_H2.2)_(South_Africa)	0.019	0.019	0.054	0.022	0.176	0.004	0.005	0.019	0.034	0.019	0.018	0.021	0.025	0.015	0.016	0.015	0.004	0.001	0.005	0.002	0.005	0.005	0.001	0.004	0.007	0.001	0.001	

*Numbers in black below the diagonal line represent the base substitution number of each site between sequences and the numbers in blue above the diagonal line represent the standard errors.

Table 4-10: Pairwise distance (p-distance) analysis of sequences using the *18S rRNA* gene

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
[1] AJ003815.1_Rhipicephalus_sanguineus_(Spain)		0.000	0.007	0.003	0.013	0.006	0.004	0.003	0.005	0.002	0.003	0.002	0.003	0.002	0.002	0.002	0.005	0.005	0.005	0.005	0.005	0.004	0.005	0.005	0.004	0.005	0.005	0.005
[2] JX987497.1_Rhipicephalus_sanguineus_(Thailand)	0.000		0.007	0.003	0.013	0.006	0.004	0.003	0.005	0.002	0.003	0.002	0.003	0.002	0.002	0.002	0.005	0.005	0.005	0.005	0.005	0.004	0.005	0.005	0.004	0.005	0.005	0.005
[3] KU198407.1_Rhipicephalus_sanguineus_(Egypt)	0.039	0.039		0.004	0.014	0.012	0.008	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
[4] MW078982.1_Rhipicephalus_sanguineus_(India)	0.009	0.009	0.011		0.014	0.011	0.005	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
[5] EU433994.1_Gehyochthonius_urticinus_(Germany)	0.148	0.148	0.188	0.156		0.026	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013
[6] U39923.1_Haemaphysalis_elliptica_(formerly_H_leachi)_(Australia)	0.011	0.011	0.038	0.031	0.227		0.004	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
[7] AF018647.1_Haemaphysalis_elliptica_(formerly_H_leachi)_(Australia)	0.014	0.014	0.052	0.021	0.151	0.004		0.005	0.006	0.005	0.005	0.004	0.005	0.005	0.004	0.005	0.002	0.003	0.003	0.003	0.003	0.001	0.002	0.002	0.002	0.003	0.002	0.002
[8] OP962712_Rhipicephalus_sanguineus_(Sample_R2.14)_(South_Africa)	0.009	0.009	0.039	0.009	0.157	0.011	0.022		0.004	0.003	0.003	0.004	0.004	0.002	0.002	0.002	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.005	0.005	0.005
[9] OP962711_Rhipicephalus_sanguineus_(Sample_R2.13)_(South_Africa)	0.017	0.017	0.040	0.009	0.165	0.011	0.031	0.016		0.005	0.005	0.005	0.004	0.005	0.005	0.005	0.007	0.006	0.006	0.006	0.007	0.006	0.006	0.006	0.006	0.006	0.006	0.006
[10] OP962710_Rhipicephalus_sanguineus_(Sample_R2.12)_(South_Africa)	0.005	0.005	0.039	0.009	0.153	0.011	0.019	0.007	0.020		0.002	0.003	0.003	0.002	0.002	0.002	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
[11] OP962709_Rhipicephalus_sanguineus_(Sample_R2.11)_(South_Africa)	0.006	0.006	0.039	0.009	0.154	0.011	0.020	0.006	0.019	0.002		0.003	0.004	0.002	0.002	0.002	0.004	0.004	0.005	0.004	0.005	0.005	0.005	0.005	0.005	0.004	0.005	0.005
[12] OP962705_Rhipicephalus_sanguineus_(Sample_R2.7)_(South_Africa)	0.002	0.002	0.039	0.009	0.150	0.011	0.016	0.014	0.022	0.007	0.009		0.004	0.003	0.003	0.003	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
[13] OP962704_Rhipicephalus_sanguineus_(Sample_R2.5)_(South_Africa)	0.010	0.010	0.039	0.009	0.158	0.011	0.024	0.011	0.014	0.010	0.011	0.015		0.003	0.003	0.003	0.006	0.005	0.006	0.005	0.006	0.005	0.005	0.006	0.006	0.005	0.005	0.005
[14] OP962703_Rhipicephalus_sanguineus_(Sample_R2.4)_(South_Africa)	0.005	0.005	0.039	0.009	0.153	0.011	0.019	0.004	0.020	0.004	0.004	0.010	0.010		0.001	0.000	0.005	0.004	0.005	0.004	0.005	0.005	0.004	0.004	0.005	0.005	0.004	0.004
[15] OP962702_Rhipicephalus_sanguineus_(Sample_R2.3)_(South_Africa)	0.002	0.002	0.039	0.009	0.151	0.011	0.016	0.005	0.020	0.002	0.002	0.007	0.010	0.001		0.001	0.004	0.005	0.005	0.005	0.005	0.004	0.004	0.004	0.005	0.005	0.004	0.004
[16] OP962701_Rhipicephalus_sanguineus_(Sample_R2.2)_(South_Africa)	0.004	0.004	0.039	0.009	0.152	0.011	0.017	0.004	0.018	0.004	0.004	0.009	0.009	0.000	0.001		0.005	0.004	0.005	0.004	0.005	0.004	0.004	0.004	0.005	0.005	0.004	0.004
[17] OP962699_Haemaphysalis_elliptica_(Sample_H2.15)_(South_Africa)	0.019	0.019	0.052	0.021	0.156	0.004	0.005	0.020	0.036	0.019	0.015	0.022	0.027	0.017	0.016	0.017		0.002	0.003	0.002	0.003	0.002	0.002	0.002	0.003	0.002	0.002	0.002
[18] OP962698_Haemaphysalis_elliptica_(Sample_H2.14)_(South_Africa)	0.020	0.020	0.052	0.021	0.157	0.004	0.006	0.020	0.032	0.017	0.016	0.022	0.023	0.016	0.017	0.016	0.005		0.003	0.001	0.003	0.003	0.002	0.002	0.003	0.000	0.002	0.001
[19] OP962697_Haemaphysalis_elliptica_(Sample_H2.13)_(South_Africa)	0.024	0.024	0.052	0.021	0.161	0.004	0.010	0.024	0.035	0.022	0.021	0.023	0.026	0.021	0.022	0.021	0.007	0.006		0.003	0.002	0.003	0.003	0.003	0.004	0.002	0.003	0.002
[20] OP962696_Haemaphysalis_elliptica_(Sample_H2.12)_(South_Africa)	0.020	0.020	0.053	0.021	0.157	0.004	0.006	0.021	0.033	0.017	0.016	0.023	0.025	0.016	0.017	0.016	0.004	0.001	0.007		0.003	0.002	0.001	0.002	0.003	0.001	0.001	0.002
[21] OP962695_Haemaphysalis_elliptica_(Sample_H2.11)_(South_Africa)	0.022	0.022	0.052	0.021	0.160	0.004	0.009	0.022	0.036	0.025	0.021	0.025	0.026	0.021	0.022	0.021	0.007	0.006	0.004	0.007		0.003	0.003	0.003	0.004	0.003	0.003	0.002
[22] OP962694_Haemaphysalis_elliptica_(Sample_H2.8)_(South_Africa)	0.015	0.015	0.052	0.021	0.152	0.004	0.001	0.021	0.032	0.017	0.019	0.019	0.022	0.017	0.015	0.016	0.004	0.006	0.010	0.005	0.009		0.002	0.002	0.002	0.003	0.002	0.002
[23] OP962693_Haemaphysalis_elliptica_(Sample_H2.7)_(South_Africa)	0.019	0.019	0.052	0.021	0.156	0.004	0.005	0.017	0.032	0.017	0.017	0.022	0.023	0.014	0.015	0.014	0.002	0.002	0.006	0.001	0.006	0.004		0.002	0.003	0.002	0.000	0.001
[24] OP962692_Haemaphysalis_elliptica_(Sample_H2.6)_(South_Africa)	0.019	0.019	0.052	0.021	0.156	0.004	0.005	0.020	0.035	0.017	0.017	0.022	0.026	0.016	0.015	0.016	0.002	0.005	0.009	0.004	0.009	0.004	0.002		0.003	0.002	0.002	0.002
[25] OP962691_Haemaphysalis_elliptica_(Sample_H2.5)_(South_Africa)	0.016	0.016	0.052	0.021	0.154	0.004	0.002	0.026	0.033	0.022	0.022	0.019	0.027	0.022	0.020	0.021	0.007	0.009	0.012	0.009	0.011	0.004	0.007	0.007		0.003	0.003	0.003
[26] OP962690_Haemaphysalis_elliptica_(Sample_H2.4)_(South_Africa)	0.020	0.020	0.052	0.021	0.158	0.004	0.006	0.021	0.033	0.017	0.016	0.021	0.025	0.017	0.019	0.017	0.005	0.000	0.005	0.001	0.006	0.006	0.002	0.005	0.009		0.002	0.001
[27] OP962689_Haemaphysalis_elliptica_(Sample_H2.3)_(South_Africa)	0.019	0.019	0.052	0.021	0.156	0.004	0.005	0.017	0.032	0.017	0.017	0.022	0.023	0.014	0.015	0.014	0.002	0.002	0.006	0.001	0.006	0.004	0.000	0.002	0.007	0.002		0.001
[28] OP962688_Haemaphysalis_elliptica_(Sample_H2.2)_(South_Africa)	0.019	0.019	0.052	0.021	0.156	0.004	0.005	0.019	0.033	0.019	0.017	0.021	0.025	0.015	0.016	0.015	0.004	0.001	0.005	0.002	0.005	0.005	0.001	0.004	0.007	0.001	0.001	

*Numbers in black below the diagonal line represent the p-distance and the numbers in blue above the diagonal line represent the standard errors.

Nucleotide position					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
OP962712	Rhipicephalus	sanguineus	(Sample R2.14)	(South Africa)	T	C	A	T	T	T	A	A	A	T	T	A	C	A	T	T	A	T	G	G
OP962711	Rhipicephalus	sanguineus	(Sample R2.13)	(South Africa)	C	.	T	C	A	.	.	.	.	A	.	C	A	G	.	.	.	.	.	.
OP962710	Rhipicephalus	sanguineus	(Sample R2.12)	(South Africa)	.	.	.	.	.	A	.	.	.	C	C	.	.	.	.	.	.	.	.	.
OP962709	Rhipicephalus	sanguineus	(Sample R2.11)	(South Africa)	.	.	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	.
OP962705	Rhipicephalus	sanguineus	(Sample R2.7)	(South Africa)	.	.	.	.	.	A	.	.	T	.	C	C	A	G	.	.	.	.	.	.
OP962704	Rhipicephalus	sanguineus	(Sample R2.5)	(South Africa)	C	T	C	A	.	.	.	.	.	.	.	C	A	G	.	.	.	.	.	.
OP962703	Rhipicephalus	sanguineus	(Sample R2.4)	(South Africa)	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.
OP962702	Rhipicephalus	sanguineus	(Sample R2.3)	(South Africa)	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.
OP962701	Rhipicephalus	sanguineus	(Sample R2.2)	(South Africa)	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.
OP962699	Haemaphysalis	elliptica	(Sample H2.15)	(South Africa)	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.
OP962698	Haemaphysalis	elliptica	(Sample H2.14)	(South Africa)	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.
OP962697	Haemaphysalis	elliptica	(Sample H2.13)	(South Africa)	C	A	T	.	.	A	.	.	T	.	C	.	.	.	.	.	.	.	.	.
OP962696	Haemaphysalis	elliptica	(Sample H2.12)	(South Africa)	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.
OP962695	Haemaphysalis	elliptica	(Sample H2.11)	(South Africa)	C	A	.	.	.	A	.	.	T	.	.	.	T	.	.	.	.	.	.	.
OP962694	Haemaphysalis	elliptica	(Sample H2.8)	(South Africa)	.	.	.	.	.	A	.	.	.	.	.	C	A	G	.	.	.	.	.	.
OP962693	Haemaphysalis	elliptica	(Sample H2.7)	(South Africa)	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.
OP962692	Haemaphysalis	elliptica	(Sample H2.6)	(South Africa)	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.
OP962691	Haemaphysalis	elliptica	(Sample H2.5)	(South Africa)	.	.	.	.	C	A	.	.	.	.	.	C	A	G	A	.	.	.	.	.
OP962690	Haemaphysalis	elliptica	(Sample H2.4)	(South Africa)	.	.	.	.	.	A	.	.	T	.	.	C	.	.	.	.	.	.	.	.
OP962689	Haemaphysalis	elliptica	(Sample H2.3)	(South Africa)	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.
OP962688	Haemaphysalis	elliptica	(Sample H2.2)	(South Africa)	.	.	.	.	.	A	.	.	T	.	.	C	.	.	.	.	.	.	.	.
U39923.1	Haemaphysalis	elliptica	(formerly H. leachi)	(Australia)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MW078982.1	Rhipicephalus	sanguineus	(India)		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KU198407.1	Rhipicephalus	sanguineus	(Egypt)		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
JX987497.1	Rhipicephalus	sanguineus	(Thailand)		.	.	.	.	.	A	.	.	.	.	.	C	A	G	.	.	.	.	.	.
AJ003815.1	Rhipicephalus	sanguineus	(Spain)		.	.	.	.	.	A	.	.	.	.	.	C	A	G	.	.	.	.	.	.
AF018647.1	Haemaphysalis	elliptica	(formerly H. leachi)	(Australia)	.	.	.	.	.	A	.	.	.	.	.	C	A	G	.	.	.	.	.	.
EU433994.1	Gehyochthonius	urticinus	(Germany)		.	.	.	.	.	A	.	.	.	.	.	C	A	G	.	.	.	C	.	.

Nucleotide position					21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
OP962712	Rhipicephalus	sanguineus	(Sample R2.14)	(South Africa)	T	T	C	C	T	T	A	G	A	T	C	G	T	T	T	C	T	T	C	C
OP962711	Rhipicephalus	sanguineus	(Sample R2.13)	(South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OP962710	Rhipicephalus	sanguineus	(Sample R2.12)	(South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OP962709	Rhipicephalus	sanguineus	(Sample R2.11)	(South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OP962705	Rhipicephalus	sanguineus	(Sample R2.7)	(South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OP962704	Rhipicephalus	sanguineus	(Sample R2.5)	(South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OP962703	Rhipicephalus	sanguineus	(Sample R2.4)	(South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OP962702	Rhipicephalus	sanguineus	(Sample R2.3)	(South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OP962701	Rhipicephalus	sanguineus	(Sample R2.2)	(South Africa)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OP962699	Haemaphysalis	elliptica	(Sample H2.15)	(South Africa)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
OP962698	Haemaphysalis	elliptica	(Sample H2.14)	(South Africa)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
OP962697	Haemaphysalis	elliptica	(Sample H2.13)	(South Africa)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
OP962696	Haemaphysalis	elliptica	(Sample H2.12)	(South Africa)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
OP962695	Haemaphysalis	elliptica	(Sample H2.11)	(South Africa)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
OP962694	Haemaphysalis	elliptica	(Sample H2.8)	(South Africa)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
OP962693	Haemaphysalis	elliptica	(Sample H2.7)	(South Africa)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
OP962692	Haemaphysalis	elliptica	(Sample H2.6)	(South Africa)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
OP962691	Haemaphysalis	elliptica	(Sample H2.5)	(South Africa)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
OP962690	Haemaphysalis	elliptica	(Sample H2.4)	(South Africa)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
OP962689	Haemaphysalis	elliptica	(Sample H2.3)	(South Africa)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
OP962688	Haemaphysalis	elliptica	(Sample H2.2)	(South Africa)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
U39923.1	Haemaphysalis	elliptica	(formerly H. leachi)	(Australia)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MW078982.1	Rhipicephalus	sanguineus	(India)		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KU198407.1	Rhipicephalus	sanguineus	(Egypt)		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
JX987497.1	Rhipicephalus	sanguineus	(Thailand)		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AJ003815.1	Rhipicephalus	sanguineus	(Spain)		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AF018647.1	Haemaphysalis	elliptica	(formerly H. leachi)	(Australia)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
EU433994.1	Gehyochthonius	urticinus	(Germany)		.	.	.	.	.	.	.	.	.	.	G	T	.	G	A	.	.	G	T	.

Figure 4-14: Nucleotide differences within the alignment sequences, from nucleotide position 1 to 40, used for phylogenetic analysis of the 18S *rRNA* gene. The full stops (.) represent nucleotides identical to the top nucleotide sequence and hyphens (-) represent an alignment gap

Table 4.11 illustrates the base substitution rates of nucleotide pairs used during phylogenetic analysis. With the removal of missing data and gaps, the average nucleotide frequencies were 24.6% for adenine, 27.2% for thymine, 21.5% for cytosine, and 26.7% for guanine.

Table 4-11: Nucleotide pairs of *18S rRNA* gene rates of substitution

From\To	A	T	C	G
A	-	0.044552743	0.044552743	0.160894514
T	0.044552743	-	0.160894514	0.044552743
C	0.044552743	0.160894514	-	0.044552743
G	0.160894514	0.044552743	0.044552743	-

A: adenine; T: thymine; C: cytosine; G: Guanine

4.3.4 Screening of tick-borne pathogens

In addition to the identification of ticks collected during this study, PCR assays were done to determine the presence of *A. phagocytophylum* (by amplification of the *16S rRNA* gene) *E. canis* (by amplification of the *16S rRNA* gene), *C. burnetti* (by amplification of the *SOD1* gene), as well as *Rickettsia* spp. (by amplification of the *gltA* gene). PCR assays were performed on tick pools (n=81) and egg batches (n=6). Additionally, blood samples (n=50) of the dog hosts were screened for the abovementioned pathogens.

There were no *E. canis* or *A. phagocytophylum* present in the tick pools, egg batches, or dog blood samples from this study. Several tick specimens harboured *C. burnetti* or *Rickettsia* spp., as illustrated in Table 4.12. There were also *C. burnetii* or *Rickettsia* spp. present in the tick egg batches, as illustrated in Table 4.13. In addition, one *R. sanguineus* tick pool (adult pool originating from Miederpark) was co-infected with both *C. burnetii* (Sample ID: C8) and *Rickettsia* spp. (Sample ID: R6), as illustrated in Table 4.12.

Table 4-12: Overall occurrence of tick-borne pathogens harboured by *R. sanguineus* and *H. elliptica* tick specimens

Location of collection	Tick species	Life stage	Tick-borne pathogens				Total pools screened
			<i>A. phagocytophylum</i>	<i>E. canis</i>	<i>C. burnetii</i>	<i>Rickettsia</i> spp.	
Baillie park	<i>H. elliptica</i>	Nymphs	0	0	0	0	2
	<i>R. sanguineus</i>	Nymphs	0	0	0	0	1
		Adults	0	0	0	0	1
Dassierand	<i>H. elliptica</i>	Nymphs	0	0	0	1 (Sample R4)	3
		Adults	0	0	0	0	2
	<i>R. sanguineus</i>	Nymphs	0	0	0	0	1
		Adults	0	0	0	0	1
Ikageng	<i>R. sanguineus</i>	Nymphs	0	0	0	0	2
		Adults	0	0	0	0	1
Miederpark	<i>H. elliptica</i>	Nymphs	0	0	1 (Sample C3)	1 (Sample R3)	10
		Adults	0	0	2 (Sample C2 and C4)	1 (Sample R1)	13
	<i>R. sanguineus</i>	Nymphs	0	0	2 (Sample C7 and C9)	0	19
		Adults	0	0	3 (Sample C5, C6 and C8*)	1 (Sample R6*)	19
Promosa	<i>H. elliptica</i>	Nymphs	0	0	0	0	1
Turfvlei	<i>H. elliptica</i>	Nymphs	0	0	0	0	1
		Adults	0	0	0	0	1
Van der Hoff Park	<i>R. sanguineus</i>	Nymphs	0	0	0	0	1
		Adults	0	0	0	0	1
Vyfhoek	<i>H. elliptica</i>	Nymphs	0	0	0	1 (Sample R5)	1
Total			0	0	8	5	81
Overall occurrence			0	0	9.88%	6.17%	

*Tick pool co-infected with *C. burnetii* (Sample C8) and *Rickettsia* spp. (Sample R6)

Table 4-13: Overall occurrence of tick-borne pathogens present in *R. sanguineus* and *H. elliptica* tick eggs

Location of collection	Tick species	Life stage	Tick-borne pathogens				Total egg batches screened
			<i>A. phagocytophylum</i>	<i>E. canis</i>	<i>C. burnetii</i>	<i>Rickettsia</i> spp.	
Ikageng	<i>R. sanguineus</i>	Eggs	0	0	0	0	1
Miederpark	<i>H. elliptica</i>	Eggs	0	0	1 (Sample C1)	1 (Sample R2)	4
Van der Hoff Park	<i>R. sanguineus</i>	Eggs	0	0	0	0	1
Total			0	0	1	1	6
Overall occurrence			0	0	16.67%	16.67%	

4.3.4.1 *Coxiella burnetii*

The presence of *C. burnetii* within tick pools, egg batches, and dog blood was screened by PCR amplification of the *SOD1* gene, where the predicted PCR product size was 257 bp (Figure 4.15). The overall occurrence of *C. burnetii* within tick pools was 9.88% (8/81), as illustrated in Table 4.12. There was a significant statistical difference ($X^2 = 2.8596$, $df = 7$, $p\text{-value} = 0.8977$) in *C. burnetii* prevalence within the tick pools based on geographical location. The *C. burnetii* were harboured by 8.82% [(3/34) 1 nymphal and 2 adult tick pools] of *H. elliptica* ticks and 10.64% [(5/47) 2 nymphal and 3 adult pools] of *R. sanguineus* ticks. All ticks harbouring *C. burnetii* originated from Miederpark (from the PAWS kennels). The overall occurrence of *C. burnetii* within egg batches was 16.67% (1/6), as illustrated in Table 4.13. There was a significant statistical difference ($X^2 = 0.6$, $df = 2$, $p\text{-value} = 0.7408$) in *C. burnetii* prevalence within the egg batches based on geographical location. *Coxiella burnetii* was present in 25% (1/4) *H. elliptica* egg batches of ticks originating from Miederpark (from the PAWS kennels). Furthermore no *C. burnetii* DNA was found in *R. sanguineus* egg batches or dog blood.

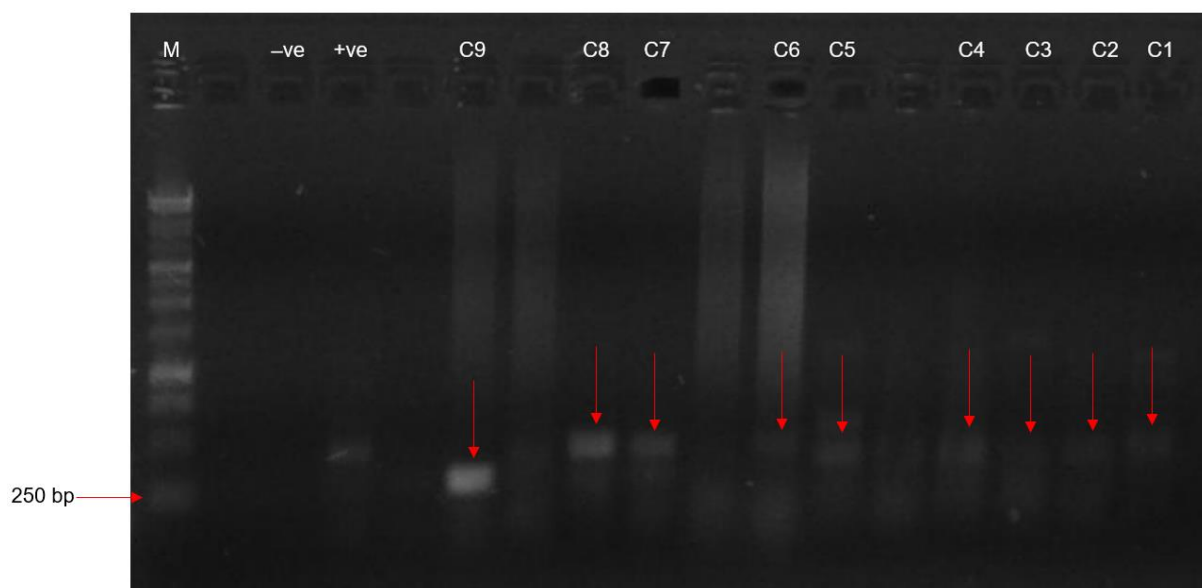


Figure 4-15: Agarose gel electrophoresis (1% w/v) of amplified DNA products of *C. burnetii*, obtained by conventional PCR targeting the *SOD1* gene, with an estimated 257 bp product size. In this electrophoresis gel, lane 1 (marked as **M**) contained the 1 kb molecular DNA marker. Lane 3 (marked as **-ve**) contained the no template control. Lane 4 (marked as **+ve**) contained the positive control. The remainder of the labelled lanes (marked as **C9** to **C1**) contained the amplified DNA products and the unlabelled lanes contained samples that were screened for *C. burnetii* DNA but it was not present. The vertical red arrows show the presence of DNA bands

One of the PCR products (Sample ID: C8) was successful after Sanger sequencing, where the sequence length was 227 bp. The remainder of the PCR products (Sample ID: C9, C7, C6, C5, C4, C3, C2, and C1) were not successful after Sanger sequencing. The sequence obtained by Sanger sequencing was submitted to GenBank on the NCBI database under the following accession number: OQ334971. The BLASTn search results revealed that the aforementioned sequence was 96.89 to 100% similar to *C. burnetii* sequences on the NCBI database (GenBank accession numbers GQ925827.1, CP040059.1, CP035112.1, CP032542.1, CP014563.1, CP014561.1, CP014559.1, CP014557.1, CP014555.1, CP014553.1, CP014551.1, CP014836.1, CP014549.1, CP014548.1, CP014565.1, CP018005.1, CP020616.1, CP018150.1, AE016828.3, CP014354.1, CP013667.1, CP107268.1, CP107247.1, CP103428.1, CP103429.1, CP103431.1, CP103435.1, CP103434.1, CP103433.1, CP103430.1, CP103425.1, CP103426.1, CP103427.1, CP103432.1, CP007555.1, LK937696.1, CP001020.1, CP001019.1, EU448143.1, CP000890.1, CP000733.1, X54627.1, M74242.1, GQ925826.1, HG825990.3, DQ379975.1). Additionally, the BLASTn search results revealed that the maximum and total scores ranged between 353 to 407, with query covers ranging between 85 to 98% and E-values ranging between 1^{-99} to 5^{-93} .

4.3.4.2 *Rickettsia* spp.

The presence of *Rickettsia* spp. within tick pools, egg batches, and dog blood was screened by PCR amplification of the *gltA* gene, where the predicted PCR product size was 381 bp (Figure 4.16). The overall occurrence of *Rickettsia* spp. within tick pools was 6.17% (5/81), as illustrated in Table 4.12. There was no significant statistical difference ($X^2 = 17.17$, $df = 7$, $p\text{-value} = 0.01633$) in *Rickettsia* spp. prevalence within the tick pools based on geographical location. *Rickettsia* spp. were harboured by 11.76% [(4/34) 3 nymphal and 1 adult pools] of *H. elliptica* ticks and 2.13% [(1/47) 1 adult pool] of *R. sanguineus* ticks. *Rickettsia* spp. harboured by *R. sanguineus* originated from Miederpark (from the PAWS kennels) [n=1 (adult pool)]. The overall occurrence of *Rickettsia* spp. within egg batches was 16.67% (1/6), as illustrated in Table 4.13. There was a significant statistical difference ($X^2 = 0.6$, $df = 2$, $p\text{-value} = 0.7408$) in *Rickettsia* spp. prevalence within the egg batches based on geographical location. *Rickettsia* spp. was present in 25% (1/4) *H. elliptica* egg batches of ticks originating from Miederpark (from the PAWS kennels). Furthermore, no *Rickettsia* spp. DNA was found in *R. sanguineus* egg batches or dog blood.

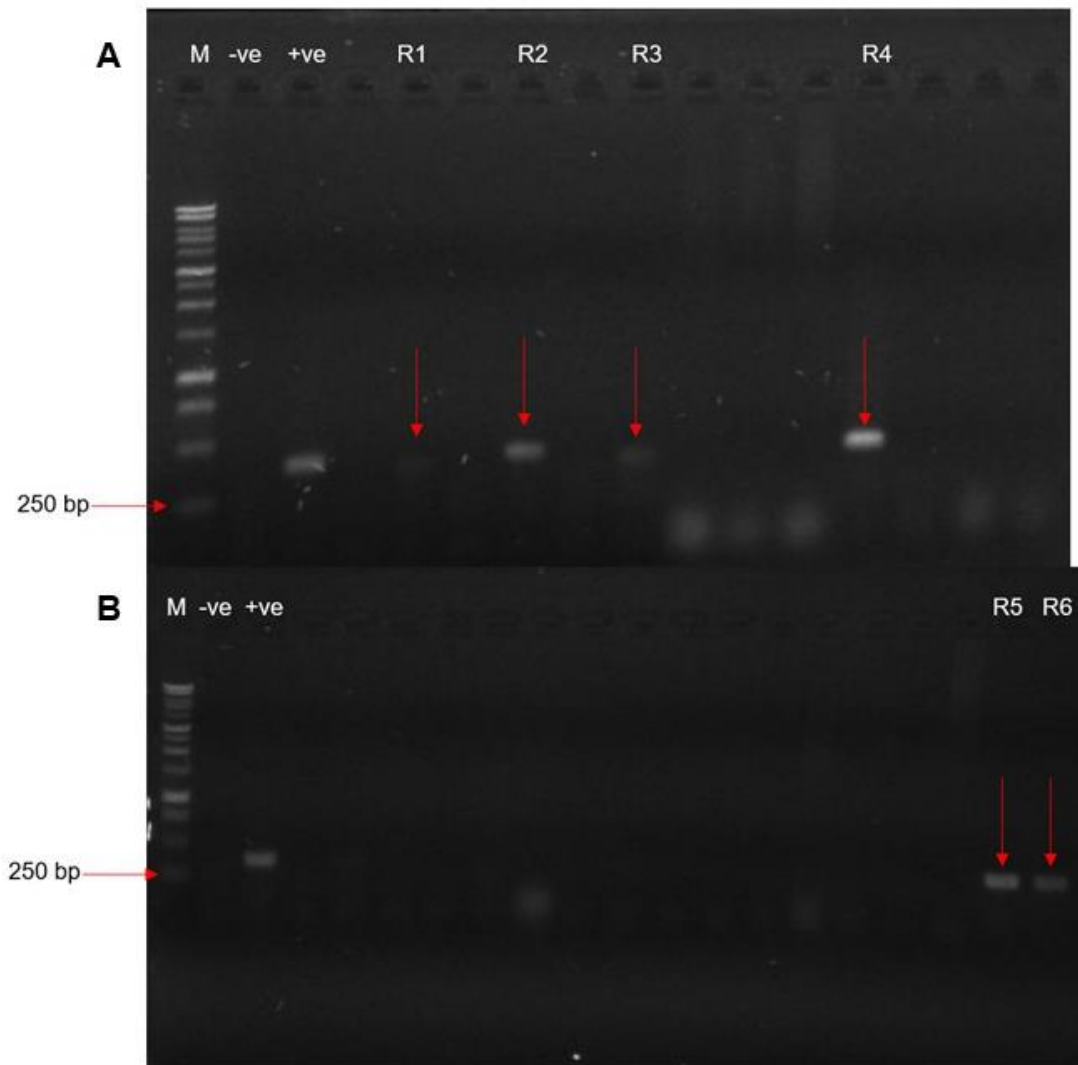


Figure 4-16: Agarose gel electrophoresis (1% w/v) of amplified DNA products of *Rickettsia* spp, obtained by conventional PCR targeting the *gltA* gene, with an estimated 381 bp product size. In electrophoresis gel 1 (marked as **A**), lane 1 (marked as **M**) contained the 1 kb molecular DNA marker. Lane 2 (marked as **-ve**) contained the no template control. Lane 3 (marked as **+ve**) contained the positive control. The remainder of the labelled lanes (marked as **R1** to **R4**) contained the amplified DNA products and the unlabelled lanes contain samples that were screened for *Rickettsia* spp. DNA but it was not present. Electrophoresis gel 2 (marked as **B**), lane 1 (marked as **M**) contained the 1kb molecular DNA marker. Lane 2 (marked as **-ve**) contained the no template control. Lane 3 (marked as **+ve**) contained the positive control. The remainder of the labelled lanes (marked as **R5** and **R6**) contained the amplified DNA products and the unlabelled lanes contained samples that were screened for *Rickettsia* spp. DNA but it was not present. On both electrophoresis gels the vertical red arrows show the presence of DNA bands

Four of the PCR products (Sample ID: R1, R2, R4, and R6) were successful after Sanger sequencing, where the sequence lengths ranged from 353 to 356 bp (Table 4.14). The sequences obtained by Sanger sequencing were submitted to GenBank on the NCBI database under the following accession numbers: OQ334972, OQ334973, OQ334974, and OQ334975. BLASTn search results revealed that two of the *Rickettsia* spp. (Sample IDs: R1 and R2) were 98.75 to 100% similar to *R. conorii* sequences on the NCBI database (GenBank accession numbers: MK295621.1, AB872795.1, DQ821855.1, KY640399.1, JN182786.1, EF177486.1, MZ351058.1, HM152564.1, MF002509.1, KY069261.1, U59730.1, MN550894.1, KU361213.1, KT261764.1, KT261763.1, KT261762.1, MT019635.1, MF166735.1, MN550895.1, AE006914.1, MF511253.1, MF511251.1, MF511249.1, MF511248.1, MF511246.1, and MF511245.1). Additionally, the BLASTn search results revealed that the maximum and total scores ranged between 610 to 636, with query covers ranging between 98.83 to 100% and E-values ranging between 1^{-171} to 8^{-178} . For the remainder of the two sequenced *Rickettsia* spp. (Sample IDs: R4 and R6) BLASTn search results revealed that they were 97.18 to 99.70% similar to *R. massiliae* sequences on the NCBI database (GenBank accession numbers: MZ420694.1, MZ420692.1, MT309037.1, KU498299.1, KT032119.1, ON109833.1, ON081515.1, ON001449.1, OM793610.1, OM777139.1, KF826286.1, U59719.1, MF002497.1, KY069259.1, MZ020770.1, MW802693.1, CP003319.1, CP000683.1, AB872797.1, AB872787.1, AB872463.1, KY640405.1, AB872810.1, KC428021.1, AB872803.1, AB872793.1, AB872789.1, MZ420698.1, MZ420696.1, and MZ420695.1). Additionally, the BLASTn search results revealed that the maximum and total scores ranged between 597 to 619, with query covers ranging between 94 to 99% and E-values ranging between 1^{-166} to 8^{-173} .

Table 4-14: *Rickettsia* spp. BLASTn results

Sample ID	Gene size (bp)	BLASTn match	Maximum score	Total score	Query cover (%)	E-value	Identity (%)	Accession number
R1	353	<i>Rickettsia conorii</i>	616-636	616-636	94-99	1 ⁻¹⁷¹ to 8 ⁻¹⁷⁸	99.15-100	MK295621.1, AB872795.1, DQ821855.1, KY640399.1, JN182786.1, EF177486.1, MZ351058.1, HM152564.1, MF002509.1, KY069261.1, U59730.1
		<i>Rickettsia conorii</i> subsp. <i>raoultii</i>	617-619	617-619	99	3 ⁻¹⁷² to 8 ⁻¹⁷³	98.57-98.58	MF166735.1, MN550895.1, MN550894.1, KU361213.1, KT261764.1, KT261763.1, KT261762.1, MT019635.1
		<i>Rickettsia conorii</i> str. Malish 7	630	630	98	4 ⁻¹⁷⁶	99.43	AE006914.1
R2	355	<i>Rickettsia conorii</i>	612-625	612-625	94-97	1 ⁻¹⁷⁰ to 6 ⁻¹⁷⁴	99.41-99.71	DQ821855.1, MK295621.1, JN182786.1, AB872795.1, MZ351058.1, MF002509.1, KY069261.1, KY640399.1, U59730.1, EF177486.1
		<i>Rickettsia conorii</i> subsp. <i>raoultii</i>	610-614	610-614	96-97	4 ⁻¹⁷¹ to 5 ⁻¹⁷⁰	98.83-98.84	MN550895.1, MN550894.1, MF511253.1, MF511251.1, MF511249.1, MF511248.1, MF511246.1, MF511245.1, KU361213.1, KT261764.1, KT261763.1, KT261762.1, MF166735.1, MT019635.1
		<i>Rickettsia conorii</i> str. Malish 7	625	625	97	2 ⁻¹⁷⁴	99.42	AE006914.1

Table 4-14: (continue)

Sample ID	Gene size (bp)	BLASTn match	Maximum score	Total score	Query cover (%)	E-value	Identity (%)	Accession number
R4	356	<i>Rickettsia massiliae</i>	597-603	597-603	96-99	1 ⁻¹⁶⁶ to 8 ⁻¹⁶⁸	97.18-97.98	MZ420694.1, MZ420692.1, MT309037.1, KU498299.1, KT032119.1, ON109833.1, ON081515.1, ON001449.1, OM793610.1, OM777139.1, KF826286.1, U59719.1, MF002497.1, KY069259.1, MZ020770.1, MW802693.1
		<i>Rickettsia massiliae</i> str. AZT80	599	599	96	1 ⁻¹⁶⁶	97.98	CP003319.1
		<i>Rickettsia massiliae</i> MTU5	599	599	96	1 ⁻¹⁶⁶	97.98	CP000683.1
R6	353	<i>Rickettsia massiliae</i>	610-619	610-619	94-98	1 ⁻¹⁷¹ to 8 ⁻¹⁷³	98.56-99.70	AB872797.1, AB872787.1, AB872463.1, KY640405.1, AB872810.1, KC428021.1, AB872803.1, AB872793.1, AB872789.1, MT309037.1, MZ420698.1, MZ420696.1, MZ420695.1, MZ420694.1, MZ420692.1, KU498299.1, KT032119.1, ON109833.1, ON081515.1, ON001449.1, OM793610.1, OM777139.1, KF826286.1, U59719.1, MF002497.1, KY069259.1, MZ020770.1, MW802693.1
		<i>Rickettsia massiliae</i> str. AZT80	614	614	97	4 ⁻¹⁷¹	98.84	CP003319.1
		<i>Rickettsia massiliae</i> MTU5	614	614	97	4 ⁻¹⁷¹	98.84	CP000683.1

4.4 Discussion

4.4.1 Morphological identification of ticks

There were two tick species, namely *R. sanguineus* and *H. elliptica*, after morphological identification. These results are similar to studies conducted by Rautenbach *et al.* (1991), where there were 99% (7337/7398) of *R. sanguineus* infections reported on dogs from the North West Province. Also, in the North West Province, there was reported prevalences of 96.62% (14226/14724) for *R. sanguineus* and 2.85% (420/14724) for *H. elliptica* infecting domestic dogs. Several studies also had similar results in other provinces of South Africa, such as Mtshali *et al.* (2017) reporting the presence of *R. sanguineus* (n=276) and *H. elliptica* (n=38) ticks infecting dogs in several provinces in South Africa, including the North West, Free State, KwaZulu Natal, and Mpumalanga. Jacobs *et al.* (2001) reported prevalences of 73.5% (20150/21636) for *R. sanguineus* and 22.4% (1290/21636) for *H. elliptica* (previously known as *H. leachi*) from dogs in the Free State Province. Kolo *et al.* (2020) reported the collection of *R. sanguineus* (n=160) from dogs in the Mpumalanga Province.

There are instances of studies conducted where *H. elliptica* was the dominant tick species collected from companion animals, where the majority of these studies were conducted at veterinary institutions. Kolo *et al.* (2016) reported the presence of *H. elliptica* (n=30) and *R. sanguineus* (n=27) from dogs in Bushbuckridge, Mpumalanga, South Africa. Horak *et al.* (2001) reported the prevalence of *H. elliptica* (n=654) from dogs in KwaZulu Natal, South Africa. Horak & Matthee (2003) reported the presence of *H. elliptica* (formerly referred to as *H. leachi*) (n=8617) and *R. sanguineus* (n=363) from dogs and cats from private properties and veterinary clinics in the Western Cape Province in South Africa. Horak (1995) reported prevalences of 30.5% (1084/3554) for *R. sanguineus* and 60.55% (2152/3554) for *H. elliptica* at a veterinary academic hospital in the Gauteng Province, South Africa. Horak *et al.* (2009) reported prevalences of 1.47% (201/13667) of *H. elliptica* from dogs and vegetation, as well as 0.18% (25/13667) of *R. sanguineus* from dogs from the Eastern Cape Province, South Africa.

In other studies, *H. elliptica* was also reported on other hosts. In a study conducted by Penzhorn *et al.* (2020), 48.84% (25/52) of *H. elliptica* was reported from jackals in the Gauteng Province, South Africa. Golezardy *et al.* (2016) reported a prevalence of 89.63% (16474/19699) from cheetahs and vegetation in the North West Province, South Africa. Petney *et al.* (2004) reported a prevalence of 0.3% (49/16599) for *H. elliptica* from striped mice in the Eastern Cape Province, South Africa. Horak *et al.* (2010) reported the presence of *H. elliptica* on several feline species in several provinces of South Africa. The aforementioned includes *H. elliptica* collected from domestic cats [(n=1798) in the Gauteng, Western Cape, Eastern Cape, and Northern Cape

Provinces], cheetahs [(n=119) in the Limpopo, Gauteng, and Free State Provinces], caracals [(n=31) from the Western Cape Province], lions [(n=53) in the Mpumalanga and Free State Provinces], and leopards [(n=58) in the Mpumalanga and Western Cape Provinces].

Of studies conducted outside of South Africa, results were reported to those similar to this study, such as a study by Sanogo *et al.* (2003), where prevalences of 77.87% (21/27) for *R. sanguineus* and 14.81% (4/27) for *H. elliptica* were reported on dogs in Kinshasa, Democratic Republic of the Congo. On the outside of the South African border, Neves *et al.* (2004) reported the presence of *R. sanguineus* and *H. elliptica* infesting dogs in the Maputo Province, southern Mozambique. During the above-mentioned study, respectively 6.9% (4/58) and 93.8% (15/16) of dogs were infected with *H. elliptica* and 94.8% (55/58) and 18.8% (3/16) of dogs were infected with *R. sanguineus* in the city and the informal settlements. Similarly, Horak *et al.* (2009) reported prevalences of 2.96% (449/15192) of *H. elliptica* from dogs and vegetation, as well as 0.89% (153/15192) of *R. sanguineus* from dogs also from the Maputo province, southern Mozambique.

Bryson *et al.* (2000) stated that although the prevalence of *R. sanguineus* and *H. elliptica* differ between studies conducted in South Africa, these tick species are very common and prevalent among domestic dogs. This may be the case as all the developmental stages of *R. sanguineus* and the adult developmental stage of *H. elliptica* prefer to feed on domestic dogs (Bryson *et al.*, 2000). Horak *et al.* (2001), Jacobs *et al.* (2001), Neves *et al.* (2004), Horak *et al.* (2010), Penzhorn *et al.* (2020), and Philip Samuel *et al.* (2021) stated that the adult developmental stage of *H. elliptica* generally infects domestic dogs, wild felids, and jackals, whereas the immature stages prefer to infect murid rodents but may feed on dogs if rodents are absent.

Most of both *R. sanguineus* and *H. elliptica* were collected from Miederpark, where the PAWS veterinary clinic is located. This was expected as all the developmental stages of *R. sanguineus* and the adult developmental stage of *H. elliptica* prefer to feed on dogs (Horak & Matthee, 2003; Neves *et al.*, 2004). Apart from the availability of dog hosts the PAWS kennels supply the perfect environmental conditions for these questing tick species to survive. The kennels themselves are suited for *R. sanguineus*, which is well adapted to survive in the crevices and cracks of the building and inside the dog's bedding (Jacobs *et al.*, 2001; Horak & Matthee, 2003; Horak *et al.*, 2009). The kennels themselves are surrounded by an open field providing plenty of vegetation for the questing *R. sanguineus* and *H. elliptica* ticks. In addition, the vegetation provides a suitable environment for rodents (Jacobs *et al.*, 2001; Ledger *et al.*, 2019; Penzhorn *et al.*, 2020; Philip Samuel *et al.*, 2021). Jacobs *et al.* (2001) suggest that the SPCA or dog kennels with an abundance of vegetation may attract rodents interested in the food of animals residing in the kennels. These rodents may be infected with *H. elliptica*, which is in the immature developmental

stages, and may subsequently feed on dogs during the adult stages. In addition, Bryson *et al.* (2000), Horak *et al.* (2001), Horak & Matthee (2003), and Abd Rani *et al.* (2011) states that developed urban communities with modernized veterinary services tend to report higher occurrences of *H. elliptica*, whereas domestic dogs in undeveloped areas or villages are mostly infected with *R. sanguineus*. Furthermore, in this study apart from the prevalence of *R. sanguineus* in Miederpark the majority of *R. sanguineus* was from dogs originating from the suburbs. This is similar to the previously mentioned results of Neves *et al.* (2004) where the prevalence of *R. sanguineus* in the Maputu province, located in southern Mozambique, was higher in the city (94.8%) than in the informal settlements surrounding the city (18.8%). Dogs that are chained or confined to small areas are more accessible for *R. sanguineus*, whereas dogs that are not confined or in contact with larger areas are more likely to be infected with *H. elliptica*. In addition, Bryson *et al.* (2000), Horak *et al.* (2001), Jacobs *et al.* (2001), Neves *et al.* (2004), Horak *et al.* (2009), and Ledger *et al.* (2019) suggest that the prevalence of *H. elliptica* may be more dependent on the availability of hosts for the immature developmental stages than the environmental conditions. Horak *et al.* (2001) states that the changing environmental conditions, especially climate change, may impact the host availability of ticks. The lifecycle of ticks relies rapidly on animal hosts in order to feed and reproduce. Rapid increases in temperature creates unsuitable habitats therefore driving the ticks and their associated hosts to a different area with more favorable environmental conditions, resulting in increases of tick populations or new niches of tick infections. Additionally, a shortfall of suitable hosts may result in incidental infections (Ledger *et al.* 2019). During this study, all adult ticks that were collected were identified as females. Kolo *et al.* (2020) suggested that this may be the case as male ticks spend more time off-host in search of mating possibilities.

Majority of ticks collected during this study were collected during the summer season, from December to February [n=171 (116 *R. sanguineus* and 55 *H. elliptica*)], followed by the second most collections in the spring season, from September to November [n=73 (52 *R. sanguineus* and 21 *H. elliptica*)]. The least number of ticks were collected during the fall season, from March to May [n=41 (37 *R. sanguineus* and 4 *H. elliptica*)], and during the winter season [n=41 (33 *R. sanguineus* and 8 *H. elliptica*)]. This is similar to a study conducted by Jacobs *et al.* (2001) where the most *H. elliptica*, in the adult developmental stage, were collected from dogs during September to November in the Free State Province, South Africa. Horak *et al.* (2002) also reported similar results to this study, where tick bites on humans from *H. elliptica* were reported mostly during January to February, in June, as well as in December in several northern provinces in South Africa. Horak *et al.* (2002) also reported the most *H. elliptica* bites on humans during July to September and then again during December in the Eastern Cape province, South Africa. Also

in the Eastern Cape Province, Guillarmod *et al.* (1987) reported the most adult *H. elliptica* ticks collected from dogs during June to July and then again in January. Horak *et al.* (2001) reported the majority of adult *H. elliptica* from dogs during January to April in the KwaZulu Natal Province, South Africa. In a study conducted by Horak & Matthee (2003) the most adult *H. elliptica* ticks were collected from dogs during August, when high rainfall was reported, as well as May to September in the Western Cape Province, South Africa. On the other hand, Petney *et al.* (2004) reported *H. elliptica* that were still in the immature stages of development was collected from striped mice mostly during the months of September to December. As mentioned previously, the distribution of *H. elliptica* during the immature developmental stages may be more dependent on the availability of hosts than it is on environmental conditions (Bryson *et al.*, 2000; Horak *et al.*, 2001; Jacobs *et al.*, 2001; Neves *et al.*, 2004; Horak *et al.*, 2009; Ledger *et al.*, 2019). In studies conducted by Horak (1982) and Jacobs *et al.* (2001), the most ticks of all the developmental stages were collected from dogs during October to May, especially in areas with summer rainfall and frost during winter. The reason could be that two or sometimes more of the tick's life cycles may be completed in these areas, as the immature stages of *R. sanguineus* ticks overwinter and enter the adult developmental stage during the spring season (Horak, 1982; Dzul-Rosado *et al.*, 2018). Horak & Matthee (2003) reported that most amount of adult *R. sanguineus* from dogs was collected during January to April in areas with winter rainfall.

4.4.2 Tick genetic identification

Even though the morphological species concept, for the identification of ticks and other organisms, are still widely used, the phylogenetic- and biological species concept are increasingly being used for identification (Dantas-Torres, 2018). Abd Rani *et al.* (2011) stated that serological or molecular techniques are especially useful for subclinical and chronic infections detection or for epidemiological investigations, whereas morphological examination methods, such as blood smears, remain the most accessible method for clinically significant and acute infections. Dantas-Torres (2018) suggests that the type of gene used for phylogenetic or biological studies needs to be considered carefully, as different genes or even different regions of the same gene tend to have different rates of evolution. The potential gene for sequencing should be variable enough to discriminate the desired organism from all species, but still, be conserved enough to compare the desired organism to closely related species. Previously there were several genes used for sequencing Ixodid ticks including the *CO1*, *18S rRNA*, *16S rRNA*, *12S rRNA*, and the *ITS2* genes (Lv *et al.*, 2014). The *CO1* gene often referred to as the barcode of life, is generally used as the DNA barcode for the identification of animals. This gene mutates fast enough to differentiate

between species that are closely related but is conserved enough between conspecifics (Hebert *et al.*, 2003; Jiao *et al.*, 2021). Generally, there are flanking regions in the *rRNA* gene sequences that are extremely conserved, which often permits the use of universal primers (Meyer *et al.*, 2010).

4.4.3 Presence of tick-borne pathogens

During this study, *C. burnetii* and *Rickettsia* spp. were detected in *R. sanguineus* and *H. elliptica* ticks and tick eggs, thus demonstrating transstadial and transovarial transmission. The ability of ticks to transmit pathogenic organisms to their host during feeding is well-known. Several tick-borne pathogens are of veterinary and zoonotic importance (Rochlin & Toledo, 2020). During this study, all of the ticks infected with tick-borne pathogens were from strays in the PAWS kennels. Abd Rani *et al.* (2011) suggested that privately owned companion animals or animals residing in kennels are less likely to be infected with tick-borne diseases, than free-roaming strays. This could be happening as housed companion animals are fed daily and have a stronger immune system than free-roaming strays living mostly off scraps. Dzul-Rosado *et al.* (2018) stated that the types of regions among ecosystems, also known as ecotones, such as backyards, or agricultural fields next to areas with a lot of vegetation, like PAWS, may be a key factor in the transmission of tick-borne pathogens. Several species of ticks, like *H. elliptica*, which are generally in the immature stages of development, prefer to feed on small mammals, mostly rodents. These small mammals may serve as reservoirs of several different tick-borne pathogens and are in constant contact with vegetative areas, like fields or jungles, the ecotones, and urban or semi-urban areas. This means that the transmission of tick-borne pathogens may potentially increase due to the accumulative contact between domestic or companion animals and the mammals or other wildlife (Abd Rani *et al.*, 2011; Rochlin & Toledo, 2020).

4.4.3.1 *Coxiella burnetii*

The overall occurrence of *C. burnetii* within tick pools was 9.88%. The *C. burnetii* were harboured by 8.82% of *H. elliptica* and 10.64% of *R. sanguineus* ticks. The overall occurrence of *C. burnetii* within egg batches was 16.67%. *Coxiella burnetii* was present in 25% of *H. elliptica* egg batches. Mtshali *et al.* (2017) reported an overall infection of 41% for *C. burnetii* in several provinces in South Africa. Respectively, the infection rates were 55% (30/55), 32% (18/56), 31% (5/16), and 100% (1/1) in the Free State, KwaZulu Natal, North West, and Mpumalanga provinces. In addition, Mtshali *et al.* (2017) reported infection rates of, respectively, 44% and 4% in *R. sanguineus* and *H. elliptica* ticks. The *C. burnetii* is documented in a wide range of livestock, companion animals, domestic animals, birds, wild mammals, and ticks (Knap *et al.*, 2019). Infection of *C. burnetii* infection in humans is also not uncommon as there are several routes of infection including the

transstadial transmission of infected arthropods that feed on humans, inhalation of contaminated aerosol particles, and the consumption of infected food or water (Mtshali *et al.*, 2017). Even though ticks are not considered to be vital in the natural cycle of *C. burnetii* to other hosts, mainly livestock, they are somewhat included in the transmission cycle of *C. burnetii* to maintain the infection in wildlife (Knap *et al.*, 2019).

4.4.3.2 Rickettsia spp.

The overall occurrence of *Rickettsia* spp. within tick pools was 6.17%. There were *Rickettsia* spp. harboured by 11.76% of *H. elliptica* and 2.13% of *R. sanguineus* ticks. The overall occurrence of *Rickettsia* spp. within egg batches was 16.67% (1/6). *Rickettsia* spp. was present in 25% *H. elliptica* egg batches. Kolo *et al.* (2016) reported the presence of 70% (7/10 positive tick pools) of *Rickettsia* spp. in ticks infecting domestic dogs in Bushbuckridge, Mapumalanga Province, South Africa, using a PCR assay. In the aforementioned study, one *H. elliptica* tick pool and one *R. sanguineus* tick pool were positive for *Rickettsia* spp. DNA, where species-specific qPCR assay specified *R. africae* in the *H. elliptica* tick pool. Mtshali *et al.* (2017) reported an overall infection of 37% for *Rickettsia* spp. in several provinces in South Africa. Respectively, the infection rates were 52% (28/54), 21% (11/52), and 38% (6/16) in the Free State, KwaZulu Natal, and North West provinces. In addition, Mtshali *et al.* (2017) reported infection rates of 35% in *R. sanguineus* ticks. Members of the *Rickettsia* genus consist of several established and emerging diseases with global distribution (Rochlin & Toledo, 2020). The majority of *Rickettsia* spp. act as endosymbionts and are present in a wide range of hosts, including ticks, mites, and fleas (Kolo *et al.*, 2016). Nicholson *et al.* (2010) suggested that specific hosts may be reservoirs of and aid to sustain *Rickettsia* spp. in natural lifecycles. Humans and several mammals are accidental hosts, where infection occurs when an infected tick, mite, or flea takes a blood meal (Mtshali *et al.*, 2017). Kolo *et al.* (2016) suggested that *R. africae* infections of humans in South Africa are not uncommon as humans often get infected at wildlife tourist centers, where infected ticks are endemic. The aforementioned is especially a problem for the tourism industry as tourists may get infected (Mtshali *et al.*, 2017).

4.4.3.3 Co-infections

During this study, there was one co-infection of *C. burnetii* (sample ID: C8) and *Rickettsia* spp. (sample ID: R6) in an *R. sanguineus* adult tick. Cabezas-Cruz *et al.* (2019) suggested that several tick-borne pathogens are transmitted by means of transstadial transmission and this may be problematic for ticks that feed on multiple hosts during their lifecycle, as the chances of obtaining a pathogen may increase. In addition, the chances of co-infections may also increase. This is especially problematic for ticks that are three-host species, including *R. sanguineus* and

H. elliptica, that require feeding on a new host for each development stage. In the presence of co-infecting pathogens, these pathogens constantly interact with one another and the microbiota of the tick. These interactions have an effect on the pathogen fitness, altering the infectivity, transmission, and virulence of these pathogens (Gomez-Chamorro *et al.*, 2021). Co-infections are of veterinary concern as they may impact the severity of the associated diseases and are difficult to diagnose (Rocha *et al.*, 2022).

Results of this study indicate that dogs admitted to PAWS may become infected with *R. sanguineus* and *H. elliptica* ticks, which is similar to results in other studies. This reveals that ticks are plentiful in environments with stray dogs with little to no tick control measures (Barker & Walker, 2014). This study also determined the presence of tick-borne pathogens responsible for diseases in animal hosts, with the potential of zoonosis (Dantas-Torres *et al.*, 2012).

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CHAPTER 5

BACTERIAL MICROBIOTA DETECTED IN TICKS INFESTING DOGS, DOG-BLOOD AND COLONY REARED DOG TICKS BY NEXT-GENERATION SEQUENCING

5.1 Introduction

Apart from the potential presence of tick-borne pathogens, ticks also harbour several symbiotic and commensal bacterial communities, collectively called the microbiome. The microbiome is composed of viruses, Archaea, and Eubacteria, as well as several eukaryotic members, including fungi, nematodes, and protozoa (Narasimhan & Fikrig, 2015; Esteve-Gassent *et al.*, 2016). Associations between the ticks and the microbiota affect several aspects of the ticks such as their vectorial competence, survival, and reproductive fitness (Narasimhan & Fikrig, 2015; Esteve-Gassent *et al.*, 2016; Cabezas-Cruz *et al.*, 2018).

The bacterial composition of ticks varies due to a lot of factors which means changes in the microbiome are inevitable (Narasimhan & Fikrig, 2015; Esteve-Gassent *et al.*, 2016; Cabezas-Cruz *et al.*, 2018; Adegoke *et al.*, 2020). One of these factors includes the tick's life cycle. Hard ticks have 4 different developmental stages including eggs, larvae, nymphs, and adults, where a blood meal from a new host is required to molt to the next developmental stage (Cabezas-Cruz *et al.*, 2018; Narasimhan *et al.*, 2021). In addition, the process of molting to the next developmental stage is complicated, and includes self-digestion and tissue regeneration (Narasimhan & Fikrig, 2015). Another factor that may influence the tick microbiome includes host ecology. After eggs hatch or ticks detach from a host to enter the next developmental stage, the ticks spend a part of their life cycle in vegetation, referred to as off-host phases. During these phases, ticks are exposed to hostile environmental conditions such as humidity and temperatures (Esteve-Gassent *et al.*, 2016; Narasimhan *et al.*, 2021). Another factor that may influence the tick microbiome includes the absence or presence of a pathogen or co-infections of several pathogens (Adegoke *et al.*, 2020; Narasimhan *et al.*, 2021).

Advancements in molecular sequencing platforms, such as Illumina or Ion torrent 16S metagenomics sequencing, 454-pyrosequencing, whole genome shotgun sequencing, and 16S rDNA clone full-length Sanger Sequencing, along with advancements in data analysis tools made it possible to identify members and gain insights into the complexity of the microbiome (Narasimhan & Fikrig, 2015; Estrada-Peña *et al.*, 2020). Additionally there are limited information

available of the bacterial communities harboured by *R. sanguineus* and *H. elliptica* ticks from natural hosts and colony dog ticks. Therefore, this chapter has determined bacterial microbiome in the two ticks infesting dogs, dog blood and in colony reared ticks by using next-generation sequencing.

5.2 Materials and methods

5.2.1 Study area

5.2.1.1 North-West Province

Detailed description of the North-West Province is given in Chapter 4 under section 4.2.1.1.

5.2.2 Sample collection

For this part of the study selected *R. sanguineus* (n=1) and *H. elliptica* (n=1) tick specimens were collected from PAWS (as described in Chapter 4, section 2.1.2) that represent the naturally infected dog hosts. Blood samples [n=4 (n=2 blood samples representing each tick species)] from dogs that were associated with the aforementioned ticks were also collected. For each tick species, one blood sample was collected from the dog host infected by the aforementioned ticks, while one blood sample was collected from a dog host infected with another tick. Furthermore, *R. sanguineus* (n=3) and *H. elliptica* (n=3) colony reared nymphal ticks, were obtained from ClinVet International, Bloemfontein, South Africa. These tick specimens were collected after they fed on gnotobiotic animal hosts. Upon collection, all of the tick specimens were transferred to collection vials and stored in 70% ethanol, whereas blood from the infected dog hosts was collected and placed on Whatman Chromatography FTA™ (Flinders Technology Associate) cards (Sigma-Aldrich, USA) (Cabezas-Cruz *et al.*, 2018) and transported to the North-West University for further analysis.

5.2.3 Tick dissections

Prior to dissections, ticks were washed in 10% Tween 20 for 1 hour. Ticks were then respectively rinsed twice and thrice with 70% ethanol and double distilled water. Each rinse was done for a period of 1 hour. During dissections, the midguts, malpighian tubules, salivary glands, ovaries, and uterus of the ticks were removed using a modified method as described by Edwards *et al.*, (2009) and Patton *et al.*, (2012). Dissections were performed under the 25X objective lens of a dissection microscope. Briefly, wax was melted onto several Petri dishes that were later used to mount the

ticks. Prior to each tick being dissected all of the surfaces (including the microscope surface) and instruments (including the previously prepared Petri dishes) were sterilized with 70% ethanol to avoid contamination. In addition, for each tick that was dissected and for each organ that was removed 4 drops of 25 μ l phosphate-buffered saline (PBS) were placed on a clean microscope slide. For each dissection, a tick was mounted onto the previously prepared Petri dishes by re-melting a small section of the wax and placing the tick dorsally onto the Petri dish. Afterward, the dorsum of the tick was cut and lifted upwards to reveal the tick insides, and the entire tick was covered with PBS to prevent tissue desiccation. The desired organ was isolated and placed on a drop of PBS on the microscope slide. Unwanted debris was then removed and the desired organ was placed on a new drop of PBS. The aforementioned step was repeated three times and finally, the organ was placed in a 1.5 ml microcentrifuge tube filled with 1 ml of PBS and stored at -20°C for further analysis. Between each tick dissection and removal of a desired organ, the surfaces and instruments were re-sterilized, and fresh drops of PBS were placed on a clean microscope slide.

5.2.4 DNA extractions

DNA was extracted from the midguts, malpighian tubules, salivary glands, ovaries, and uterus of naturally infected dog ticks, dogs' blood, and colony dog ticks using the DNeasy Blood & Tissue Kit (Qiagen, DE) by following the manufactures instructions. Briefly, the samples were placed into individual 1.5 ml microcentrifuge tubes along with 180 μ l Buffer ATL and 20 μ l proteinase K. The tubes were then placed into the TissueLyser LT (Qiagen, DE) and the contents were mixed for 10 minutes at 1800 oscillations/minute. Subsequently, 200 μ l of Buffer AL was added to each tube and vortexed to mix the contents. This was followed by incubation for 10 minutes at 56°C. After incubation 200 μ l of 97% ethanol was added to each tube and vortexed to mix the contents. Subsequently, the mixture was transferred to a DNeasy Mini spin column placed inside a 2 ml collection tube. This was followed by centrifuging for 1 minute at 8000 rpm. The collection tube containing the flow-through was discarded and the spin column was placed inside a new collection tube. Afterward, 500 μ l of Buffer AW2 was added. The tubes were then centrifuged at 14 000 rpm for 3 minutes. The collection tube containing the flow-through was discarded and the spin column was placed inside a new 1.5 ml microcentrifuge tube. Finally, DNA elution was done by adding 200 μ l Buffer AE to the spin column center. This was followed by incubation at room temperature for 1 min and centrifuging the tubes at 8000 rpm for 1 min. The spin column was then discarded. Nano-Drop was used to confirm the presence and concentration, as well as assess the purity of DNA. Afterward, DNA samples were stored at -20°C until further use.

5.2.5 16S rRNA library preparation

5.2.5.1 First-stage PCR (Amplicon PCR)

During the first stage PCR, the amplicon primers sequences were gene-specific targeting the V3 and V4 region of the 16S rRNA gene. Additionally, the primers contained Illumina adapter overhanging sequences. The first-stage PCR used the 341F forward primer (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). The adapters used during this step consisted of the Forward overhanging (TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG) and Reverse overhanging (GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G) adapters (Illumina Inc., 2013). PCR reaction mixtures were prepared in a 69-well 0.2 ml PCR plate covered with Microseal 'A' film. Individual PCR reaction mixtures had a final volume of 25 µl and were made up of 2.5 µl of the microbial DNA, 5 µl of the 1 µM Amplicon PCR forward primer, 5 µl of the 1 µM Amplicon PCR Reverse primer, 12.5 µl of the 2X KAPA HiFi Hotstart ReadyMix [containing KAPA HiFi HotStart DNA Polymerase (1 U/µl), GC Buffer (5X), Fidelity Buffer (5X), as well as high-quality dNTPs (10 mM each)] (KAPA Biosystems, USA). First-stage PCR was thermally cycled under the following conditions: initial denaturation at 95°C for 3 minutes, followed by 25 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds, this was followed by final extension at 72°C for 5 minutes and infinite final hold at 4°C. Visualization was done by running 1 µl of the PCR product on the Bioanalyzer DNA 1000 chip (Agilent Technologies, DE) to determine the size of PCR fragments.

5.2.5.2 First-stage PCR clean-up

Amplicon PCR plate was sealed and centrifuged at 3 500 rpm for 1 minute at 20°C to collect condensation. Afterward, the Amplicon PCR plate seal was removed and discarded. The AMPure XP beads were equilibrated to room temperature for 30 minutes before use. As soon as the beads reached room temperature it was vortexed for 30 seconds, to ensure even distribution (Illumina Inc., 2013). Subsequently, 20 µl of the beads were transferred to each Amplicon PCR plate well and the mixture was resuspended by pipetting the mixture up and down a total of 10 times. Afterward, the samples were incubated at room temperature for 5 minutes. During incubation the samples were stationary. The Amplicon PCR plate was then placed on a magnetic stand for 2 minutes. When the supernatant was transparent, it was removed from the Amplicon PCR well and discarded. While the Amplicon PCR plate remained on the magnetic stand, the beads were washed twice with 200 µl of freshly prepared 80% ethanol. After incubation for 30 seconds, the supernatant was removed and discarded. Subsequently, the Amplicon PCR plate was left for a

period of 10 minutes to allow the samples to air-dry. The Amplicon PCR plate was taken off the magnetic stand, with DNA eluted in 52.5 μ l of 10 mM Tris buffer (pH of 8.5). The DNA in each well was gently resuspended and incubated at room temperature for 2 minutes. The plate was placed back onto the magnetic stand and allowed to stand for 2 minutes. Once the supernatant was transparent, 50 μ l of supernatant from each individual well was transferred to a new sterilized 96-well PCR plate for the second stage PCR.

5.2.5.3 Second-stage PCR (Index PCR)

For the second stage PCR, 5 μ l from the 50 μ l purified PCR amplicons were transferred to a new sterilized 96-well plate. The 96-well plate containing the remaining 45 μ l purified PCR amplicons was stored at -20°C for future analysis.

The 96-well plate with the 5 μ l purified PCR amplicons was placed in the TruSeq Index Plate Fixture. The TruSeq Index Plate Fixture was arranged as follows: Index 1 primer tubes (the tubes with a yellow solution and orange cap) were arranged horizontally to the 96-well plate where the columns of the plate are numbered 1 to 12, whereas the Index 2 primer tubes (the tubes with a transparent solution and white cap) were arranged vertically to the 96-well plate where the rows of the plate are labeled A to H, as illustrated in Figure 5.1 (Illumina Inc., 2013).

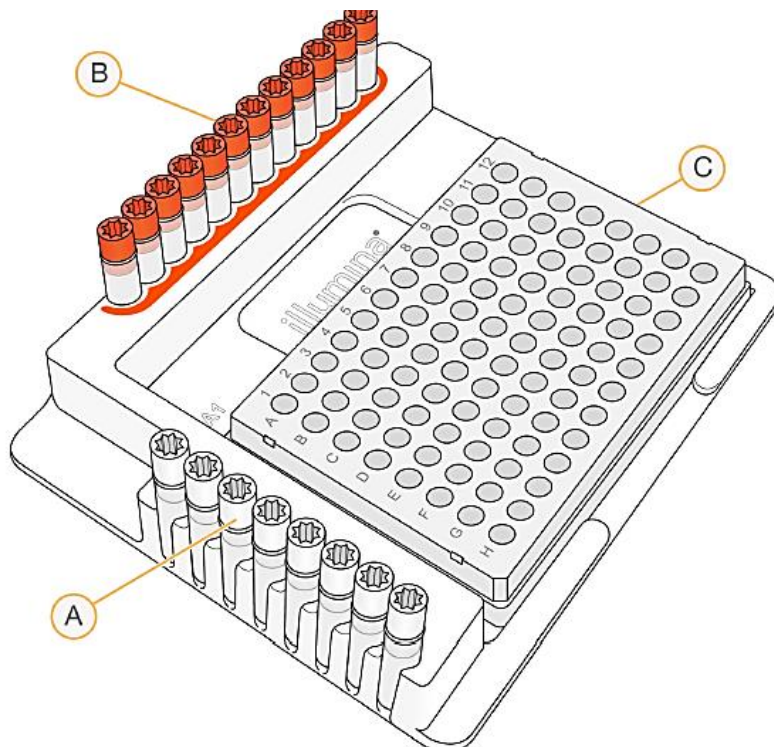


Figure 5-1: Setup of the TruSeq Index Plate Fixture, Where **A** illustrates the Index 2 primers, **B** illustrates the Index 1 primers and **C** illustrates the 96-well plate (Illumina Inc., 2013).

A PCR reaction mixture with a final volume of 50 µl was made up in each well of the 96-well plate. The PCR reaction mixture consisted of the 5 µl DNA (this was the purified PCR amplicons from the First stage PCR clean-up), 25 µl 2x KAPA HiFi HotStart ReadyMix [containing KAPA HiFi HotStart DNA Polymerase (1 U/µl), high-quality dNTPs (10 mM each), CG Buffer (5X) and Fidelity Buffer (5X)] (KAPA Biosystems, USA), 5 µl of Nextera XT Index Primer 1 (N7xx), 5 µl of Nextera XT Primer 2 (S5xx), and 10 µl of PCR grade water. Each PCR reaction mixture was resuspended 10 times by pipetting the volume in each well of the 96-well plate up and down and the plate was sealed with Microseal 'A'. Subsequently, the 96-well plate was centrifuged at 3 500 rpm at 20°C for 1 minute. Second-stage PCR consisted of the following conditions: initial denaturation at 95°C for 3 minutes, followed by 8 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds, this was followed by final extension at 72°C for 5 minutes and infinite final hold at 4°C.

5.2.5.4 Second stage PCR clean-up

Index PCR plate was sealed and centrifuged at 2 500 rpm for 1 minute at 20°C to collect condensation. The seal was removed and discarded. Similar to the First-stage PCR cleanup, the AMPure XP beads were equilibrated to room temperature for 30 minutes and vortexed for 30 seconds to ensure even distribution (Illumina Inc., 2013). As soon as the beads reached room temperature it was vortexed for 30 seconds, to ensure even distribution (Illumina Inc., 2013). Subsequently, 56 µl of the beads were transferred to each well of the Index PCR plate and the mixture was resuspended 10 times. Afterward, the samples were incubated at room temperature for 5 minutes while remaining stationary. The Index PCR plate was placed on a magnetic stand for 2 minutes. The supernatant was removed and discarded when it was transparent. The beads were washed twice with 200 µl of freshly prepared 80% ethanol, incubated for 30 minutes and the supernatant was removed and discarded. The Index PCR plate was left for 10 minutes for the samples to air-dry. The Index PCR plate was removed from the magnetic stand, with DNA eluted in 27.5 µl of 10 mM Tris buffer (pH of 8.5). The DNA was resuspended and incubated at room temperature for 2 minutes. The Index PCR plate was placed back onto the magnetic stand for 2 minutes. Once the supernatant was transparent, 50 µl of supernatant from each individual well was transferred to a new sterilized 96-well PCR plate.

5.2.5.5 Library quantification, normalization, and pooling

The DNA concentration, in ng/μl, was determined by using the Qubit 4 Fluorometer (Thermo Fisher Scientific, ZA). The following formula was used to determine the concentration of each library in nM:

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

After the determination of DNA concentrations, to prepare the library the DNA of each sample was diluted to a final concentration of 4 nM by using 10 mM Tris buffer (pH of 8.5). Pooling libraries with special indices were prepared by mixing 5 μl of the diluted DNA from every individual library.

5.2.5.6 Denaturation of library and sample loading into MiSeq

Prior to denaturation of the library, heat blocks were pre-set to 96°C, an ice-water bath consisting of 3 parts ice and 1 part water was prepared, and the MiSeq reagent cartridge (Illumina, USA) was left outside until it reached room temperature. Subsequently, for each individual sample 5 μl of freshly prepared diluted 0.2 N NaOH and 5 μl of the 4 nM pooled library DNA were mixed together in a microcentrifuge tube. Afterward, the tubes were vortexed and centrifuged at 2 500 rpm for a period of 1 minute. In order to let the DNA denature and form single strands (Illumina Inc., 2013), the samples were incubated at room temperature for a period of 5 minutes. Subsequently, 10 μl of the denatured DNA was added to 990 μl pre-chilled HT1. The denatured DNA was then temporarily placed on ice while the final dilution step was done.

5.2.5.7 Denaturation and dilution of PhiX control

Dilution of the PhiX library to 4 nM was done by combining 2 μl of the 10 nM PhiX library with 3 μl of 10 mM Tris buffer (pH 8.5). Subsequently, 5 μl of 0.2 N NaOH was added to a microcentrifuge tube containing 5 μl of the 4 nM PhiX library. For the PhiX library to denature and produce single strands (Illumina Inc., 2013), the microcentrifuge tubes were vortexed and incubated at room temperature for a period of 5 minutes. Afterward, 20 pM PhiX library was prepared by the addition of 10 μl of the denatured PhiX library with 990 μl pre-chilled HT1 into microcentrifuge tubes. Each tube was then inverted several times, and pulse centrifuged and finally, the tubes were placed on ice.

5.2.5.8 Amplicon library and PhiX control combination

To allow the combination of the two libraries, 30 μl of the denatured diluted PhiX control was added with 570 μl of the denatured diluted amplicon library into microcentrifuge tubes. The

combined libraries were then placed on ice prior to the heat denaturation step. During the heat denaturation step, the tubes were incubated in a heat block at 96°C for a period of 2 minutes, mixed by inverting twice, and then placed on ice for a period of 5 minutes. The samples were then transferred to the MiSeq flow cell (Illumina, USA). By making use of the MiSeq system metagenomic workflow option, the samples were observed with MiSeq Reporter Software (MRS) (Illumina Inc., 2013).

5.2.6 16S metabarcoding data analysis

Prior to data analysis Oracle VirtualBox version 6.1.3.2 and QIIME 2 Core version 2022.2.1 were downloaded and installed. Raw sequences were retrieved from the MiSeq system in FASTQ format. The raw sequences were submitted to the Sequence Read Archive (SRA) of the National Centre of Biotechnology Information (NCBI) database under the Bioproject ID: PRJNA981132 (for the dog blood sample types) and PRJNA980979 (for the tick specimen sample types).

5.2.6.1 Metadata file construction and validation

A metadata file, containing the information of the samples used for downstream analysis, was created in a spreadsheet and the metadata file was validated using the Keemei plugin (Rideout *et al.*, 2016) in Google spreadsheets.

5.2.6.2 Quality control

The quality of the raw reads was visualized using FastQC version 0.118 (Brown *et al.*, 2017) and MultiQC version 1.14 (Ewels *et al.*, 2016). To trim or crop the sequences at the low-quality ends and remove adapters, Trimmomatic version 0.32 (Bolger *et al.*, 2014) was used. Subsequently, the reads were imported into Quantitative Insights into Microbial Ecology 2, or QIIME 2 (Bolyen *et al.*, 2019), as an artifact. Using the cutadapt plugin (Martin, 2011) the primers were removed from the reads, whereas reads that did not contain the primers that corresponded to the V3 to V4 region of the 16S *rRNA* gene were removed. Subsequently, the reads were denoised into amplicon sequence variants, or ASVs, using the DADA2 plugin (Callahan *et al.*, 2016).

5.2.6.3 Taxonomic classification of ASVs

The SILVA 132 reference database (Quast *et al.*, 2012; Glöckner *et al.*, 2017) was obtained, trained, and subsequently used to assign taxonomy to the ASVs. Briefly, the reference database FASTA file with representative sequences of the 16S *rRNA* gene, with 97 to 100% similarity, as well as the table file with taxonomy, from phylum up to genus level, were imported into the QIIME 2 environment. Subsequently, the V3 to V4 domain was singled out of the 16S *rRNA* gene data,

the classifier was trained using naïve Bayes (Pedregosa *et al.*, 2011), and taxonomic identification was done using the feature classifier plugin (Bokulich *et al.*, 2018) resulting in a feature table.

5.2.6.4 Statistical analysis

The resulting feature table was filtered out to exclude rare ASVs that had a low number of counts across the dataset, such as singletons and doubletons. Additionally, the feature table was further filtered to exclude contaminants, including mitochondria, and unclassified ASVs, that were possibly chimeric sequences (Xia & Sun, 2023). After the feature table was filtered the ASVs sequences were subset and summarised to match the same sample types. The filtered feature table was then collapsed by different taxonomic levels from level 2 (phylum) to level 6 (genus) according to abundance. Lastly, the tables were exported from QIIME2 and imported into the open-source programming language R version 2023.03.1 (Allaire, 2012) where alpha- and beta-diversity analysis was done. The ggplot2 version 3.4.2 (Gómez-Rubio, 2017), tidyverse version 2.0.0 (Wickham *et al.*, 2019), vegan version 2.6-4 (Dixon, 2003), gplots version 3.1.3 (Gatto *et al.*, 2015), venneuler version 1.1-3 (Wilkinson, 2011), reshape version 0.8.9 (Wickham, 2007), devtools version 2.4.5 (Wickham & Bryan, 2023), stringi version 1.7.12 (Gagolewski, 2022), irlba version 2.3.5.1 (Tsuyuzaki *et al.*, 2020), magrittr version 2.0.3 (Lemenkova, 2020), pkgconfig version 2.0.3 (Lowndes *et al.*, 2017), igraph version 1.4.2 (Csardi & Nepusz, 2006), huge version 1.3.5 (T. Zhao *et al.*, 2012), VGAM version 1.1-8 (Yee, 2010), phyloseq version 1.44.0 (McMurdie & Holmes, 2013), biomformat version 1.28.0 (Y. Zhao *et al.*, 2021), tidyr version 1.3.0 (Z. Ross *et al.*, 2017), and reshape version 0.8.9 (Wickham, 2007) R packages were installed and used for producing and visualizing of bar graphs, heatmaps, and venn diagrams.

5.3 Results

The concentrations of the DNA used for this part of the study ranged between 3.481 to 10.862 ng/μl. There was a total of 1 945 967 sequence counts imported into QIIME 2. After trimming the sequences with cutadapt, 1 867 788 sequence counts remained. After denoising with DADA2, there were 7689 features and a total frequency of 1 678 022. The results of DADA2 of each sample are summarized in Table 5.1. After filtering out unclassified and contaminant ASVs, there were 7689 features and a total of 1 489 814 frequencies remaining, which were subsequently compared to a reference database for sequences that were 97 to 100% similar. There were 1450 bacterial communities classified from phylum to genus level (Appendix 5).

Table 5-1: Results of denoising sequences with DADA2

Sample ID	Sample type	Input sequences*	Sequences filtered*	Input sequences past filter (%)	Sequences denoised*	Non-chimeric sequences*	Input non-chimeric sequences (%)
HF1	<i>H. elliptica</i> from gnotobiotic host (number 1)	102034	100357	98.36	99762	95193	93.3
HF2	<i>H. elliptica</i> from gnotobiotic host (number 2)	88202	86093	97.61	85506	81554	92.46
HF5	<i>H. elliptica</i> from gnotobiotic host (number 5)	86795	85288	98.26	84576	82166	94.67
RF1	<i>R. sanguineus</i> from gnotobiotic host (number 1)	84138	82901	98.53	82268	76632	91.08
RF3	<i>R. sanguineus</i> from gnotobiotic host (number 3)	84462	82876	98.12	82425	77209	91.41
RF4	<i>R. sanguineus</i> from gnotobiotic host (number 4)	62227	61270	98.46	60716	58008	93.22
I2	Blood from dog host infected by <i>R. sanguineus</i> (infected by tick number 2)	87034	84576	97.18	83826	76568	87.97
I3	Blood from dog host infected by <i>R. sanguineus</i> (infected by tick number 3)	109303	107488	98.34	107081	97679	89.37
I29	Blood from dog host infected by <i>H. elliptica</i> (infected by tick number 29)	105141	102439	97.43	101988	92729	88.19
I12	Blood from dog host infected by <i>H. elliptica</i> (infected by tick number 12)	73163	71364	97.54	70826	64794	88.56
HG12	Salivary gland from <i>H. elliptica</i> (tick number 12)	97680	94706	96.96	94163	86676	88.73
RG2	Salivary gland from <i>R. sanguineus</i> (tick number 2)	180799	176421	97.58	175536	156805	86.73
HO12	Ovaries from <i>H. elliptica</i> (tick number 12)	96071	93543	97.37	92757	84899	88.37
RO2	Ovaries from <i>R. sanguineus</i> (tick number 2)	79807	76757	96.18	76186	70684	88.57
HM12	Midgut from <i>H. elliptica</i> (tick number 12)	93285	91063	97.62	90570	83848	89.88
RM7	Midgut from <i>R. sanguineus</i> (tick number 7)	86407	84868	98.22	84411	77848	90.09
HP12	Malpighian tubules from <i>H. elliptica</i> (tick number 12)	86223	84605	98.12	84121	78487	91.03
RP2	Malpighian tubules from <i>R. sanguineus</i> (tick number 2)	90499	88050	97.29	87116	80181	88.6
HU12	Uterus from <i>H. elliptica</i> (tick number 12)	86027	83816	97.43	83273	77046	89.56
RU2	Uterus from <i>R. sanguineus</i> (tick number 2)	88491	86289	97.51	85698	79016	89.29

*Refers to the number (n) of sequence reads

5.3.1 Bacterial communities identified and classified by sequence alignment

During metagenomic analysis, there were a total of 46 phyla identified. The dominating bacterial phyla are illustrated in Figure 5.2. All of the bacterial phyla that had a relative abundance across all of the sample types of less than 1% or that were either unclassified or uncultured were grouped together and labeled as other. The dominating bacterial phyla were identified as Proteobacteria (54.02%), Bacteroidetes (18.03%), and Actinobacteria (11.76%), followed by Firmicutes (4.17%), Verrucomicrobia (3.44%), Patescibacteria (2.71%), Fusobacteria (1.26%), Cyanobacteria (1.01%), and Planctomycetes (0.89%).

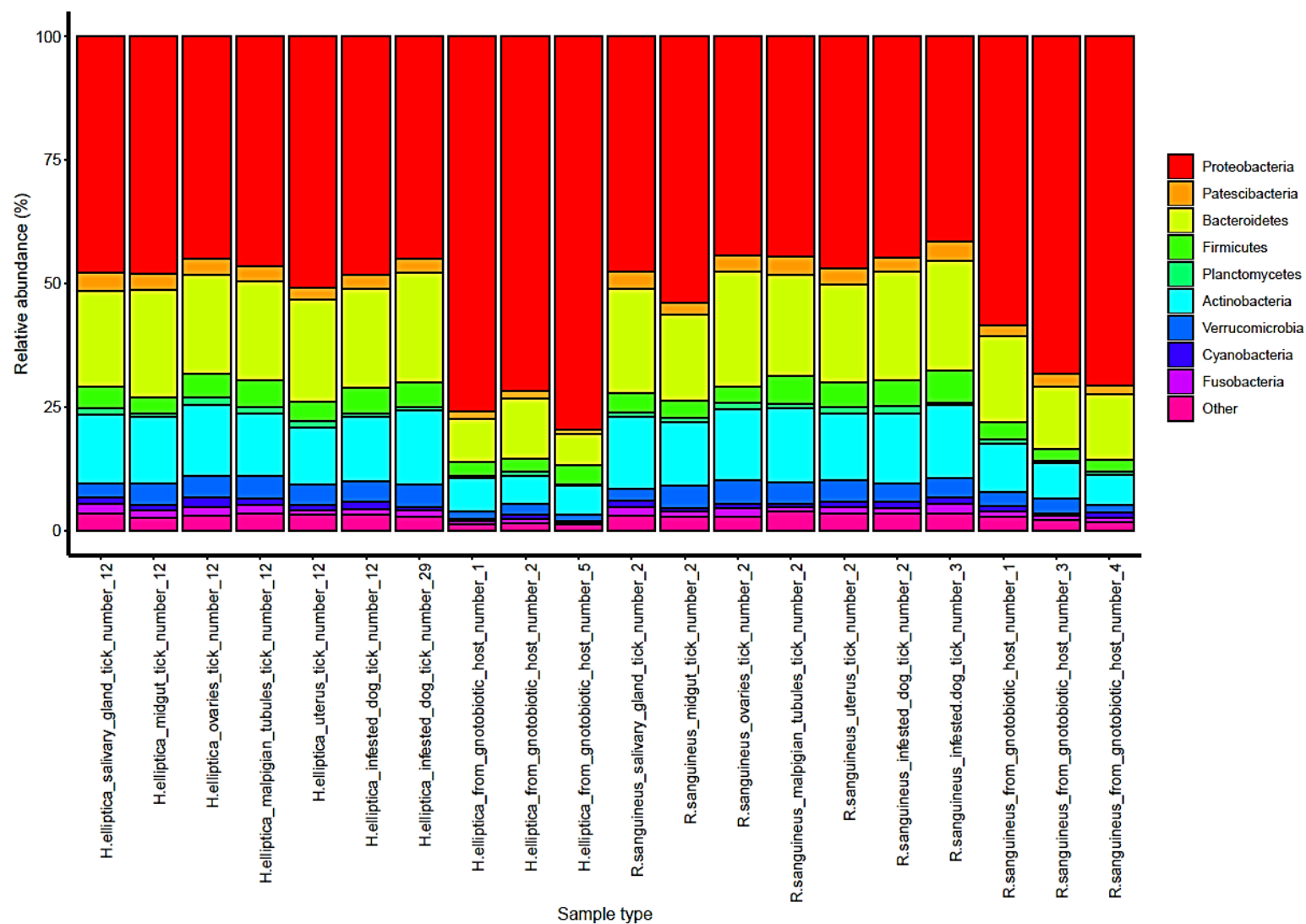


Figure 5-2: Bacterial phyla detected from different tick samples and dog blood

During metagenomic analysis, there were a total of 141 classes identified. The dominating bacterial classes are illustrated in Figure 5.3. All of the bacterial classes that had a relative abundance across all of the sample types of less than 1% or that were either unclassified or uncultured were grouped together and labeled as other. The dominating bacterial classes were identified as Gammaproteobacteria (41.61%), Bacteroidia (17.93%), Alphaproteobacteria (11.20%), and Actinobacteria (11.11%), followed by Verrucomicrobiae (3.44%), Bacilli (1.90%), Clostridia (1.76%), Parcubacteria (1.70%), Fusobacteriia (1.26%), Deltaproteobacteria (1.10%), Oxyphotobacteria (0.95%), and Planctomycetacia (0.64%).

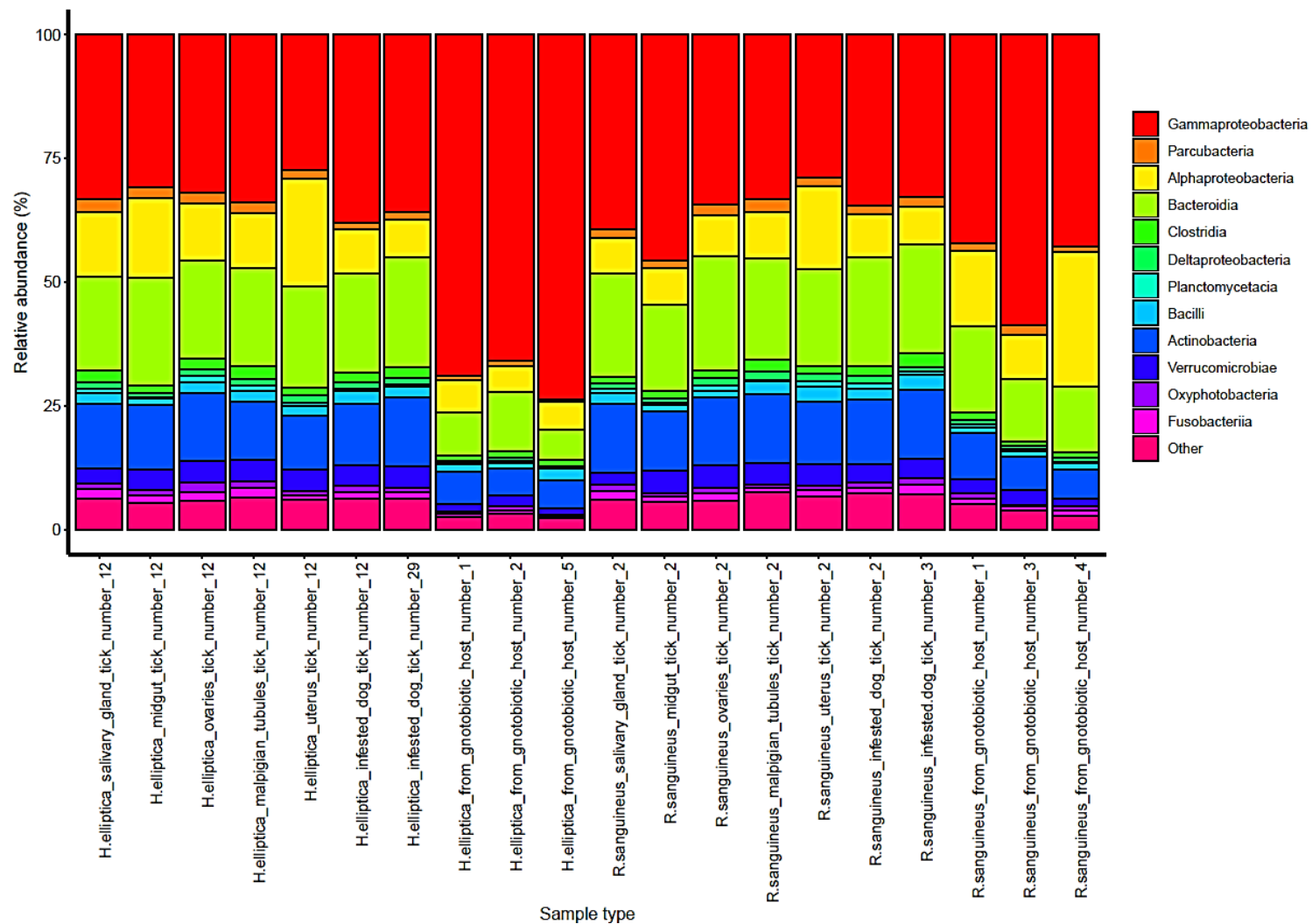


Figure 5-3: Bacterial classes detected from different tick samples and dog blood

During metagenomic analysis, there were a total of 393 orders identified. The dominating bacterial orders are illustrated in Figure 5.4. All of the bacterial orders that had a relative abundance across all of the sample types of less than 1% or that were either unclassified or uncultured were grouped together and labeled as other. The dominating orders were identified as Betaproteobacteriales (14.78%) and Coxiellales (14.40%), followed by Flavobacteriales (7.44%), Cytophagales (5.84%), Enterobacteriales (5.61%), Frankiales (5.40%), Micrococcales (4.36%), Rickettsiales (2.96%), Pseudomonadales (2.56%), Verrucomicrobiales (2.09%), Sphingomonadales (1.95%), Aeromonadales (1.83%), Clostridiales (1.76%), Chitinophagales (1.76%), Bacteroidales (1.50%), Rhizobiales (1.44%), Bacillales (1.31%), Fusobacteriales (1.26%), Rhodobacterales (1.19%), Sphingobacteriales (1.04%), Lactobacillales (0.58%), Propionibacteriales (0.50%), Acetobacterales (0.49%), Thalassobaculales (0.46%), Reyranelles (0.42%), Nostocales (0.26%), and Legionellales (0.17%).

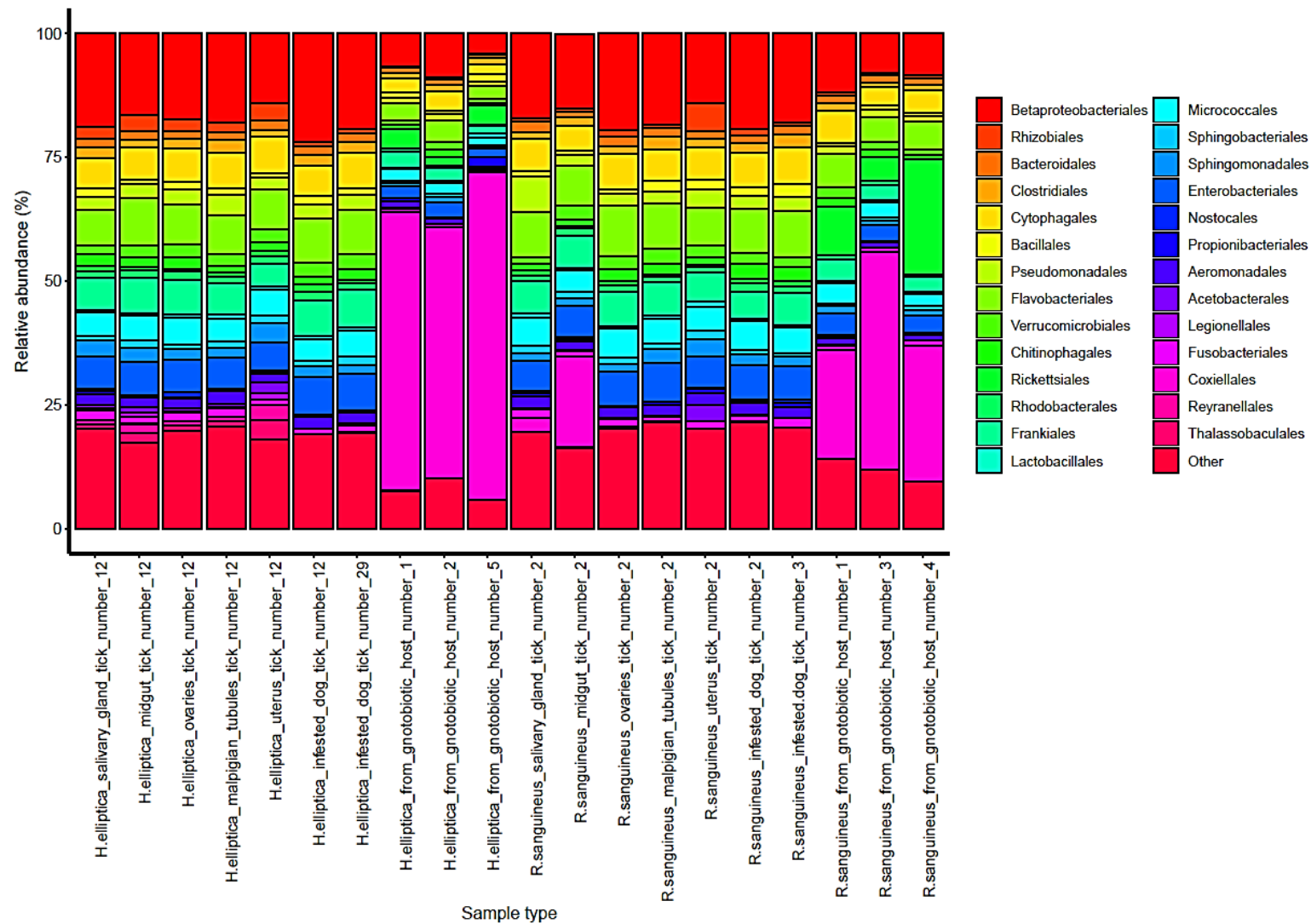


Figure 5-4: Bacterial orders detected from different tick samples and dog blood

During metagenomic analysis, there were a total of 778 families identified. The dominating bacterial families are illustrated in Figure 5.5. All of the bacterial families that had a relative abundance across all of the sample types of less than 1% or that were either unclassified or uncultured were grouped together and labeled as other. The dominating bacterial families were identified as Coxiellaceae (14.40%) and Burkholderiaceae (12.12%), followed by Enterobacteriaceae (5.61%), Sporichthyaceae (5.32%), Spirosomaceae (5.13%), Flavobacteriaceae (4.41%), Microbacteriaceae (4.21%), Rickettsiaceae (2.89%), Crocinitomicaceae (2.58%), Sphingomonadaceae (1.95%), Aeromonadaceae (1.83%), Pseudomonadaceae (1.58%), Chitinophagaceae (1.57%), Rubritaleaceae (1.46%), Rhodocyclaceae (1.28%), Rhodobacteraceae (1.19%), Fusobacteriaceae (1.01%), Moraxellaceae (0.99%), Bacillaceae (0.81%), Clostridiaceae (0.61%), Paludibacteraceae (0.59%), Oceanibaculaceae (0.46%), Propionibacteriaceae (0.42%), Reyraneliaceae (0.42%), Microscillaceae (0.41%), Beijerinckiaceae (0.41%), Staphylococcaceae (0.31%), Acetobacteraceae (0.27%), Xanthobacteraceae (0.22%), and Legionellaceae (0.17%).

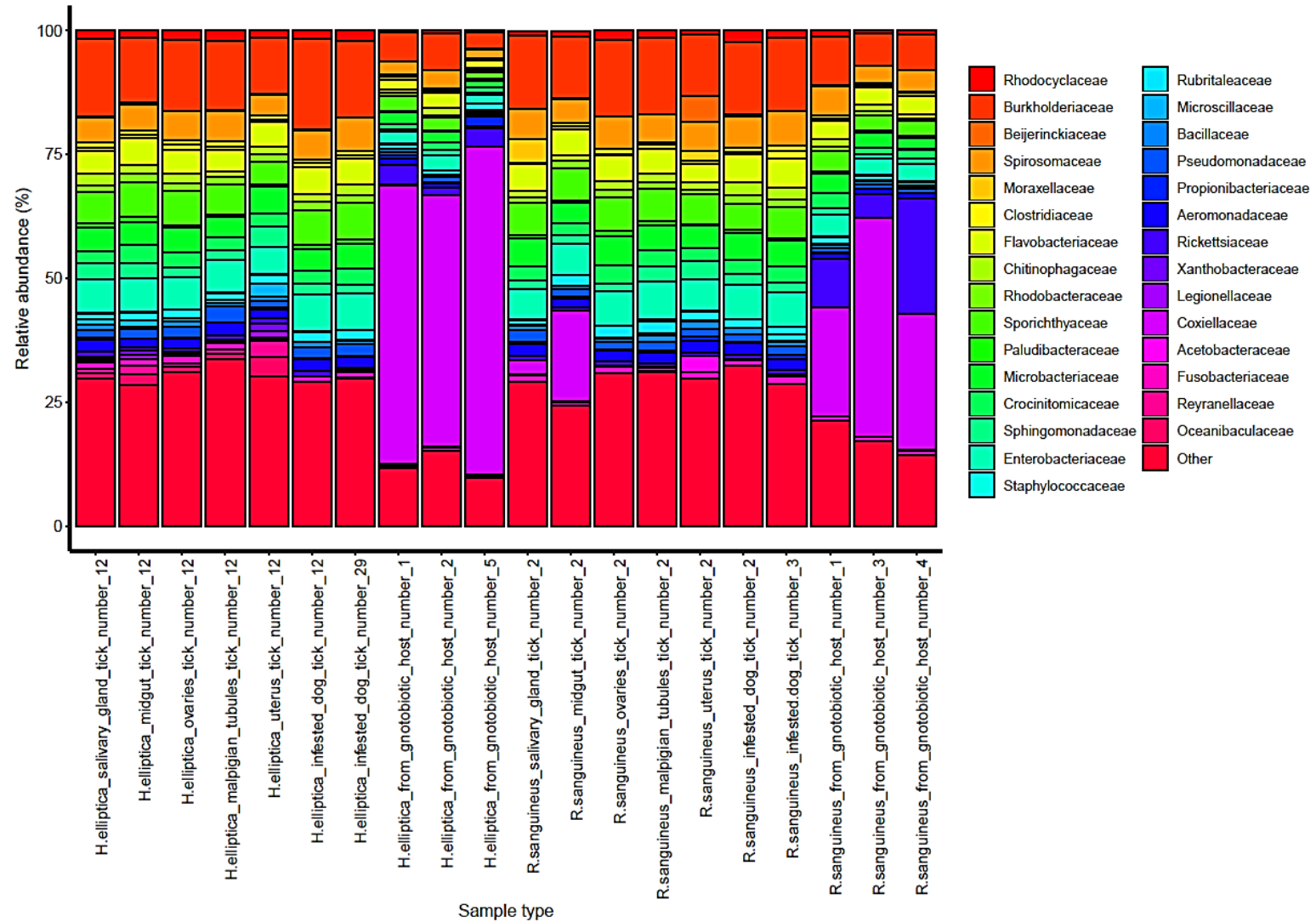


Figure 5-5: Bacterial families detected from different tick samples and dog blood

During metagenomic analysis, there were a total of 1450 genera identified. The dominating bacterial genera are illustrated in Figure 5.6. All of the bacterial genera that had a relative abundance across all of the sample types of less than 1% or that were either unclassified or uncultured were grouped together and labeled as other. The dominating bacterial genera were identified as *Coxiella* (14.40%), followed by *Pseudarcicella* (4.87%), *Limnohabitans* (4.63%), *Flavobacterium* (4.21%), *Polynucleobacter* (3.23%), *Rickettsia* (2.82%), *Fluviicola* (2.56%), *Aeromonas* (1.79%), *Pseudomonas* (1.48%), *Luteolibacter* (1.41%), *Sediminibacterium* (1.18%), *Fusobacterium* (0.98%), *Bacillus* (0.80%), *Acinetobacter* (0.78%), *Paludibacter* (0.55%), *Oceanibaculum* (0.46%), *Reyranella* (0.41%), *Cutibacterium* (0.34%), *Staphylococcus* (0.30%), *Methylobacterium* (0.17%), *Legionella* (0.16%), and *Roseomonas* (0.11%).

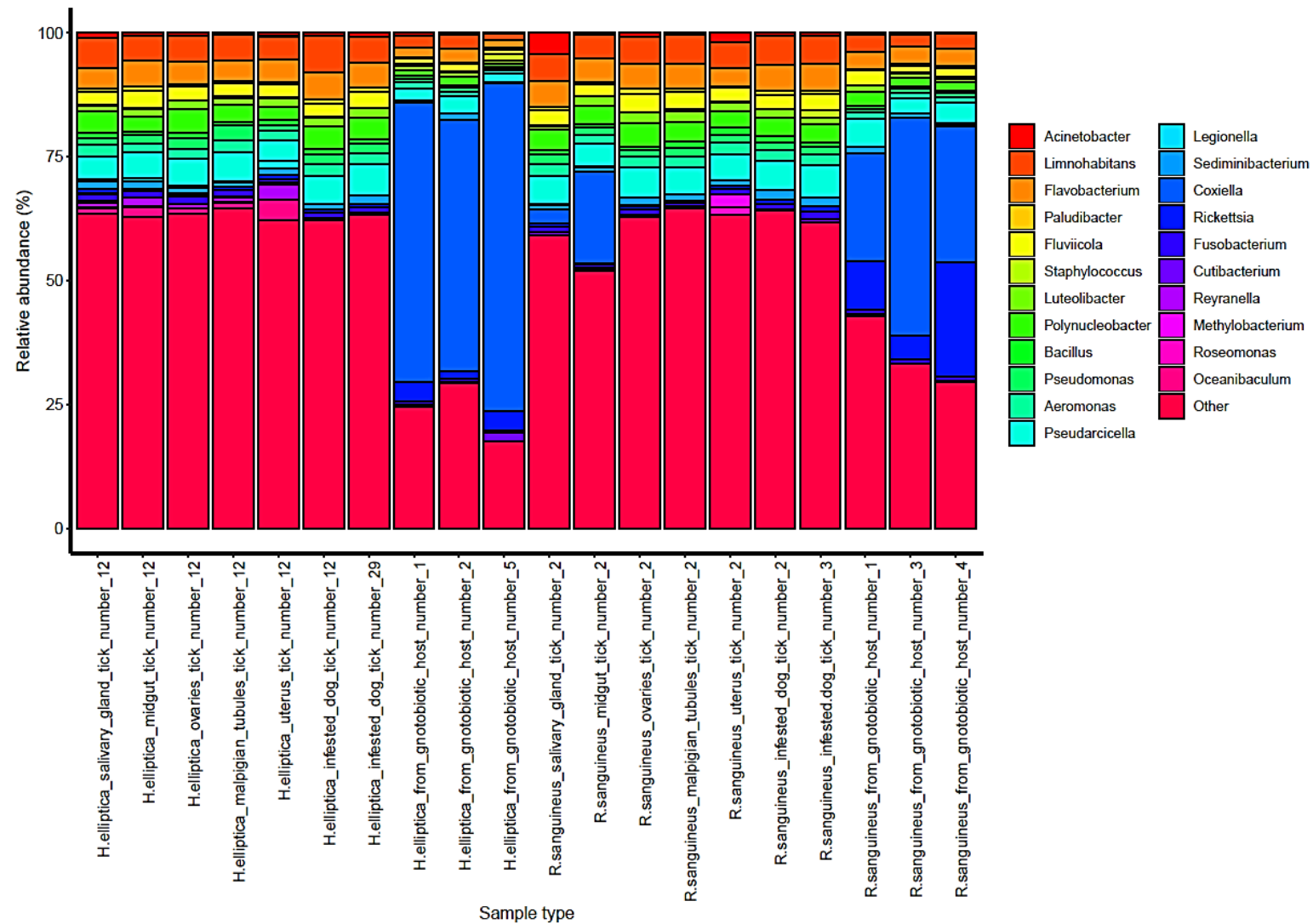


Figure 5-6: Bacterial genera detected from different tick samples and dog blood

5.3.2 Alpha and beta diversity of the two dog ticks

To determine the evenness and richness of the species within the sample types, alpha diversity analysis was done (Table 5.2). For alpha analyses the chao1 confidence interval and the number of observed features for all the sample types were similar ranging between 552 to 803. The values of the Shannon diversity index ranged between 3.45 to 8.03. The Simpson diversity index values were less than one for all of the sample types, ranging between 0.69 to 0.99, which is an indication of no diversity between the samples.

Table 5-2: Alpha diversity indices of different tick samples and dog blood

Sample ID	Sample information	Chao1 confidence interval	Number of observed features	Shannon diversity	Simpson diversity
HF1	<i>H. elliptica</i> from gnotobiotic host (number 1)	627	627	4.35	0.69
HF2	<i>H. elliptica</i> from gnotobiotic host (number 2)	607	607	4.88	0.75
HF5	<i>H. elliptica</i> from gnotobiotic host (number 5)	548	548	3.45	0.58
RF1	<i>R. sanguineus</i> from gnotobiotic host (number 1)	677	677	6.51	0.94
RF3	<i>R. sanguineus</i> from gnotobiotic host (number 3)	605	605	5.24	0.80
RF4	<i>R. sanguineus</i> from gnotobiotic host (number 4)	552	552	5.21	0.87
I2	Blood from dog host infected by <i>R. sanguineus</i> (infected by tick number 2)	758	758	8.03	0.99
I3	Blood from dog host infected by <i>R. sanguineus</i> (infected by tick number 3)	684	684	7.88	0.99
I29	Blood from dog host infected by <i>H. elliptica</i> (infected by tick number 29)	691	691	7.88	0.99
I12	Blood from dog host infected by <i>H. elliptica</i> (infected by tick number 12)	671	671	7.80	0.99
HG12	Salivary gland from <i>H. elliptica</i> (tick number 12)	666	666	7.92	0.99
RG2	Salivary gland from <i>R. sanguineus</i> (tick number 2)	803	803	7.91	0.99
HO12	Ovaries from <i>H. elliptica</i> (tick number 12)	668	668	7.86	0.99
RO2	Ovaries from <i>R. sanguineus</i> (tick number 2)	652	652	7.83	0.99
HM12	Midgut from <i>H. elliptica</i> (tick number 12)	672	672	7.74	0.99
RM7	Midgut from <i>R. sanguineus</i> (tick number 7)	676	676	7.06	0.96
HP12	Malpighian tubules from <i>H. elliptica</i> (tick number 12)	699	699	7.93	0.99
RP2	Malpighian tubules from <i>R. sanguineus</i> (tick number 2)	672	672	7.87	0.99
HU12	Uterus from <i>H. elliptica</i> (tick number 12)	651	651	7.69	0.99
RU2	Uterus from <i>R. sanguineus</i> (tick number 2)	748	748	7.90	0.99

To determine the sample types differentiation, beta diversity analysis was done. Heatmaps were created of the classes and genera relative abundances. As illustrated in Figure 3.7 and Figure 3.8, respectively, at class and genera levels the relative abundances between the *R. sanguineus* and *H. elliptica* of the colony ticks from gnotobiotic hosts were similar, whereas the relative abundances of the *R. sanguineus* and *H. elliptica* ticks and their natural dog hosts was similar. These similarities between the relative abundances show that there are similarities between the species richness. On the other hand, the relative abundances between the colony ticks from gnotobiotic hosts and the ticks collected from ticks and their natural hosts are different. These differences between the relative abundances show that there is a differentiation between the species richness. Respectively, at class and genus level there was a high abundance of Gammaproteobacteria, in all the sample types, and high abundances of *Coxiella* spp. between the colony ticks from gnotobiotic hosts.

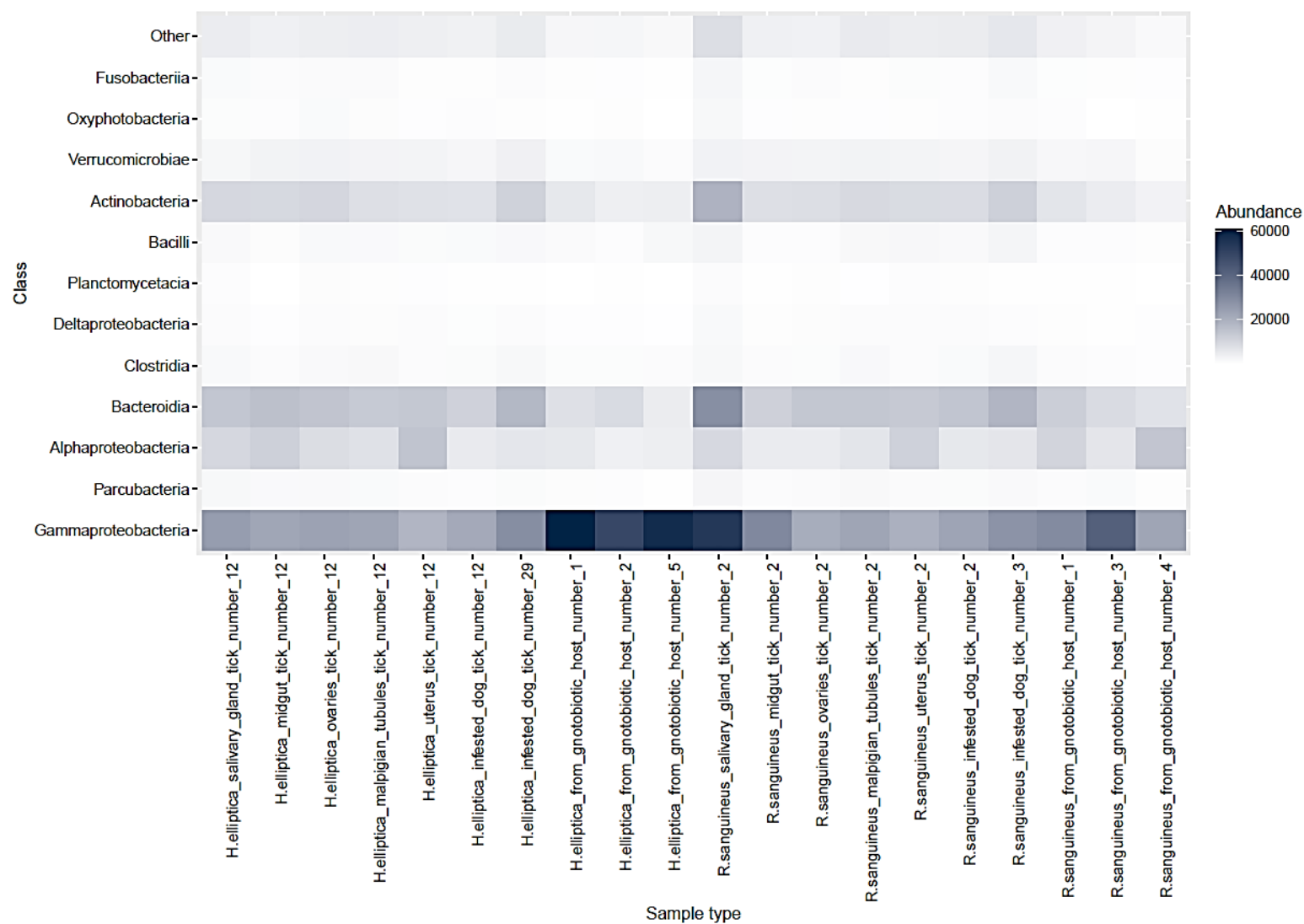


Figure 5-7: Heatmaps at the class level to determine bacterial abundance

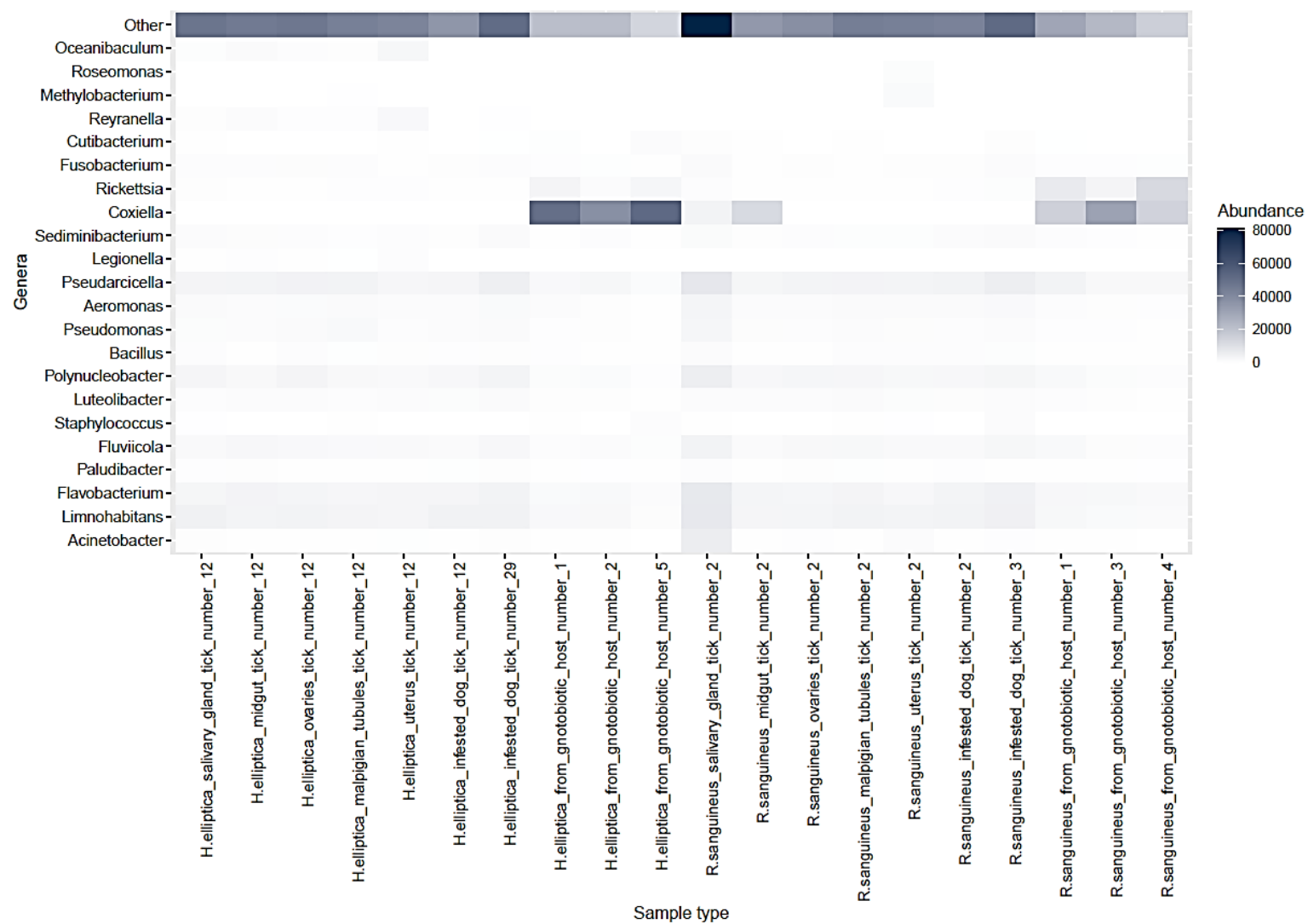


Figure 5-8: Heatmaps at the genus level to determine bacterial abundance

The construction of Venn diagrams was done of the bacterial genera between the different sample types to determine the mutual species richness. Between *R. sanguineus* and *H. elliptica* colony ticks collected from gnotobiotic hosts, there were 353 shared bacterial genera (Figure 5.9). Between *R. sanguineus* and *H. elliptica* ticks collected from natural dog hosts, there were 520 shared bacterial genera (Figure 5.10). There were 439 shared bacterial genera between *R. sanguineus* collected from gnotobiotic hosts and naturally infected hosts (Figure 5.11). There were 300 shared bacterial genera between the naturally collected *R. sanguineus* and their associated dog host (Figure 5.12). Between the midgut, salivary gland, uterus, mapigian tubules, and ovaries of the dissected *R. sanguineus*, collected from natural dog hosts, there were 111 shared bacterial genera (Figure 5.13). There were 421 shared bacterial genera between *H. elliptica* collected from gnotobiotic hosts and naturally infected hosts (as illustrated in Figure 5.14). There were 289 shared bacterial genera between the naturally collected *H. elliptica* and their associated dog host (Figure 5.15). Between the midgut, salivary gland, uterus, mapigian tubules, and ovaries of the dissected *H. elliptica*, collected from natural dog hosts, there were 120 shared bacterial genera (Figure 5.16).

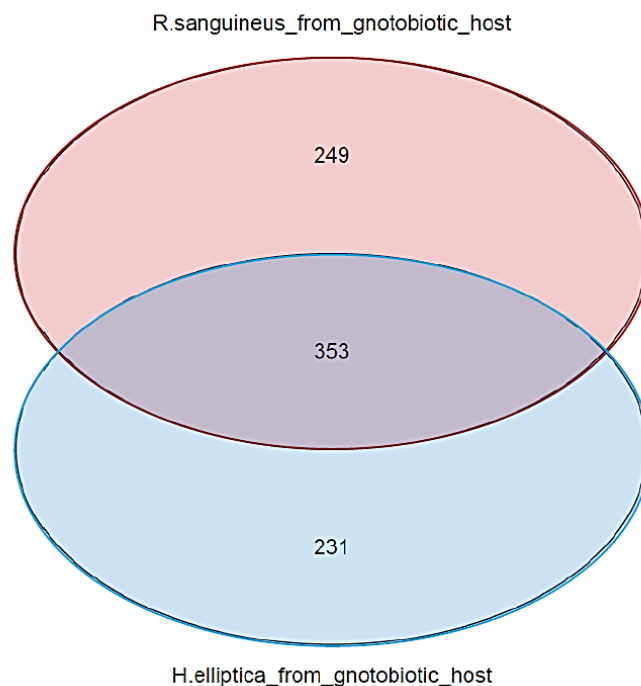


Figure 5-9: Venn diagram indicating the shared bacterial genera between *R. sanguineus* and *H. elliptica* collected from gnotobiotic hosts

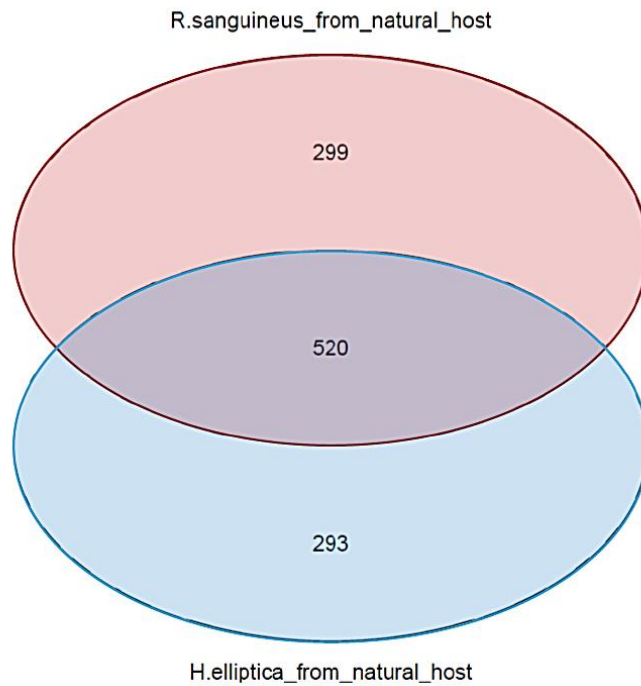


Figure 5-10: Venn diagram indicating the shared bacterial genera between *R. sanguineus* and *H. elliptica* collected from natural hosts

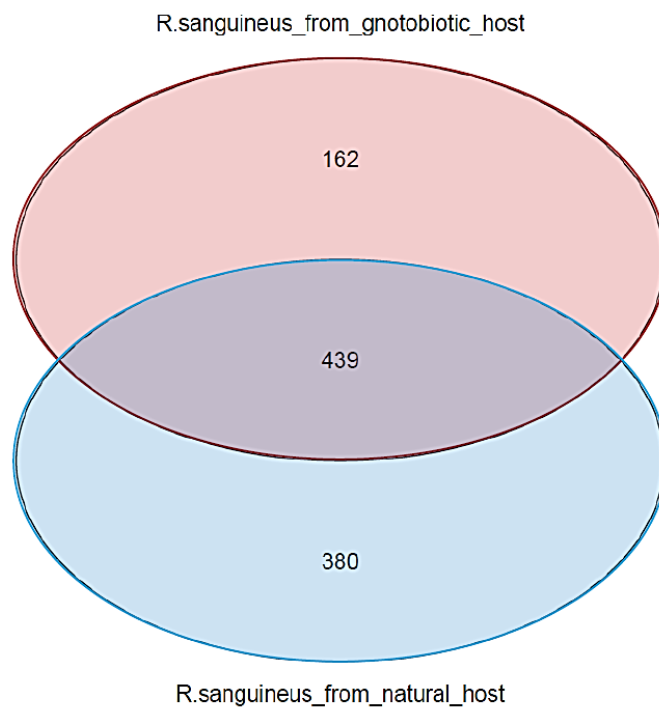


Figure 5-11: Venn diagram indicating the shared bacterial genera between *R. sanguineus* collected from gnotobiotic hosts and naturally infected hosts

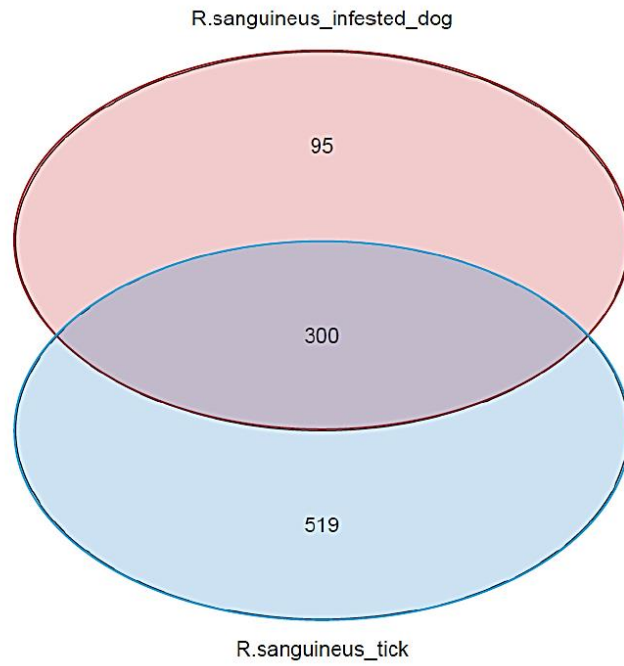


Figure 5-12: Venn diagram indicating the shared bacterial genera between naturally collected *R. sanguineus* and their associated dog host

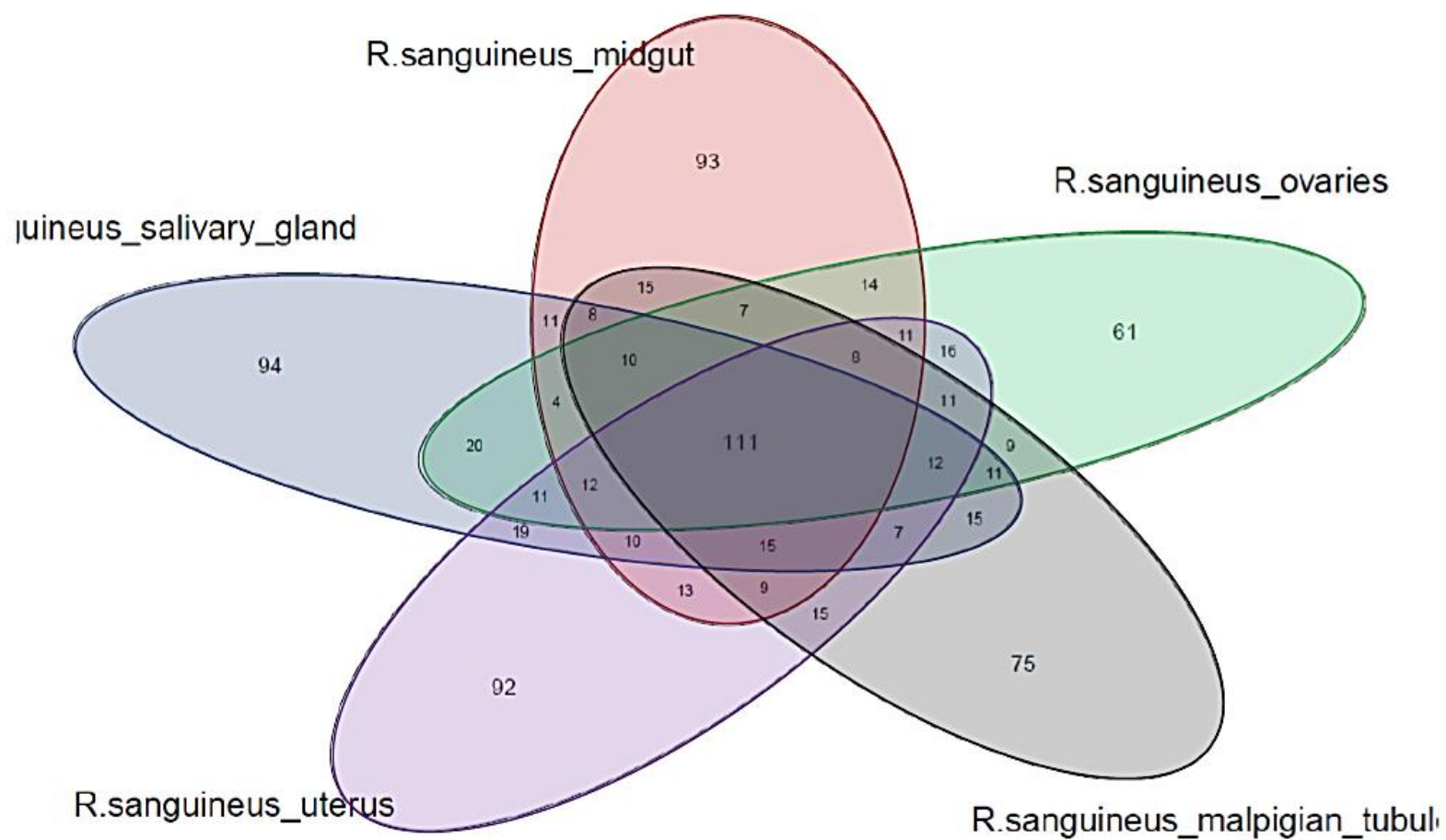


Figure 5-13: Venn diagram indicating the shared bacterial genera between the midgut, ovaries, salivary gland, uterus, and malpighian tubules of *R. sanguineus* collected from naturally infected dog hosts

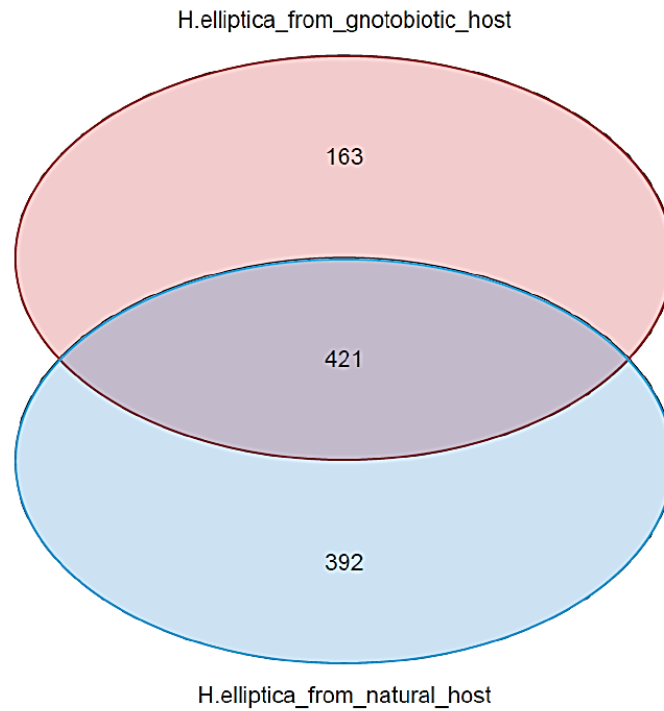


Figure 5-14: Venn diagram indicating the shared bacterial genera between *H. elliptica* collected from gnotobiotic hosts and naturally infected hosts

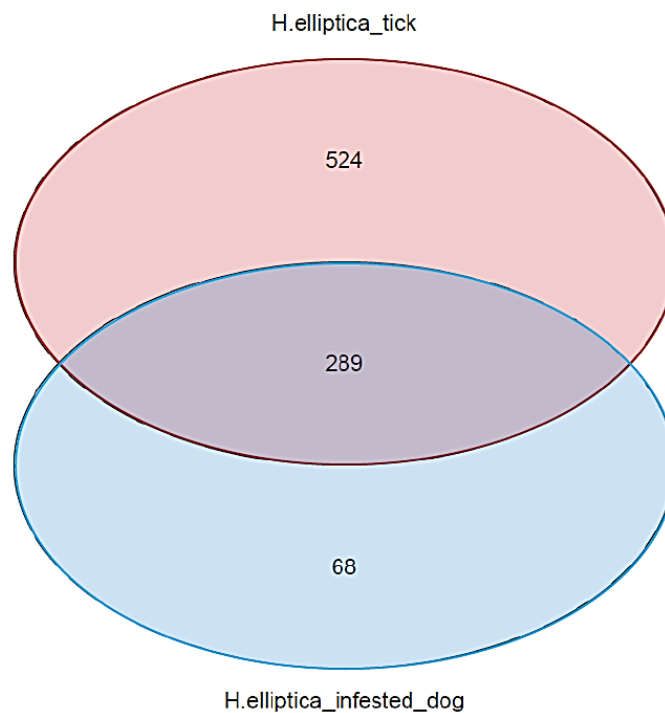


Figure 5-15: Venn diagram indicating the shared bacterial genera between naturally collected *H. elliptica* and their associated dog host

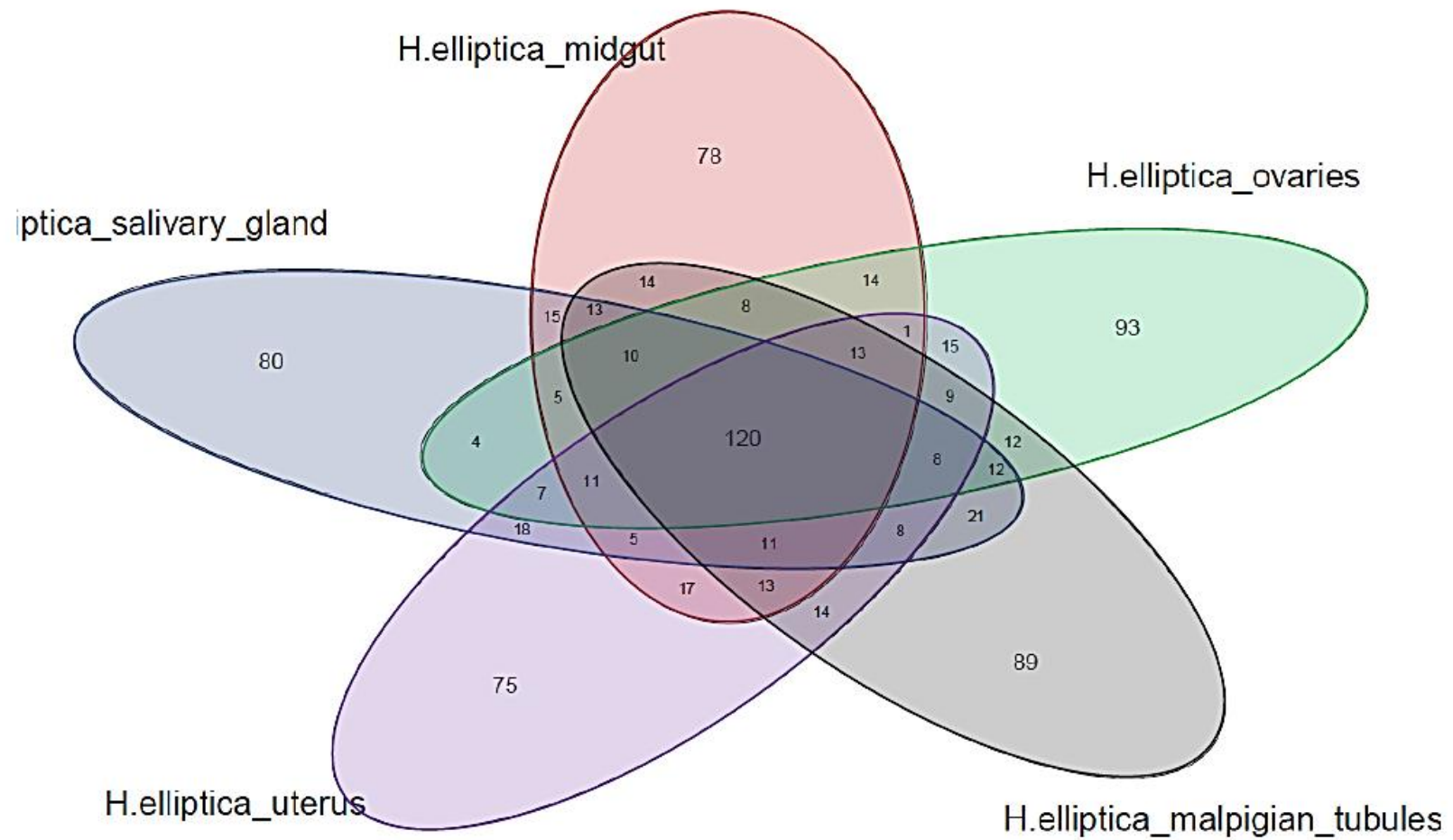


Figure 5-16: Venn diagram indicating the shared bacterial genera between the midgut, ovaries, salivary gland, uterus, and malpighian tubules of *H. elliptica* collected from naturally infected dog hosts

Several bacterial genera were detected that are of veterinary, medical, and ecological importance including *Prevotella*, *Bdellovibrio*, *Acinetobacter*, *Clostridium*, *Pajaroellobacter*, *Acidovorax*, *Anaerococcus*, *Streptococcus*, *Gemella*, *Flavobacterium*, *Staphylococcus*, *Chryseobacterium*, *Alcaligenes*, *Arcobacter*, *Cloacibacillus*, *Bacillus*, *Treponema*, *Leuconostoc*, *Pseudomonas*, *Achromobacter*, *Morganella*, *Tenacibaculum*, *Vibrio*, *Desulfovibrio*, *Rhizobacter*, *Rickettsiella*, *Bacteroides*, *Sphingomonas*, *Aeromonas*, *Ruminococcus*, *Comamonas*, *Veillonella*, *Facklamia*, *Lactococcus*, *Legionella*, *Abiotrophia*, *Psychrobacter*, *Erysipelothrix*, *Helcococcus*, *Coxiella*, *Actinomyces*, *Rickettsia*, *Serratia*, *Fusobacterium*, *Listeria*, *Streptomyces*, *Sutterella*, *Bordetella*, *Mycobacterium*, *Rothia*, *Finegoldia*, *Neisseria*, *Prevotella*, *Dysgonomonas*, *Klebsiella*, *Roseomonas*, *Leptospira*, *Moraxella*, *Enterococcus*, *Anaplasma*, *Proteus*, *Capnocytophaga*, *Francisella*, *Plesiomonas*, *Streptobacillus*, and *Ehrlichia* (Table 5.3).

Table 5-3: Bacterial genera detected from several ticks' samples and dog blood of medical, veterinary, and ecological importance

Genus	Sample type detected from	Significance	Reference
<i>Prevotella</i>	• <i>H. elliptica</i> from gnotobiotic host • Blood from dog host infected by <i>R. sanguineus</i> • Malpighian tubules from <i>R. sanguineus</i> • <i>H. elliptica</i> from gnotobiotic host	Several species are associated with periodontal disease.	Könönen & Gursoy (2022)
<i>Bdellovibrio</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Malpighian tubules from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Predators of pathogenic bacteria that infect plants and humans but are not pathogenic to the host. (Cavallo <i>et al.</i> , 2021) suggests that certain species may be used as "living antibiotics".	Evans <i>et al.</i> (2007) and Cavallo <i>et al.</i> , (2021)
<i>Acinetobacter</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Several species are pathogenic and are widely known for being hospital-acquired infections.	Munoz-Price & Weinstein (2008)

Table 5-3: (continue)

Genus	Sample type detected from	Significance	Reference
<i>Clostridium</i>	• Malpighian tubules from <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i>	Responsible for several diseases of animals and humans.	Stevens <i>et al.</i> (2012)
<i>Pajaroellobacter</i>	• Blood from dog host infected by <i>H. elliptica</i> • Ovaries from <i>R. sanguineus</i>	The only documented species is <i>P. abortibovis</i> , responsible for epizootic bovine abortion.	Brooks <i>et al.</i> (2016)
<i>Acidovorax</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host number 1 • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Several species are plant pathogens.	Hu & Young (1998)
<i>Anaerococcus</i>	• <i>H. elliptica</i> from gnotobiotic host • Midgut from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i>	Responsible for several infections of humans and animals such as urinary tract infections, soft tissue infections, pleural empyema, chronic ulcers, and blood infections.	Murphy & Frick (2013)

Table 5-3: (continue)

Genus	Sample type detected from	Significance	Reference
<i>Streptococcus</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Contain pathogenic and non-pathogenic species. Non-pathogenic species are a part of the commensal human microbiota of the upper respiratory tract, mouth, intestines, and skin. Pathogenic species are associated with strep throat, meningitis, pink eye, bacterial pneumonia, erysipelas, endocarditis, and necrotizing fasciitis.	Krzyściak <i>et al.</i> (2013)
<i>Gemella</i>	• Blood from dog host infected by <i>R. sanguineus</i> • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i>	One species, namely <i>G. haemolysans</i> are associated with pulmonary exacerbations in humans diagnosed with cystic fibrosis.	Khan <i>et al.</i> (2004)
<i>Flavobacterium</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Several species are responsible for diseases of freshwater fish.	Loch & Faisal (2015)

Table 5-3: (continue)

Genus	Sample type detected from	Significance	Reference
<i>Staphylococcus</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Several species are associated with diseases of humans and animals, including toxic shock syndrome, skin infections, pneumonia, bacteremia, sepsis, food poisoning, endocarditis, and bone infections.	Tenover & Gorwitz (2006)
<i>Chryseobacterium</i>	• <i>H. elliptica</i> from gnotobiotic host • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Several species are responsible for infections in humans and animals. One species namely, <i>C. indologenes</i> is described as an uncommon human pathogen and is often hospital-acquired, responsible for central venous catheter-associated infections, bacteremia, meningitis, peritonitis, cystitis, pneumonia, pyelonephritis, and empyema. In addition, <i>C. indologenes</i> may be pathogenic to several soft tick species. Other <i>Chryseobacterium</i> spp. is responsible for frog, turtle, and fish diseases. Other <i>Chryseobacterium</i> spp. may disintegrate the exoskeletons of insects and feathers of birds.	Burešová et al. (2006), Chou et al. (2011), Loch & Faisal (2015), and Page et al. (2019)
<i>Alcaligenes</i>	• <i>H. elliptica</i> from gnotobiotic host • Malpighian tubules from <i>H. elliptica</i> • <i>R. sanguineus</i> from gnotobiotic host • Uterus from <i>R. sanguineus</i>	Members are rarely responsible for opportunistic infections, such as blood poisoning.	Malek-Marín et al. (2009) and Huang, (2020)

Table 5-3: (continue)

Genus	Sample type detected from	Significance	Reference
<i>Arcobacter</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Certain species are pathogenic to humans and animals. In particular, <i>A. butzleri</i> and <i>A. cryaerophilus</i> are responsible for enteritis and uncommonly bacteremia.	Doudah <i>et al.</i> (2012)
<i>Cloacibacillus</i>	• Salivary gland from <i>H. elliptica</i>	Potentially pathogenic to humans and may be associated with bacteremia.	Domingo <i>et al.</i> (2015)
<i>Bacillus</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	There are mainly two pathogenic species, namely <i>B. cereus</i> responsible for food poisoning, and <i>B. anthracis</i> responsible for anthrax.	Vilas-Bôas <i>et al.</i> (2007)

Table 5-3: (continue)

Genus	Sample type detected from	Significance	Reference
<i>Treponema</i>	• <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Blood from dog host infected by <i>R. sanguineus</i>	Several species are considered pathogenic to humans and animals. Mainly, <i>T. pallidum</i> is associated with yaws, bejel, and syphilis, as well as <i>T. carateum</i> is responsible for pinta.	Miao & Fieldsteel, (1980) and Heymans <i>et al.</i> (2010)
<i>Leuconostoc</i>	• <i>H. elliptica</i> from gnotobiotic host • Blood from dog host infected by <i>R. sanguineus</i>	Some species are potential human pathogens.	Vagiakou-Voudris <i>et al.</i> (2002)
<i>Pseudomonas</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Several species are pathogens of humans and animals. Infectious species include <i>P. aeruginosa</i> , <i>P. oryzihabitans</i> , and <i>P. plecoglossicida</i> which are mainly hospital-acquired infections and are associated with bloodstream infections. Several species of this genus are also plant pathogens, including <i>P. syringae</i> .	Silby <i>et al.</i> (2011)
<i>Achromobacter</i>	• Salivary gland from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i>	Several species are opportunistic pathogens with immunosuppressive conditions, such as kidney failure, cystic fibrosis, and cancer.	Swenson & Sadikot (2015)
<i>Morganella</i>	• <i>H. elliptica</i> from gnotobiotic host • Blood from dog host infected by <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i>	Several species are opportunistic pathogens of humans responsible for cellulitis, sepsis, chorioamnionitis, abscess, and purple urine bag syndrome	Liu <i>et al.</i> (2016)

Table 5-3: (continue)

Genus	Sample type detected from	Significance	Reference
<i>Tenacibaculum</i>	Ovaries from <i>R. sanguineus</i>	Several species are opportunistic pathogens of fish, including <i>T. maritimum</i> , <i>T. soleae</i> , <i>T. discolor</i> , <i>T. gallaicum</i> , and <i>T. dicentrarchi</i> , responsible for tenacibaculosis.	Avendaño-Herrera <i>et al.</i> (2006)
<i>Vibrio</i>	Uterus from <i>R. sanguineus</i>	Several species are human and animal pathogens. In humans, they are responsible for gastroenteritis and sometimes sepsis. In animals, members of this genus cause disease in shellfish and fish.	Baker-Austin <i>et al.</i> (2018)
<i>Desulfovibrio</i>	- Malpighian tubules from <i>H. elliptica</i> - Uterus from <i>H. elliptica</i> - Blood from dog host infected by <i>H. elliptica</i> - Blood from dog host infected by <i>R. sanguineus</i> - Midgut from <i>R. sanguineus</i> - Ovaries from <i>R. sanguineus</i>	Responsible for several diseases, including Parkinson's disease, bacteremia, and inflammatory bowel disease.	Fan <i>et al.</i> (2022)
<i>Rhizobacter</i>	<i>H. elliptica</i> from gnotobiotic host - Midgut from <i>H. elliptica</i> - Ovaries from <i>H. elliptica</i> - Blood from dog host infected by <i>H. elliptica</i> - Blood from dog host infected by <i>R. sanguineus</i> <i>R. sanguineus</i> from gnotobiotic host - Malpighian tubules from <i>R. sanguineus</i> - Uterus from <i>R. sanguineus</i>	Members of this genus are plant pathogens. It is responsible for producing tumors in a wide range of plant hosts.	Caballo-Ponce <i>et al.</i> (2017)
<i>Rickettsiella</i>	Ovaries from <i>H. elliptica</i>	Members of this genus are pathogenic to mammals and are responsible for a rickettsial disease infecting Japanese beetle larvae and associated scarab larvae.	Bouchon <i>et al.</i> (2011)

Table 5-3: (continue)

Genus	Sample type detected from	Significance	Reference
<i>Bacteroides</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i>	Even though members of this genus are substantial in the microbiota of the gastrointestinal tract of mammals, several species are opportunistic human pathogens. They are often responsible for the infection of appendicitis, gastrointestinal surgery, and peritoneal cavity through impeding phagocytosis, abscess development, or inactivating beta-lactam antibiotics.	Wexler (2007)
<i>Sphingomonas</i>	• Blood from dog host infected by <i>H. elliptica</i>	Certain species are harmful to humans, mainly by resulting in non-life-threatening hospital-acquired infections.	Ryan & Adley (2010)
<i>Aeromonas</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Most of these species are associated with diseases of humans. Mainly they are responsible for wound infections, diarrheal disease, or systemic disease in immunocompromised individuals.	Janda & Abbott (2010)

Table 5-3: (continue)

Genus	Sample type detected from	Significance	Reference
<i>Ruminococcus</i>	• <i>H. elliptica</i> from gnotobiotic host • Ovaries from <i>H. elliptica</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i>	One species, namely <i>R. gnavus</i> , is documented to cause Crohn's disease.	Henke <i>et al.</i> (2019)
<i>Comamonas</i>	• Midgut from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • <i>R. sanguineus</i> from gnotobiotic host	Two species namely <i>C. kerstersii</i> and <i>C. testosteroni</i> are documented to cause infections, such as endocarditis and septicemia, in humans.	Ryan <i>et al.</i> (2022)
<i>Veillonella</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Although members of this genus are a normal part of the microbiota in mammalian oral mucus and intestines, several species are responsible for human infections. These species are responsible for endocarditis and osteomyelitis.	Mitsui <i>et al.</i> (2018)
<i>Facklamia</i>	• Malpighian tubules from <i>R. sanguineus</i>	Several species are associated with bacteremia from CNS disease, necrotizing fasciitis, chorioamnionitis, and endocarditis.	Rahmati <i>et al.</i> (2017)
<i>Lactococcus</i>	• <i>R. sanguineus</i> from gnotobiotic host	Several species are implicated to cause diseases mainly of fish.	Rao <i>et al.</i> (2022)

Table 5-3: (continue)

Genus	Sample type detected from	Significance	Reference
<i>Legionella</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	The majority of species of this genus are pathogenic and are responsible for legionellosis.	Carratalà & Garcia-Vidal (2010)
<i>Abiotrophia</i>	• <i>H. elliptica</i> from gnotobiotic host • Blood from dog host infected by <i>R. sanguineus</i>	Certain species are documented to cause endocarditis.	Senn <i>et al.</i> (2006)
<i>Psychrobacter</i>	• Salivary gland from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i>	Certain species cause human infections, like endocarditis or peritonitis.	Deschaght <i>et al.</i> (2012)
<i>Erysipelothrix</i>	• <i>H. elliptica</i> from gnotobiotic host • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	<i>Certain species are pathogens of humans and animals. In humans, E. rhusiopathiae is responsible for erysipeloid. In poultry and domestic pigs, E. rhusiopathiae causes erysipelas. The E. tonsillarum is also documented as a pathogen of dogs, resulting in endocarditis.</i>	Stackebrandt <i>et al.</i> (2006)

Table 5.3: (continued)

Genus	Sample type detected from	Significance	Reference
<i>Helcococcus</i>	• <i>H. elliptica</i> from gnotobiotic host • Blood from dog host infected by <i>R. sanguineus</i>	Several species cause illness in animals and humans. Often associated with wound infections or endocarditis.	Chow & Clarridge III (2014)
<i>Coxiella</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i>	The majority of these species are non-pathogenic and are harboured by ticks. <i>Coxiella</i> -like endosymbionts are required by ticks to aid in reproduction and survival. Certain species, like <i>C. burnetti</i> , are responsible for Q fever in humans and animals.	Duron <i>et al.</i> (2018)
<i>Actinomyces</i>	• <i>H. elliptica</i> from gnotobiotic host • Malpighian tubules from <i>H. elliptica</i> • Malpighian tubules from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Several species are opportunistic pathogens of humans and livestock. Infections result in actinomycosis, endocarditis, periodontal disease, oral-cervicofacial disease, lymphadenopathy, thoracic disease, and abdominal disease.	Könönen & Wade (2015)
<i>Rickettsia</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Several species are well-documented pathogens of humans and animals, and others are non-pathogenic arthropod symbionts. The majority of diseases associated with <i>Rickettsia</i> spp. include Queensland tick typhus, Flinders Island spotted fever, rickettsialpox, Rocky Mountain spotted fever, African tick-bite fever, and boutonneuse fever.	Perlman <i>et al.</i> (2006)

Table 5.3: (continued)

Genus	Sample type detected from	Significance	Reference
<i>Serratia</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Several species are opportunistic pathogens of humans and animals. In particular, <i>S. marcescens</i> , are a hospital-acquired infection responsible for endocarditis, pneumonia, and urinary tract infections. Other species, including <i>S. odoriferae</i> , <i>S. plymuthica</i> , <i>S. rubidaea</i> , and <i>S. liquefaciens</i> cause endocarditis and osteomyelitis.	Mahlen (2011)
<i>Fusobacterium</i>	• <i>H. elliptica</i> from gnotobiotic host • Salivary gland from <i>H. elliptica</i> • Midgut from <i>H. elliptica</i> • Ovaries from <i>H. elliptica</i> • Malpighian tubules from <i>H. elliptica</i> • Uterus from <i>H. elliptica</i> • Blood from dog host infected by <i>H. elliptica</i> • Blood from dog host infected by <i>R. sanguineus</i> • <i>R. sanguineus</i> from gnotobiotic host • Malpighian tubules from <i>R. sanguineus</i> • Midgut from <i>R. sanguineus</i> • Ovaries from <i>R. sanguineus</i> • Uterus from <i>R. sanguineus</i>	Several species are human pathogens. Infection results in topical skin ulcers, periodontal diseases, colorectal cancer, Lemierre's syndrome, neck infections, oral infections, and head infections.	Roberts (2000)
<i>Listeria</i>	• Malpighian tubules from <i>H. elliptica</i>	Several species are pathogens of humans and are associated with listeriosis.	Ramaswamy et al. (2007)

Table 5.3: (continued)

Genus	Sample type detected from	Significance	Reference
<i>Streptomyces</i>	Malpighian tubules from <i>H. elliptica</i>	Several members of this genus are uncommon pathogens of humans and plants. Infections of humans by <i>S. sudanensis</i> and <i>S. somaliensis</i> and in plants by <i>S. scabies</i> , <i>S. cavaresiae</i> , <i>S. turgidiscabies</i> , and <i>S. acidiscabies</i> result in mycetoma.	Watve <i>et al.</i> (2001)
<i>Sutterella</i>	<i>H. elliptica</i> from gnotobiotic host - Salivary gland from <i>H. elliptica</i> - Ovaries from <i>H. elliptica</i> <i>R. sanguineus</i> from gnotobiotic host - Malpighian tubules from <i>R. sanguineus</i> - Midgut from <i>R. sanguineus</i> - Uterus from <i>R. sanguineus</i>	Imbalances of the occurrence of certain species are harmful to humans, resulting in autism spectrum disorder, Crohn's disease, and irritable bowel disease.	Lorber (2013)
<i>Bordetella</i>	<i>R. sanguineus</i> from gnotobiotic host - Uterus from <i>R. sanguineus</i>	All species may cause infection in humans and animals. Several species are associated with kennel cough in animals, especially domestic dogs. In addition, members of this genus are harmful to immunocompromised humans.	Mattoo <i>et al.</i> (2001)
<i>Mycobacterium</i>	- Salivary gland from <i>H. elliptica</i> - Malpighian tubules from <i>H. elliptica</i>	Several species are pathogenic to mammals. Generally, these species are associated with tuberculosis and leprosy.	Getahun <i>et al.</i> (2015)
<i>Rothia</i>	Salivary gland from <i>H. elliptica</i>	Certain species cause diseases in humans, such as intestinal metaplasia and gastric atrophy.	Fox & Wang (2007)
<i>Finegoldia</i>	<i>H. elliptica</i> from gnotobiotic host - Malpighian tubules from <i>H. elliptica</i> - Blood from dog host infected by <i>R. sanguineus</i>	Opportunistic human pathogens.	Levy <i>et al.</i> (2009)

Table 5.3: (continued)

Genus	Sample type detected from	Significance	Reference
<i>Neisseria</i>	Blood from dog host infected by <i>R. sanguineus</i>	Several species are pathogens of humans. Mainly, <i>N. gonorrhoeae</i> is associated with gonorrhea, and <i>N. meningitidis</i> are associated with meningococcal septicaemia and bacterial meningitis.	Rotman & Seifert (2014)
<i>Prevotella</i>	<i>H. elliptica</i> from gnotobiotic host - Blood from dog host infected by <i>H. elliptica</i> <i>R. sanguineus</i> from gnotobiotic host - Midgut from <i>R. sanguineus</i>	Several species cause infection in humans and animals. Infections often result in sinusitis, aspiration pneumonia, chronic otitis media, lung abscess, and pulmonary empyema.	Ley (2016)
<i>Dysgonomonas</i>	<i>R. sanguineus</i> from gnotobiotic host - Malpighian tubules from <i>R. sanguineus</i>	Several species may cause gastroenteritis when infection occurs in immunocompromised patients.	Hofstad <i>et al.</i> (2000)
<i>Klebsiella</i>	<i>H. elliptica</i> from gnotobiotic host number 5 - Salivary gland from <i>H. elliptica</i> (tick number 12) - Blood from dog host infected by <i>H. elliptica</i> - Blood from dog host infected by <i>R. sanguineus</i> - Malpighian tubules from <i>R. sanguineus</i> - Uterus from <i>R. sanguineus</i>	Several species are opportunistic pathogens of humans and animals. Infections generally result in spondyloarthropathies, ankylosing spondylitis, soft tissue infections, pneumonia, peritonitis, urinary tract infections, diarrhea, sepsis, and meningitis.	Paczosa & Meccas (2016)
<i>Roseomonas</i>	- Ovaries from <i>H. elliptica</i> - Blood from dog host infected by <i>R. sanguineus</i> - Malpighian tubules from <i>R. sanguineus</i>	Several species are opportunistic pathogens of humans.	Ioannou <i>et al.</i> (2020)
<i>Leptospira</i>	- Midgut from <i>H. elliptica</i> - Blood from dog host infected by <i>R. sanguineus</i> <i>R. sanguineus</i> from gnotobiotic host	Several species are responsible for leptospirosis in humans and animals.	Bharti <i>et al.</i> (2003)

Table 5.3: (continued)

Genus	Sample type detected from	Significance	Reference
<i>Moraxella</i>	Midgut from <i>R. sanguineus</i>	Several species are opportunistic pathogens of humans and animals. Mainly, <i>M. catarrhalis</i> are associated with pneumonia and tracheobronchitis in humans. Also in humans, <i>M. lacunata</i> infections result in blepharoconjunctivitis. In cattle, <i>M. bovis</i> infections result in bovine keratoconjunctivitis.	Postma <i>et al.</i> (2008)
<i>Enterococcus</i>	Uterus from <i>R. sanguineus</i>	Several species are pathogenic to humans. Infections result in spontaneous bacterial peritonitis, urinary tract infections, meningitis, bacteremia, diverticulitis, and bacterial endocarditis.	García Solache & Rice (2019)
<i>Anaplasma</i>	<i>R. sanguineus</i> from gnotobiotic host	Members of this genus are tick-borne pathogens of humans and animals and are associated with anaplasmosis.	Hosseini-Vasoukolaei <i>et al.</i> (2014)
<i>Proteus</i>	Malpighian tubules from <i>H. elliptica</i>	Several pathogens are opportunistic human pathogens. Infection results in urinary tract infections and wound infections.	Hamilton <i>et al.</i> (2018)
<i>Capnocytophaga</i>	<i>H. elliptica</i> from gnotobiotic host - Malpighian tubules from <i>R. sanguineus</i>	Several species are opportunistic pathogens of animals and humans and are mainly known to cause periodontal disease.	Jan da <i>et al.</i> (2006)
<i>Francisella</i>	<i>H. elliptica</i> from gnotobiotic host - Midgut from <i>H. elliptica</i> - Malpighian tubules from <i>H. elliptica</i> - Uterus from <i>H. elliptica</i> - Blood from dog host infected by <i>R. sanguineus</i> - Uterus from <i>R. sanguineus</i>	Several pathogens are pathogenic to humans and animals. The <i>F. tularensis</i> is associated with rabbit fever, whereas <i>F. philomiragia</i> and <i>F. novicida</i> are associated with invasive systemic infections and sepsis.	Keim <i>et al.</i> (2007)
<i>Plesiomonas</i>	- Blood from dog host infected by <i>R. sanguineus</i> - Midgut from <i>R. sanguineus</i>	Several species are pathogens of humans and animals. Infection results in gastroenteritis or diarrheal infection.	Shigematsu <i>et al.</i> (2000)

Table 5.3: (continued)

Genus	Sample type detected from	Significance	Reference
<i>Streptobacillus</i>	Blood from dog host infected by <i>H. elliptica</i>	The <i>S. moniliformis</i> is associated with Haverhill fever.	Elliott (2007)
<i>Ehrlichia</i>	Blood from dog host infected by <i>H. elliptica</i>	A well-documented tick-transmitted genus of zoonotic pathogens associated with ehrlichiosis.	Dumler (2005)

5.4 Discussion

Several studies document the bacterial communities of ticks by using metabarcoding targeting the 16S *rRNA* gene. Estrada-Peña *et al.* (2020) suggest that identifying and determining bacterial communities and their abundances are generally done utilizing Next-generation sequencing using 16S metabarcoding. There is limited information available on the bacterial communities that are specifically harboured by *R. sanguineus* and *H. elliptica* ticks. One of the aforementioned studies was conducted by René-Martellet *et al.* (2017), where the dominating bacterial communities specifically detected from *R. sanguineus* ticks were members of the *Rickettsia* genus in the nymphal ticks, as well as members of the *Rickettsia*, *Coxiella*, *Acinetobacter*, and *Bacillus* genera of the adult ticks. In another study by Ravi *et al.* (2019) the dominating bacterial communities of *R. sanguineus* were from the genus *Coxiella*. Also similar to the current study, Brinkmann *et al.* (2019) reported the presence of *Rickettsia*, *Coxiella*, *Hemolivia*, *Francisella*, *Theileria*, *Borrelia*, and *Babesia* from *R. sanguineus* and several other tick species, including *R. bursa*, *H. aegyptium*, *H. marginatum*, *H. excavatum*, *Ha. parva* and *D. marginatus* were collected from naturally infected hosts. The dominating bacterial genera detected by Brinkmann *et al.* (2019) were *Coxiella* with a prevalence of 40%, followed by *Francisella* with a prevalence of 22.5%, and *Rickettsia* with a prevalence of 17.5%. René-Martellet *et al.* (2017) reported the presence of members from the *Bacillus*, *Rickettsia*, and *Coxiella* genera of *R. sanguineus* ticks. Cabezas-Cruz *et al.* (2018) stated that the dominating bacterial genera of *Rhipicephalus* ticks include *Variovorax* and *Bacteroides*, whereas the dominating bacterial genera of *Haemaphysalis* ticks include *Agrobacterium*, *Kineococcus*, and *Cyanothece*. In another study that was not based on 16S metabarcoding, da Silva Costa *et al.* (2011) reported the presence of *R. rickettsii* in the ovaries of *R. sanguineus* ticks using histological methods.

Several studies determined the bacterial communities of multiple tick species that were similar to this study but with a difference in the relative abundances. One of the aforementioned studies was from Adegoke *et al.* (2020) that detected the presence of bacterial members of the Bacteroidota, Proteobacteria, Actinobacteriota, and Firmicutes phyla in *H. anatolicum* ticks, along with high abundances of bacterial communities from the *Cutibacterium*, *Staphylococcus*, *Sphingomonas*, and *Corynebacterium* genera. In another study, Papa *et al.* (2020) reported bacterial genera, from several tick species (including *Haemaphysalis*, *Rhipicephalus*, *Dermacentor*, and *Ixodes*), with high abundances of *Proteobacteria* (75.13%), *Actinobacteria* (14.96%), *Firmicutes* (4.81%), and *Bacteroidetes* (3.76%). Papa *et al.* (2020) reported a high abundance of members of the *Coxiella* genus from several tick species. Papa *et al.* (2020) also reported the presence of *Chloroflexi*, *Fusobacteria*, *Cyanobacteria*, *Spirochaetes*, and *Tenericutes* (with combined abundances of less than 2%). Khoo *et al.* (2016) reported the

presence of *Leptospira* spp., *Rickettsia* spp., *Klebsiella* spp., *Coxiella* spp., *Anaplasma* spp., *Acinetobacter* spp., *Ehrlichia* spp., *Staphylococcus* spp., *Mycobacteria* spp., and *Pseudomonas* spp. from several ticks of the *Haemaphysalis* genus collected from domestic animals. Results of the study performed by Lejal *et al.* (2020) were also similar to this study demonstrating that the dominant bacterial genera of *I. ricinus* ticks were members of the *Reyranella*, *Pseudomonas*, *Deinococcus*, *Acinetobacter*, *Polaromonas*, *Halomonas*, *Photobacterium*, *Shewanella*, *Escherichia-Shigella*, and *Tepidimonas*. A study by Karim *et al.* (2017) reported that the 6 dominant bacterial classes were Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacilli, Actinobacteria, and Clostridia in *Rhipicephalus*, *Haemaphysalis*, *Hyalomma*, *Ornithodoros*, and *Argas* tick genera collected from naturally infected livestock hosts. Karim *et al.* (2017) reported that the bacterial families with high abundances, that were similar to this study were Rickettsiaceae, Staphylococcaceae, Coxiellaceae, Enterobacteriaceae, and Clostridiaceae. Karim *et al.* (2017) also reported bacterial genera with high abundances that were similar to this study, including *Staphylococcus*, *Ralstonia*, *Corynebacterium*, and *Clostridium*. Karim *et al.* (2017) stated that these bacterial genera are commonly detected in ticks' microbiome studies and may be acquired from the environment or host surface that ticks are in contact with. Tekin *et al.* (2017) reported the presence of *Shigella* spp., *Francisella* spp., *Rickettsia* spp., and *Coxiella* spp. from *Dermacentor marginatus* and *Rhipicephalus annulatus* collected from humans. Rynkiewicz *et al.* (2015) reported the presence of members from the *Bartonella*, *Francisella*, and *Rickettsia* genera of *Dermacentor variabilis* and *Ixodes scapularis* ticks collected from rodents. Vayssier-Taussat *et al.* (2013) reported the presence of members from the *Rickettsia*, *Anaplasma*, *Francisella*, *Ehrlichia*, *Borrelia*, and *Coxiella* genera from *Ixodes ricinus* ticks collected from vegetation. In a study conducted by Ross *et al.* (2018), it was demonstrated that several bacterial communities, including *Pseudomonas*, *Bacillus*, and Enterobacteriaceae were present internally and on the surface of *I. scapularis* ticks.

Karim *et al.* (2017), Cabezas-Cruz *et al.* (2018), Landesman *et al.* (2019), and Lejal *et al.* (2020) suggest that the bacterial profile differences of several tick species may be a result of differences in the tick's geographical location, developmental stage, host type, and genotype, as well as the health status of the ticks. In addition, Papa *et al.* (2020) and Narasimhan *et al.* (2021) state that ticks may acquire bacteria from the host when taking a blood meal. Regier *et al.* (2019) state that several species of hard ticks, including *R. sanguineus* and *H. elliptica*, feed three times on different hosts during their life cycle when compared to other species that only follow one or two-host life cycle, thus increasing the chances of transmitting certain bacterial communities to or acquiring certain bacterial communities from hosts. Regier *et al.* (2019) and Narasimhan *et al.* (2021) also state that certain bacterial communities may be transmitted through transstadial

transmission during the ticks' different life stages or transovarial transmission from the engorged female ticks to the eggs. Apart from acquiring certain bacteria during feeding Narasimhan *et al.* (2021) states that questing ticks may acquire specific bacterial communities from soil environments during time spent off-host. Generally, these bacterial communities are members of the Actinobacteria, Verrucomicrobia, Plantomycetes, Bacteroidetes, Betaproteobacteria, Alphaproteobacteria, Deltaproteobacteria, and Gammaproteobacteria phyla that are present in the soil in very low abundances. Narasimhan *et al.* (2021) suggest that several of the bacterial communities acquired from soil may be vital to the tick's microbiome, while other bacterial communities may only be contaminants.

This study has demonstrated that the bacterial communities of the ticks collected from naturally infected dog hosts and the bacterial communities in the dog host blood were similar in terms of identity and relative abundance. In addition, it was demonstrated that the bacterial communities of ticks from naturally infected dog hosts and the bacterial communities from ticks from gnotobiotic hosts were also similar in terms of identity but had different relative abundances. One of the major differences between the ticks collected from natural hosts and gnotobiotic hosts was that the relative abundances of *Coxiella* spp. and *Rickettsia* spp. were very high in the ticks collected from gnotobiotic hosts. Cabezas-Cruz *et al.* (2018) suggested that there is limited information available on bacterial communities of ticks collected from gnotobiotic hosts or of bacterial communities harboured by ticks where different factors are compared. One of the above-mentioned studies was conducted by Regier *et al.* (2019), who compared the bacterial communities of ticks collected from different hosts, specifically of ticks collected from wild animal hosts and ticks collected from companion animals. Regier *et al.* (2019) demonstrated that ticks infecting wild animals had higher abundances of Rickettsiaceae, Anaplasmataceae, Spirochaetaceae, and Bartonellaceae, whereas the ticks infecting companion animals had higher abundances of Francisellaceae and Coxiellaceae. Regier *et al.* (2019) also demonstrated that ticks infecting wild animals had high abundances of Lachnospiraceae, Ruminococcaceae, and Carnobacteriaceae which are bacterial members generally inhabiting the animal intestines, indicating that ticks feeding mainly on wild animals may have bacterial communities that are beneficial towards the host. Specifically, Regier *et al.* (2019) reported the presence of members from the *Rickettsia* genus (19.7%) of ticks infecting wild animals including deer, raccoons, boar, as well as domestic dogs. Secondly, they reported the presence of members from the *Coxiella* genus (3.9%) of ticks infecting raccoons and domesticated dogs. Regier *et al.* (2019) also reported the presence of members from the *Anaplasma* genus (19.7%) from ticks infecting wild animals, including boar and roe deer, as well as domesticated dogs. Lastly, they reported the presence of members from the *Staphylococcus* genus (10.5%) of ticks infecting wild animals, including boar and raccoons, as well as

domesticated dogs. Alpha diversity results of the study by Regier *et al.* (2019) demonstrated that the abundance of dominant bacterial communities of ticks infecting wild animals was significantly higher compared to the ticks infecting companion animals. The aforementioned demonstrated that the wild animals had a higher species richness of bacterial communities. Additionally, Regier *et al.* (2019) reported the presence of co-infections (11.8%) within several ticks infecting wild animals by using NGS assays. The aforementioned include a co-infection between *Staphylococcus* spp., *Anaplasma* spp., and *Spirochaetaceae*, another co-infection was between *Staphylococcus* spp., *Rickettsia* spp., and *Coxiella* spp., another co-infection was between *Rickettsia* spp., *Anaplasma* spp., and *Spirochaetaceae*, another co-infection was between *Anaplasma* spp. and *Bartonella* spp., another co-infection was between *Anaplasma* spp. and *Spirochaetaceae*, and lastly there was an infection between *Anaplasma* spp. and *Rickettsia* spp. Papa *et al.* (2020) suggested that co-infections are observed with more ease by using NGS technologies when compared to other conventional or culture-dependent methods for bacterial community detection. In another study conducted by Chicana *et al.* (2019), where the bacterial communities of several tick species, including ticks from the *Ixodes*, *Dermacentor*, and *Haemaphysalis* genera, were compared it was revealed that the ticks feeding on a more diverse group of hosts had more diverse bacterial communities than ticks that fed on a specific host or the ticks that were nest-dwelling. Both studies conducted by Swei & Kwan (2017) and Chicana *et al.* (2019) demonstrated that the host type influences the abundance and members of the microbiome. In another study conducted by Estrada-Peña *et al.* (2020), the bacterial communities between one experimental *Borrelia burgdorferi*-infected larval *Ixodes scapularis* tick with two uninfected *Ixodes scapularis* nymphs were compared. The results of the aforementioned study demonstrated that the identities of the bacterial communities between the infected and uninfected ticks were similar but there was an immense difference between the abundances of the bacterial communities. Estrada-Peña *et al.* (2020) suggested that the composition of bacterial communities forming the microbiome of ticks during the different developmental stages tends to be different. They further stated that the differences in microbial abundances between the different developmental stages of ticks are visibly different but regardless of these differences each bacterial member has a functional role. In the study conducted by Swei & Kwan (2017), it was demonstrated that the *I. pacificus* ticks in the nymphal developmental stage, that fed on lizards during the larval developmental stage had higher abundances of *Rickettsia* spp. as well as a lower species richness of the overall bacterial communities when compared to the *I. pacificus* that fed on *Peromyscus* mice during the larval developmental stage. In a study performed by Hawlena *et al.* (2013), where the bacterial communities of two tick species, namely *D. variabilis* and *I. scapularis*, feeding on rodents were compared, it was revealed that the main factor of the difference between the bacterial communities was due to the different tick species.

Cabezas-Cruz *et al.* (2018) and Narasimhan *et al.* (2021) suggested that in addition to ticks, other obligate blood-feeders, including tsetse flies and bed bugs, require several bacterial endosymbionts to supply B vitamins that are limited or absent in blood, including riboflavin, biotin, and folate. In ticks, these bacteria are mainly *Coxiella*-like endosymbionts, *Rickettsia* spp., *Candidatus Midichloria*, and *Francisella* spp., whereas *Wolbachia* are mainly the endosymbiont in bed bugs, and *Wigglesworthia* are mainly the endosymbiont in tsetse flies. One of the main endosymbionts of particular importance to ticks are members of the *Rickettsia* genus. Results of this study, revealed that *Rickettsia* was one of the dominating genera from all of the sample types from *R. sanguineus* and *H. elliptica* collected from both naturally infected and gnotobiotic hosts. Several *Rickettsia* spp. are important due to the nutritional benefit they supply, mainly the B vitamins that are lacking in ticks' diet. Apart from the blood that lacks vital nutrients for insects that are provided by certain endosymbionts, several insects are in environments that have limited nutrients. In the case of the aforementioned, the endosymbionts may provide necessary nutrients to the insect host (Gurung *et al.*, 2019). The bacterial endosymbionts that are of particular importance to providing nutrients to the insect hosts in nutrient-poor environments are diazotrophic bacteria, including several members from the Firmicutes and Proteobacteria phyla, that fix atmospheric nitrogen. An example of one of the most important interactions between nitrogen-fixing endosymbionts and the insect hosts is between several bacterial members of the Enterobacteriaceae family and the wood-boring beetles originating from Italy or the *Dendroctonus* spp. of bark beetles originating from the USA and Mexico, where the bacterial endosymbionts provide the beetles with nutrients (Geib *et al.*, 2009; Morales-Jiménez *et al.*, 2012). Several nitrogen-fixing endosymbionts were detected in this study, including *Pantoea*, *Citrobacter*, *Pectobacteria*, and *Klebsiella*, which have important interactions with the *Ceratitis capitata* medflies that aid with reproduction and development (Behar *et al.*, 2005). In addition, to supply the ticks with nutrients to aid with their nutritional needs, the endosymbionts also support the ticks with the temperature pressure of the surrounding environment and aid to protect the ticks from certain pathogenic organisms (Gurung *et al.*, 2019).

Several studies document the presence of endosymbionts in ticks. Some of the aforementioned studies were conducted by Al-Khafaji *et al.* (2020) and Oliver *et al.* (2021) where *R. buchneri* was detected in *I. scapularis* midgut, ovaries, and salivary glands. In other studies, conducted by Bagheri *et al.* (2017) and Kwan *et al.* (2017), *R. buchneri*-like endosymbionts were detected in *I. pacificus* midguts and ovaries. Several endosymbionts, including members of the *Rickettsia*, *Candidatus Midichloria*, *Spiroplasma*, and *Francisella* genera were detected in *I. ricinus* malpighian tubules, ovaries, salivary glands, and trachea in studies conducted by Hernández-Jarguín *et al.* (2018) and Olivieri *et al.* (2019). In a study conducted by Gall *et al.* (2016),

Arsenophonus spp., *Francisella* spp., *R. belli*, and *R. peacockii* were detected in *D. andersoni* midguts and salivary glands. Studies conducted by Klyachko *et al.* (2007) and Maldonado-Ruiz *et al.* (2021) detected the presence of *Rickettsia* spp. and *Coxiella*-like endosymbionts in the *A. americanum* midgut, ovaries, muscles, malpighian tubules, and salivary glands. In other studies, by Budachetri *et al.* (2014) and Budachetri *et al.* (2018), *Candidatus* Midichloria mitochondrii and *Francisella*-like endosymbionts were detected in *A. maculatum* ovaries, midguts, and salivary glands. *Francisella*-like and *Coxiella*-like endosymbionts were detected in *H. longicornis* ovaries and Malpighian tubules in studies conducted by M. Wang *et al.* (2018) and Y. Wang *et al.* (2018). Additionally, Karim *et al.* (2017) reported the presence of *Coxiella*, *Rickettsia*, and *Francisella*, which was similar to this study. Several studies, such as those conducted by Van Treuren *et al.* (2015), Thapa *et al.* (2019), and Chauhan *et al.* (2020) demonstrate that the endosymbiont's relative abundance is higher as the *Ixodes* spp. ticks progress from the one to the next developmental stage. Hunter *et al.* (2015) and Bonnet *et al.* (2017) suggest that several endosymbionts, such as *Rickettsia* spp., are of such importance that female adult ticks adapted the ability to transmit them to their offspring through transovarial transmission. The aforementioned is further suggested by studies performed by Van Treuren *et al.* (2015), Zolnik *et al.* (2016), Tokarz *et al.* (2019), and Chauhan *et al.* (2020) where it was demonstrated that the female adult ticks had a higher relative abundance of *Rickettsia* spp. than the male ticks. Additionally, several *Rickettsia* spp. are documented as defensive endosymbionts of ticks. Bonnet *et al.* (2017) stated that in *Dermacentor andersoni* ticks, *R. peacockii* serves as a maternally-acquired endosymbiont that obstructs the transovarial transmission and development of *R. rickettsii*. Another study by Macaluso *et al.* (2002) reported that the co-infection between *R. rhipicephali* and *R. montana* was resisted in *D. variabilis* ticks' ovaries. Similarly, the study by Gall *et al.* (2016) demonstrated that *A. marginale* infection rates of *D. andersoni* were negatively impacted by the presence of *R. belli*, serving as a maternally inherited endosymbiont. In another study by Steiner *et al.* (2008), it was demonstrated that the presence of the maternally-inherited *R. buchneri* in *I. scapularis* male ticks displayed lower infection rates of *B. burgdorferi* when compared to *I. scapularis* ticks without the endosymbiont. Generally, several bacterial members of the *Rickettsia*, *Coxiella*, and *Francisella* genera are some of the most vital endosymbionts of ticks. In this study it was demonstrated that *Coxiella* spp. were detected in *R. sanguineus* and *H. elliptica* ticks collected both from naturally infected and gnotobiotic hosts. However, the relative abundance of *Coxiella* spp. of ticks collected from the gnotobiotic hosts was significantly higher than the ticks collected from naturally infected hosts. Similar to *Rickettsia* spp. it is suggested that several members of the *Coxiella* genus evolved to be endosymbionts of certain tick species (Bonnet *et al.*, 2017). This is commonly demonstrated by the interactions associated between *Coxiella*-LE and certain species of ticks. *Coxiella*-LE was

previously reported in the ovaries, to ensure the transovarial transmission from the female adult ticks to the eggs, and the Malpighian tubules, which may be linked to aiding the tick's host with excretion, nutrition, or osmoregulation (Klyachko *et al.*, 2007; Machado-Ferreira *et al.*, 2011; Lalzar *et al.*, 2014). Even though *Francisella*-LE is mostly undocumented in other arthropods, it is not uncommon in ticks and is considered an alternative obligate symbiont of several species of ticks (Gerhart *et al.*, 2016; Bonnet *et al.*, 2017; Duron *et al.*, 2017).

Bonnet *et al.* (2017) suggested that if certain non-pathogenic bacterial communities are present in ticks, they may influence the diversity and abundance of certain pathogenic bacterial communities. In certain cases, resources are limited, such as specific host tissue or certain nutrients, causing non-pathogenic and pathogenic bacterial communities to be in direct competition, resulting in both bacterial communities nullifying each other. Bonnet *et al.* (2017) also suggest that in addition, to influencing the diversity and abundance, non-pathogenic bacterial communities may also influence the transmission of pathogenic bacterial communities to the vertebrate host when ticks take a blood meal. The non-pathogenic bacterial communities may release molecules that either immunosuppress the tick host, therefore, aiding the growth of pathogenic bacterial communities or inhibiting the development of pathogenic bacterial communities within the tick host. Narasimhan & Fikrig (2015) stated that the gut of ticks serves as the point of access for pathogenic organisms and the bacterial communities colonizing the gut therefore influence the pathogenic organisms. Several studies focus on the influence that the diversity and abundance of bacterial communities have on the tick gut. One of these studies was conducted by Narasimhan *et al.* (2014), where *I. scapularis* ticks were kept in sterile containers during different developmental stages of their life cycle, to avoid contamination of external bacterial communities. This resulted in low abundances of *B. burgdorferi* and their engorgement weight was higher when compared to ticks collected from natural environments. Additionally, Narasimhan *et al.* (2014) demonstrated that *I. scapularis* that took a blood meal from mice, treated with antibiotics, resulted in low abundance of *B. burgdorferi* and improved feeding. In contrast, a study by Abraham *et al.* (2017) demonstrated that certain bacterial communities may facilitate the infections of pathogenic organisms, where it was demonstrated that *A. phagocytophylum* alters the bacterial communities of the gut to support infection.

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CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The *R. sanguineus* was more prevalent than *H. elliptica* across reported countries. The data obtained from the systematic revealed that *R. sanguineus* has a global distribution, whereas *H. elliptica* was only reported in the African continent, particularly in South Africa and several of its neighboring countries. The *R. sanguineus* has the ability to infest several hosts and has excellent adaptation abilities. The global distribution of *R. sanguineus* and the presence of *H. elliptica* in countries close to South Africa may indicate that these ticks are transported from one geographic location to another by several means. The presence of various haemopathogens (bacterial and parasitic) which have been detected from these dog ticks, of the systematic review is cause for concern as imported ticks may potentially harbour haemopathogens resulting in increased prevalence of infections or creating a new niche of infection.

Furthermore, this study demonstrated that *R. sanguineus* and *H. elliptica* ticks infest dogs residing in or admitted to the Potchefstroom Animal Welfare Society (PAWS). This is common as domestic dogs are the preferred hosts of *R. sanguineus*, whereas *H. elliptica* feed on dogs during their adult developmental stage. This data suggests that the PAWS kennels serve as an adequate habitat for both tick species due to the availability of dog hosts and the surrounding vegetation making it perfect for the on-host and off-host developmental stages of these ticks. This study also demonstrated that ticks carry more pathogens than dog hosts, therefore proving that the first hypothesis can be accepted. Several of these ticks were infected with *C. burnetii* or *Rickettsia* spp. which are considered as pathogens of veterinary importance.

Lastly, this study revealed the presence of several bacterial communities harboured by ticks collected by and shared between *R. sanguineus* and *H. elliptica* ticks from naturally infected dog hosts, dog blood, and *R. sanguineus* and *H. elliptica* ticks from gnotobiotic hosts. Several of these bacterial genera were of medical, veterinary, and ecological significance. The ticks naturally infesting dogs and their dog hosts had larger bacterial populations than the colony ticks, therefore proving that the second hypothesis can be accepted. Additionally, several bacterial communities were taxonomically similar among all the sample types but the relative abundances of the bacterial communities of both the dog blood and ticks from natural dog hosts differed compared to the ticks from gnotobiotic hosts, specifically among the *Rickettsia* spp. and *Coxiella* spp. that

were very high in the tick specimens collected from gnotobiotic hosts and where several species are considered as endosymbionts of the ticks.

6.2 Recommendations

- Future studies should use 18S metabarcoding to detect abundance of tick-borne eukaryotic haemoparasites.
- Further 16S rRNA metabarcoding study should be conducted to determine bacterial communities in tick fluid or secretions, such as haemolymph, saliva and faeces and from different developmental stages of ticks including, egg, larva, nymph, and adult stages.

ANNEXURES

Appendix 3A: JBI quality assessment checklist for prevalence studies



JBI Critical Appraisal Checklist for Studies Reporting Prevalence Data

Reviewer _____ Date _____

Author _____ Year _____ Record Number _____

	Yes	No	Unclear	Not applicable
1. Was the sample frame appropriate to address the target population?	☐	☐	☐	☐
2. Were study participants sampled in an appropriate way?	☐	☐	☐	☐
3. Was the sample size adequate?	☐	☐	☐	☐
4. Were the study subjects and the setting described in detail?	☐	☐	☐	☐
5. Was the data analysis conducted with sufficient coverage of the identified sample?	☐	☐	☐	☐
6. Were valid methods used for the identification of the condition?	☐	☐	☐	☐
7. Was the condition measured in a standard, reliable way for all participants?	☐	☐	☐	☐
8. Was there appropriate statistical analysis?	☐	☐	☐	☐
9. Was the response rate adequate, and if not, was the low response rate managed appropriately?	☐	☐	☐	☐

Overall appraisal: Include ☐ Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)

Appendix 3B: JBI quality assessment score of each study included in the systematic review

Study reference	JBI quality assessment question number									Quality assessment score (n/9) ^a	overall score (%)
	1	2	3	4	5	6	7	8	9		
Ramos <i>et al.</i> (2014)	1	1	1	1	1	1	1	1	NA	8	88.9
Horak <i>et al.</i> (2009)	1	1	1	0	0	1	1	1	NA	6	66.7
L. L. Chao <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
Dall'Agnol <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
A. B. Silva <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
Chisu <i>et al.</i> (2019)	1	1	1	0	0	1	1	1	NA	6	66.7
Manoj <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Dzul-Rosado <i>et al.</i> (2021)	1	1	1	1	1	1	1	1	NA	8	88.9
Carvalho <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
M. A. B. Santos <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
Vila <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
Mediannikov <i>et al.</i> (2012)	1	1	1	1	1	1	1	1	NA	8	88.9
Trotta <i>et al.</i> (2012)	1	1	1	1	1	1	1	1	NA	8	88.9
Ybañez <i>et al.</i> (2012)	1	1	1	1	1	1	1	1	NA	8	88.9
R. A. Ramos, Giannelli, <i>et al.</i> (2014)	1	1	1	0	0	1	0	1	NA	5	55.6
Beck <i>et al.</i> (2014)	1	1	1	1	0	1	1	1	NA	7	77.8
Maia <i>et al.</i> (2014)	1	1	1	0	0	1	1	1	NA	6	66.7
Chisu <i>et al.</i> (2014)	1	1	1	1	0	1	1	1	NA	7	77.8
Cicuttin <i>et al.</i> (2014)	1	1	1	1	1	1	1	1	NA	8	88.9
Iwakami <i>et al.</i> (2014)	1	1	1	1	0	1	1	1	NA	7	77.8
Barbieri <i>et al.</i> (2014)	1	1	1	1	1	1	1	1	NA	8	88.9
Cicuttin <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Ionita <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Almazán <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Witter <i>et al.</i> (2016)	1	1	1	0	0	1	1	1	NA	6	66.7
Cetinkaya <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Azmi <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Golezardy <i>et al.</i> (2016)	1	1	1	0	0	1	1	1	NA	6	66.7
C. B. da Silva <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Scarpulla <i>et al.</i> (2016)	1	1	1	0	0	1	1	1	NA	6	66.7
Campos-Calderón <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Rogovskyy <i>et al.</i> (2017)	1	1	1	0	0	1	1	1	NA	6	66.7
Licari <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
de Sousa <i>et al.</i> (2018)	1	1	1	1	0	1	1	1	NA	7	77.8
Springer <i>et al.</i> (2018)	1	1	1	1		1	1	1	NA	7	77.8
Hornok <i>et al.</i> (2018)	1	1	1	0	0	1	1	1	NA	6	66.7
L. L. Chao <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
G. M. B. de Oliveira <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Chandra <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9

Penzhorn <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Barradas <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Forlano <i>et al.</i> (2005)	1	1	1	1	1	1	1	1	NA	8	88.9
Rojas <i>et al.</i> (2014)	1	1	1	1	1	1	1	1	NA	8	88.9
Thomas <i>et al.</i> (2016)	1	1	1	1	0	1	1	1	NA	7	77.8
Mediannikov <i>et al.</i> (2011)	1	1	1	1	1	1	1	1	NA	8	88.9
Hsu <i>et al.</i> (2011)	1	1	1	1	1	1	1	1	NA	8	88.9
Bessas <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Sipin <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
González <i>et al.</i> (2004)	1	1	1	1	1	1	1	1	NA	8	88.9
Zabó <i>et al.</i> (2007)	1	1	1	1	0	1	1	1	NA	7	77.8
M'ghirbi & Bouattour (2008)	1	1	1	1	1	1	1	1	NA	8	88.9
Silveira <i>et al.</i> (2009)	1	1	1	1	1	1	1	1	NA	8	88.9
Brianti <i>et al.</i> (2010)	1	1	1	1	1	1	1	1	NA	8	88.9
Bermúdez <i>et al.</i> (2011)	1	1	1	1	1	1	1	1	NA	8	88.9
René <i>et al.</i> (2012)	1	1	1	1	1	1	1	1	NA	8	88.9
Loftis <i>et al.</i> (2013)	1	1	1	1	1	1	1	1	NA	8	88.9
Giannelli <i>et al.</i> (2013)	1	1	1	0	1	1	0	1	NA	6	66.7
Aktas (2014)	1	1	1	0	0	1	1	1	NA	6	66.7
Sándor <i>et al.</i> (2014)	1	1	1	1	0	1	1	1	NA	7	77.8
Cao <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Low <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
de Toledo Vieira <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
Little <i>et al.</i> (2018)	1	1	1	1	0	1	1	1	NA	7	77.8
Oskam <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
Monje <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Alieva <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Ghafar & Amer (2012)	1	1	1	1	1	1	1	1	NA	8	88.9
Ebani <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Sarani <i>et al.</i> (2014)	1	1	1	1	1	1	1	1	NA	8	88.9
Hayati <i>et al.</i> (2020)	1	1	1	1	0	1	1	1	NA	7	77.8
Kumsa <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
De Matos <i>et al.</i> (2008)	1	1	1	1	1	1	1	1	NA	8	88.9
Aydin <i>et al.</i> (2020)	1	1	1	0	0	1	1	1	NA	6	66.7
Billeter <i>et al.</i> (2012)	1	1	1	1	1	1	1	1	NA	8	88.9
Jacobs <i>et al.</i> (2001)	1	1	1	1	1	1	1	1	NA	8	88.9
Inokuma <i>et al.</i> (2001)	1	1	1	1	1	1	1	1	NA	8	88.9
Andoh <i>et al.</i> (2013)	1	1	1	1	1	1	1	1	NA	8	88.9
Wallach <i>et al.</i> (2008)	1	1	1	1	0	1	1	1	NA	7	77.8
Sosa-Gutiérrez <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Watanabe <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Bogunović <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
H. A. Santos <i>et al.</i> (2013)	1	1	1	1	1	1	1	1	NA	8	88.9
Adamu <i>et al.</i> (2014)	1	1	1	1	1	1	1	1	NA	8	88.9

Rautenbach <i>et al.</i> (1991)	1	1	1	1	1	1	1	1	NA	8	88.9
J. C. P. de Oliveira <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Ferreira <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
J. S. Costa <i>et al.</i> (2019)	1	1	1	0	0	1	1	1	NA	6	66.7
Sthitmatee <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Potkonjak <i>et al.</i> (2013)	1	1	1	1	1	1	1	1	NA	8	88.9
Díaz-Medina <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Cordeiro <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Sparagano <i>et al.</i> (2003)	1	1	1	1	1	1	1	1	NA	8	88.9
Yuasa <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
Horak (1995)	1	1	1	1	1	1	1	1	NA	8	88.9
Jacques <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Aktas <i>et al.</i> (2009)	1	1	1	1	0	1	1	1	NA	7	77.8
Aktas <i>et al.</i> (2013)	1	1	1	1	1	1	1	1	NA	8	88.9
de Almeida <i>et al.</i> (2013)	1	1	1	1	1	1	1	1	NA	8	88.9
De Waal <i>et al.</i> (2020)	1	1	1	1	0	1	1	1	NA	7	77.8
Khamassi Khbou <i>et al.</i> (2021)	1	1	1	1	1	1	1	1	NA	8	88.9
Augustine <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
Balinandi <i>et al.</i> (2020)	1	1	1	1	0	1	1	1	NA	7	77.8
Tsai <i>et al.</i> (2011)	1	1	1	1	1	1	1	1	NA	8	88.9
Burroughs <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
L. L. Chao <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
L.-L. Chao <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
Konto <i>et al.</i> (2014)	1	1	1	1	1	1	1	1	NA	8	88.9
Ayan <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
I. N. da Costa <i>et al.</i> (2021)	1	1	1	1	0	1	1	1	NA	7	77.8
Dantas-Torres <i>et al.</i> (2010)	1	1	1	1	1	1	1	1	NA	8	88.9
A. P. D. Costa <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Araujo <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Arroyave <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Dantas-Torres <i>et al.</i> (2009)	1	1	1	1	1	1	1	1	NA	8	88.9
J. C. P. Oliveira <i>et al.</i> (2021)	1	1	1	1	1	1	1	1	NA	8	88.9
Rozental <i>et al.</i> (2002)	1	1	1	1	1	1	1	1	NA	8	88.9
Cordeiro <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Campos, Cunha, Machado, Telleria, <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Campos, Cunha, Machado, Nadal, <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
de Farias Rotondano <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Dantas-Torres (2009)	1	1	1	1	1	1	1	1	NA	8	88.9
Nascimento <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Dumitrache <i>et al.</i> (2014)	1	1	1	1	0	1	1	1	NA	7	77.8
Petney <i>et al.</i> (2004)	1	1	1	1	0	1	1	1	NA	7	77.8
Neves <i>et al.</i> (2004)	1	1	1	1	1	1	1	1	NA	8	88.9
Bryson <i>et al.</i> (2000)	1	1	1	1	1	1	1	1	NA	8	88.9

Kumsa <i>et al.</i> (2012)	1	1	1	1	0	1	1	1	NA	7	77.8
Roshan <i>et al.</i> (2021)	1	1	1	1	1	1	1	1	NA	8	88.9
Ferrell <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
Fonseca <i>et al.</i> (2020)	1	1	1	1	0	1	1	1	NA	7	77.8
Galaviz-Silva <i>et al.</i> (2013)	1	1	1	1	1	1	1	1	NA	8	88.9
Greay <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Soares <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Hazihan <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
Gillingham <i>et al.</i> (2020)	1	1	1	1	0	1	1	1	NA	7	77.8
Shimada <i>et al.</i> (2003)	1	1	1	1	0	1	1	1	NA	7	77.8
Papazahariadou <i>et al.</i> (2003)	1	1	1	1	1	1	1	1	NA	8	88.9
Pacheco <i>et al.</i> (2011)	1	1	1	1	1	1	1	1	NA	8	88.9
Toledo <i>et al.</i> (2011)	1	1	1	1	1	1	1	1	NA	8	88.9
Fritz <i>et al.</i> (2012)	1	1	1	1	1	1	1	1	NA	8	88.9
Szabó <i>et al.</i> (2010)	1	1	1	1	1	1	1	1	NA	8	88.9
Motaghipisheh <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Banafshi <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
Rahmani-Varmale <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
Goldberg <i>et al.</i> (2002)	1	1	1	1	1	1	1	1	NA	8	88.9
Sabatini <i>et al.</i> (2010)	1	1	1	1	0	1	1	1	NA	7	77.8
A. P. Costa <i>et al.</i> (2013)	1	1	1	1	1	1	1	1	NA	8	88.9
Szabó <i>et al.</i> (2001)	1	1	1	1	1	1	1	1	NA	8	88.9
Kamani <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
Kamani <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
Klimpel <i>et al.</i> (2010)	1	1	1	1	1	1	1	1	NA	8	88.9
Kolo <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Köseoglu <i>et al.</i> (2021)	1	1	1	1	1	1	1	1	NA	8	88.9
Laatamna <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Laatamna <i>et al.</i> (2021)	1	1	1	1	1	1	1	1	NA	8	88.9
Latrofa <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
Satta <i>et al.</i> (2011)	1	1	1	1	1	1	1	1	NA	8	88.9
Lee <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Leulmi <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Lineberry <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Marquez <i>et al.</i> (2008)	1	1	1	1	1	1	1	1	NA	8	88.9
Ramezani <i>et al.</i> (2014)	1	1	1	1	0	1	1	1	NA	7	77.8
Kolo <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Mirzaei <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Cervantes S <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Rodriquez <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Mumcuoglu <i>et al.</i> (1993)	1	1	1	1	1	1	1	1	NA	8	88.9
Melo <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Aktas & Özübek (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
Aktas & Özübek (2018)	1	1	1	1	1	1	1	1	NA	8	88.9

Cevidanes <i>et al.</i> (2021)	1	1	1	1	1	1	1	1	NA	8	88.9
Ndip <i>et al.</i> (2010)	1	1	1	1	1	1	1	1	NA	8	88.9
V. L. Nguyen <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
V. L. Nguyen <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Nijhof <i>et al.</i> (2007)	1	1	1	1	0	1	1	1	NA	7	77.8
Hassissen <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
P. B. de Oliveira <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
Oyarzún-Ruiz <i>et al.</i> (2021)	1	1	1	1	1	1	1	1	NA	8	88.9
Davari <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
J. Zhang <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
Cicculli <i>et al.</i> (2019)	1	1	1	1	0	1	1	1	NA	7	77.8
Díaz-Sánchez <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Sharifdini <i>et al.</i> (2021)	1	1	1	1	1	1	1	1	NA	8	88.9
Gehrke <i>et al.</i> (2009)	1	1	1	1	1	1	1	1	NA	8	88.9
Márquez & Millán (2009)	1	1	1	1	1	1	1	1	NA	8	88.9
Kamani <i>et al.</i> (2013)	1	1	1	1	1	1	1	1	NA	8	88.9
Ereqat <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Diarra <i>et al.</i> (2017)	1	1	1	1	0	1	1	1	NA	7	77.8
Quintero V <i>et al.</i> (2017)	1	1	1	1	0	1	1	1	NA	7	77.8
F. B. Costa <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
Agu <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Proboste <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Qablan <i>et al.</i> (2012)	1	1	1	1	1	1	1	1	NA	8	88.9
R. A. N. Ramos <i>et al.</i> (2013)	1	1	1	1	1	1	0	1	NA	7	77.8
René-Martellet <i>et al.</i> (2015)	1	1	1	0	0	1	1	1	NA	6	66.7
Medeiros-Silva <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Li <i>et al.</i> (2015)	1	1	1	0	0	1	1	1	NA	6	66.7
Xu <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Pennisi <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Andersson <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
Greay <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
Maurelli <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
Livanova <i>et al.</i> (2018)	1	1	1	1	0	1	1	1	NA	7	77.8
Galay <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
Regier <i>et al.</i> (2019)	1	1	1	1	0	1	1	1	NA	7	77.8
Checa <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
Ortega-Morales <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
Saleh <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
Choe <i>et al.</i> (2011)	1	1	1	1	0	1	1	1	NA	7	77.8
M. A. B. Santos <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
Minervino <i>et al.</i> (2015)	1	1	1	1	0	1	1	1	NA	7	77.8
Solano-Gallego <i>et al.</i> (2012)	1	1	1	1	1	1	1	1	NA	8	88.9
Tarasevich <i>et al.</i> (1991)	1	1	1	1	1	1	1	1	NA	8	88.9
López-Pérez <i>et al.</i> (2022)	1	1	1	0	0	1	1	1	NA	6	66.7

Temoche <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
Tsai <i>et al.</i> (2012)	1	1	1	0	0	1	1	1	NA	6	66.7
Medrano-Bugarini <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
Merino <i>et al.</i> (2005)	1	1	1	1	1	1	1	1	NA	8	88.9
Moerbeck <i>et al.</i> (2016)	1	1	1	0	0	1	1	1	NA	6	66.7
Poo-Muñoz <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Durden <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Aktas & Özübek (2017b)	1	1	1	0	0	1	1	1	NA	6	66.7
Low <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
Prakash, Low, Tan, <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
Prakash, Low, Vinnie-Siow, <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
B. C. M. Oliveira <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
Beeler <i>et al.</i> (2011)	1	1	1	0	0	1	1	1	NA	6	66.7
L. Zhang <i>et al.</i> (2012)	1	1	1	0	0	1	1	1	NA	6	66.7
Horak <i>et al.</i> (2010)	1	1	1	1	1	1	1	1	NA	8	88.9
Henning <i>et al.</i> (2014)	1	1	1	0	0	1	1	1	NA	6	66.7
Araes-Santos <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Shoorijeh <i>et al.</i> (2008)	1	1	1	1	1	1	1	1	NA	8	88.9
Mengfan <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Kiouani <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Vieira <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
Wells <i>et al.</i> (2012)	1	1	1	1	1	1	1	1	NA	8	88.9
Lucero-Velasco <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
V. N. Ramos <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
B. R. D. Silva <i>et al.</i> (2017)	1	1	1	1	1	1	1	1	NA	8	88.9
Zanet <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Zheng <i>et al.</i> (2019)	1	1	1	0	0	1	1	1	NA	6	66.7
Khrouf <i>et al.</i> (2014)	1	1	1	1	1	1	1	1	NA	8	88.9
Yaglom <i>et al.</i> (2018)	1	1	1	1	1	1	1	1	NA	8	88.9
Pieracci <i>et al.</i> (2019)	1	1	1	1	1	1	1	1	NA	8	88.9
H. M. Nguyen <i>et al.</i> (2020)	1	1	1	1	1	1	1	1	NA	8	88.9
Diyas & Rajakaruna (2015)	1	1	1	1	0	1	1	1	NA	7	77.8
Miranda & Mattar (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Fryxell <i>et al.</i> (2012)	1	1	1	1	1	1	1	1	NA	8	88.9
Dantas-Torres <i>et al.</i> (2012)	1	1	1	0	0	1	1	1	NA	6	66.7
Pat-Nah <i>et al.</i> (2015)	1	1	1	1	1	1	1	1	NA	8	88.9
Persichetti <i>et al.</i> (2016)	1	1	1	1	1	1	1	1	NA	8	88.9
Özübek & Aktas (2018)	1	1	1	0	0	1	1	1	NA	6	66.7

^aTotal score out of 9; n: Number; NA: Not applicable

Appendix 5

Phylum	Class	Order	Family	Genus
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	C39
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales		
Patescibacteria	Parcubacteria			
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	uncultured
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	
Patescibacteria	Parcubacteria	uncultured bacterium	uncultured bacterium	uncultured bacterium
Proteobacteria	Alphaproteobacteria			
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 7
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	
Bacteroidetes	Bacteroidia	Bacteroidales	Dysgonomonadaceae	uncultured
Proteobacteria	Gammaproteobacteria	Xanthomonadales		
Epsilonbacteraeota	Campylobacteria	Campylobacterales	Thiovulaceae	Sulfurimonas
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio
Firmicutes	Clostridia	DTU014	uncultured bacterium	uncultured bacterium
Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	uncultured
Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Arcicella
Firmicutes	Bacilli	Bacillales		
Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardaceae	Saccharopolyspora
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Desulfobulbus
Kiritimatiellaeota	Kiritimatiellae	WCHB1-41		
Actinobacteria	Thermoleophilia	Gaiellales		
Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto 7
Acidobacteria	Subgroup 17			
Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	uncultured
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Pajaroellobacter

Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Prostheco bacter
Patescibacteria	Parcubacteria	Candidatus Vogelbacteria	Candidatus Vogelbacteria bacterium RIFOXYB1	Candidatus Vogelbacteria bacterium RIFOXYB1
Patescibacteria	Parcubacteria	Candidatus Adlerbacteria	metagenome	metagenome
FCPU426	uncultured bacterium	uncultured bacterium	uncultured bacterium	uncultured bacterium
Nanoarchaeaeota	Woese archaeia	Candidatus Pacearchaeota archaeon CG1	Candidatus Pacearchaeota archaeon CG1	Candidatus Pacearchaeota archaeon CG1
Verrucomicrobia	Verrucomicrobiae	Opitutales	Opitutaceae	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Pelosinus
Proteobacteria	Deltaproteobacteria	NB1-j	uncultured organism	uncultured organism
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	
Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	
Planctomycetes	Phycisphaerae	Tepidisphaerales	WD2101 soil group	uncultured bacterium
Actinobacteria	Thermoleophilia	Gaiellales	uncultured	
Proteobacteria	Alphaproteobacteria	Rickettsiales		
Chloroflexi	Dehalococcoidia	S085	metagenome	metagenome
Actinobacteria	Actinobacteria			
Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Aquaspirillaceae	Rivicola
Planctomycetes	vadinHA49	uncultured Antarctic bacterium LB3-13	uncultured Antarctic bacterium LB3- 13	uncultured Antarctic bacterium LB3- 13
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	
Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl clade
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Neisseriaceae	
Proteobacteria	Gammaproteobacteria			
Nanoarchaeaeota	Woese archaeia	Candidatus Staskawiczbacteria bacterium RIFOXYA2	Candidatus Staskawiczbacteria bacterium RIFOXYA2	Candidatus Staskawiczbacteria bacterium RIFOXYA2
Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29 marine group
Bacteroidetes	Bacteroidia			
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans
Proteobacteria	Alphaproteobacteria	Sneathiellales	Sneathiellaceae	Sneathiella

Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Acidovorax
Planctomycetes	vadinHA49			
Cyanobacteria	Melainabacteria	Obscuribacterales		
Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	ADurb.Bin063-1
Firmicutes	Clostridia	Clostridiales	Family XI	Anaerococcus
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
Bacteroidetes	Bacteroidia	Bacteroidales	Paludibacteraceae	
Lentisphaerae	Oligosphaeria	Oligosphaerales	Lenti-02	metagenome
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Neisseriaceae	uncultured
Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	WCHB1-32
Patescibacteria	Gracilbacteria	Absconditabacteriales (SR1)		
Chloroflexi	Anaerolineae	Caldilineales	Caldilineaceae	uncultured bacterium
Firmicutes	Bacilli	Bacillales	Family XII	Exiguobacterium
Patescibacteria	Parcubacteria	Candidatus Kaiserbacteria		
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	uncultured
Proteobacteria	Deltaproteobacteria	Oligoflexales	Oligoflexaceae	uncultured
Firmicutes	Bacilli	Bacillales	Family XI	Gemella
Patescibacteria	Gracilbacteria	Absconditabacteriales (SR1)		
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacterium
Proteobacteria	Gammaproteobacteria	JG36-TzT-191		
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Gallionellaceae	Candidatus Nitrotoga
Chlamydiae	Chlamydiae	Chlamydiales	cvE6	uncultured bacterium
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Pir4 lineage
Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae	Candidatus Koribacter
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Uliginosibacterium
Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	
Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	BSV13
Bacteroidetes	Bacteroidia	Chitinophagales	Saprospiraceae	uncultured
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Methylophilus
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Arenimonas
Bacteroidetes	Bacteroidia	Bacteroidales	Paludibacteraceae	Paludibacter

Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Muricauda sp. LR3
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Aquabacterium
Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	Azospirillum
Proteobacteria	Alphaproteobacteria	Holosporales	Holosporaceae	Holospora
Patescibacteria	Parcubacteria	Candidatus Nomurabacteria		
Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Spirochaeta 2
Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	
Proteobacteria	Gammaproteobacteria	Diplorickettsiales	Diplorickettsiaceae	Aquicella
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Thermomonas
Patescibacteria				
Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	uncultured
Proteobacteria	Gammaproteobacteria	Methylococcales	Methylomonaceae	
Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Phascolarctobacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Curvibacter
Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	Gemmata
Acidobacteria	Blastocatellia (Subgroup 4)	Pyrinomonadales	Pyrinomonadaceae	RB41
Bacteroidetes	Bacteroidia	Chitinophagales	uncultured	uncultured Cytophagales bacterium
Proteobacteria	Alphaproteobacteria	Dongiales	Dongiaceae	Dongia
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	OM27 clade
Bacteroidetes	Bacteroidia	Flavobacteriales	Crocinitomicaceae	Fluviicola
Patescibacteria	Parcubacteria	Candidatus Campbellbacteria		
Bacteroidetes	Bacteroidia	Sphingobacteriales	env.OPS 17	
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	
Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	DEV007	
Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae R-7 group

Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas
Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	uncultured
Proteobacteria	Alphaproteobacteria	Rhizobiales		
Planctomycetes	Phycisphaerae	Tepidisphaerales	WD2101 soil group	
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Marivita
Armatimonadetes	Fimbriimonadia	Fimbriimonadales	Fimbriimonadaceae	
Cyanobacteria	Oxyphotobacteria			
Proteobacteria	Gammaproteobacteria	Cellvibrionales	Haliaceae	OM60(NOR5) clade
Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	Pedosphaera
Patescibacteria	Saccharimonadia	Saccharimonadales		
Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	
Nitrospirae	Thermodesulfovibrionia	uncultured	uncultured bacterium	uncultured bacterium
Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	uncultured
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus
Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium
Actinobacteria	Actinobacteria	Micrococcales		
Cyanobacteria	Oxyphotobacteria	Limnotrichales	Limnotrichaceae	Limnothrix
Cyanobacteria	Oxyphotobacteria	Nostocales		
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Alcaligenes
Acidobacteria	c5LKS83	uncultured bacterium	uncultured bacterium	uncultured bacterium
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	
Patescibacteria	Parcubacteria	uncultured soil bacterium	uncultured soil bacterium	uncultured soil bacterium
Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter
Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	
Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides
Lentisphaerae	Lentisphaeria	Victivallales	PRD18C08	uncultured bacterium
Verrucomicrobia	Verrucomicrobiae	Opitutales	Opitutaceae	Alterococcus
Nanoarchaeaeota	Woesearchaeia			
Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae (Subgroup 3)	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Hydrogenophaga
Acidobacteria	Subgroup 6			

Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Lacihabitans
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	Luteolibacter
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Confluentibacter
Patescibacteria	ABY1	Candidatus Uhrbacteria	uncultured bacterium	uncultured bacterium
Proteobacteria	Deltaproteobacteria	Deltaproteobacteria Incertae Sedis	Syntrophorhabdaceae	Syntrophorhabdus
Patescibacteria	Parcubacteria	Candidatus Adlerbacteria		
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Paucibacter
Elusimicrobia	Endomicrobia	Endomicrobiales	Endomicrobiaceae	Candidatus Endomicrobium
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	
Zixibacteria	uncultured bacterium	uncultured bacterium	uncultured bacterium	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio
Gemmatimonadetes	BD2-11 terrestrial group			
Patescibacteria	Gracilibacteria	uncultured soil bacterium	uncultured soil bacterium	uncultured soil bacterium
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Pirellula
Chloroflexi	Anaerolineae	Caldilineales	Caldilineaceae	uncultured
Acidobacteria				
Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	
Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Nitrosomonas
Epsilonbacteraeota	Campylobacteria	Campylobacterales	Arcobacteraceae	Arcobacter
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoracaceae	Peredibacter
Nanoarchaeaetota	Woesearchaeia	uncultured euryarchaeote	uncultured euryarchaeote	uncultured euryarchaeote
Proteobacteria	Deltaproteobacteria	MBNT15		
Planctomycetes	Phycisphaerae	mle1-8	uncultured bacterium	uncultured bacterium
Planctomycetes	OM190			
Proteobacteria	Deltaproteobacteria			
Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	Ohtaekwangia
Firmicutes	Clostridia	Clostridiales	Family XII	Acidaminobacter
Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadaceae	
Proteobacteria	Deltaproteobacteria	Myxococcales	Sandaracinaceae	uncultured

Patescibacteria	Gracilibacteria			
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Delftia
Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	
Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Tibeticola
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	
Proteobacteria	Alphaproteobacteria	Paracaedibacterales	Paracaedibacteraceae	Candidatus Paracaedibacter
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Polyangium
Verrucomicrobia	Verrucomicrobiae	Methylacidiphilales	Methylacidiphilaceae	uncultured
Chloroflexi	SL56 marine group			
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus
Actinobacteria	Acidimicrobiia	IMCC26256	uncultured bacterium Acidimicrobidae	uncultured bacterium Acidimicrobidae
Omnitrophicaeota	uncultured beta proteobacterium	uncultured beta proteobacterium	uncultured beta proteobacterium	uncultured beta proteobacterium
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	uncultured
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	
Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured cyanobacterium	uncultured cyanobacterium
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Caproiciproducens
Bacteroidetes	Bacteroidia	Bacteroidales	M2PB4-65 termite group	
Patescibacteria	Parcubacteria	Candidatus Nomurabacteria	uncultured bacterium	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Methylotenera
Planctomycetes	Phycisphaerae	Phycisphaerales	Phycisphaeraceae	SM1A02
Firmicutes	Bacilli	Bacillales	Planococcaceae	Paenisporosarcina
Synergistetes	Synergistia	Synergistales	Synergistaceae	Cloacibacillus
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	
Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Salinispira
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Magnetospirillaceae	Magnetospirillum
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Flavisolibacter
Patescibacteria	WS6 (Dojkabacteria)			
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	

Proteobacteria	Deltaproteobacteria	Oligoflexales	Oligoflexaceae	Silvanigrella
Bacteroidetes	Bacteroidia	Bacteroidales		
Actinobacteria	Actinobacteria	Propionibacteriales	Nocardoidaceae	
Acidobacteria	Holophagae	Holophagales	Holophagaceae	
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	
Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Polynucleobacter
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Thauera
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Aquaspirillaceae	Aquaspirillum
Actinobacteria	Acidimicrobiia	IMCC26256		
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	OM43 clade
Planctomycetes	Phycisphaerae	Phycisphaerales	Phycisphaeraceae	CL500-3
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus
Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Holdemanella
Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Salinicoccus
Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	
Patescibacteria	Parcubacteria	Candidatus Doudnabacteria	Candidatus Doudnabacteria bacterium RIFCSPHIGHO2	Candidatus Doudnabacteria bacterium RIFCSPHIGHO2
WPS-2	bacterium ADurb.Bin236	bacterium ADurb.Bin236	bacterium ADurb.Bin236	bacterium ADurb.Bin236
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc
Acidobacteria	Subgroup 6	Unknown Order	Unknown Family	Luteitalea
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Agromyces
Proteobacteria	Gammaproteobacteria	Methylococcales	Methylomonaceae	Methylomicrobium
Actinobacteria	Actinobacteria	PeM15		
Patescibacteria	Parcubacteria	Candidatus Moranbacteria		
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Rheinheimera
Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Sulfuritalea

Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Anaerolinea
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga
Proteobacteria	Deltaproteobacteria	Desulfuromonadales		
Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	T34	uncultured beta proteobacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Achromobacter
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Morganella
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	alpha cluster
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum
Bacteroidetes	Bacteroidia	Cytophagales		
Omnitrophicaeota	uncultured organism	uncultured organism	uncultured organism	uncultured organism
Patescibacteria	Saccharimonadia	Saccharimonadales	wastewater metagenome	wastewater metagenome
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-014
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Alloiococcus
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Sulfuricellaceae	Ferritrophicum
Actinobacteria	Acidimicrobiia			
Omnitrophicaeota	uncultured Banisveld landfill bacterium BVC56	uncultured Banisveld landfill bacterium BVC56	uncultured Banisveld landfill bacterium BVC56	uncultured Banisveld landfill bacterium BVC56
Proteobacteria	Gammaproteobacteria	Methylococcales	Methylomonaceae	
Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Aerococcus
Verrucomicrobia	Verrucomicrobiae	Opitutales	Opitutaceae	IMCC26134
Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	Limisphaera
Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
Planctomycetes	Planctomycetacia	Planctomycetales	Schlesneriaceae	Planctopirus
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	uncultured
Proteobacteria	Alphaproteobacteria	Rickettsiales	SM2D12	
Proteobacteria	Deltaproteobacteria	Myxococcales		
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium 1
Proteobacteria	Deltaproteobacteria	NB1-j		

Bacteroidetes	Ignavibacteria	OPB56	uncultured soil bacterium	uncultured soil bacterium
Armatimonadetes	uncultured			
Proteobacteria	Alphaproteobacteria	Paracaedibacterales	Paracaedibacteraceae	Candidatus Captivus
Bacteroidetes	Bacteroidia	Bacteroidales	Tannerellaceae	Macellibacteroides
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloligellaceae	Methyloceanibacter
Bacteroidetes	Bacteroidia	Sphingobacteriales	Z4MB62	
Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacterales Incertae Sedis	uncultured
Firmicutes	Clostridia	Clostridiales	Hellobacteriaceae	Hydrogenispora
Gemmatimonadetes	Longimicrobia	Longimicrobiales	Longimicrobiaceae	uncultured bacterium
Chloroflexi	OLB14			
Planctomycetes	Planctomycetacia	Planctomycetales	uncultured	metagenome
Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto 1
Proteobacteria	Gammaproteobacteria	Methylococcales	Methylococcaceae	
Firmicutes	Clostridia	Clostridiales	Family XII	
Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio
Cyanobacteria	Oxyphotobacteria	Nostocales	Phormidiaceae	Kamptomena PCC-6407
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Confluentimicrobium
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	
WS4	uncultured bacterium	uncultured bacterium	uncultured bacterium	uncultured bacterium
Bacteroidetes	Bacteroidia	Flavobacteriales		
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Hydrogenophilaceae	uncultured
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	
Omnitrophicaeota	Omnitrophia	Omnitrophales	Omnitrophaceae	Candidatus Omnitrophus
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Rhizobacter
Patescibacteria	ABY1	Candidatus Falkowbacteria	uncultured Parcubacteria group bacterium	uncultured Parcubacteria group bacterium
Chlamydiae	Chlamydiae	Chlamydiales	Parachlamydiaceae	Neochlamydia
Bacteroidetes	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	metagenome

Proteobacteria	Gammaproteobacteria	Methylococcales	Methylomonaceae	uncultured
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Ferruginibacter
Spirochaetes	Leptospirae	Leptospirales	Leptospiraceae	Turneriella
Proteobacteria				
Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	MND1
Patescibacteria	Parcubacteria	Candidatus Vogelbacteria	Candidatus Vogelbacteria bacterium RIFOXYD1	Candidatus Vogelbacteria bacterium RIFOXYD1
Hydrogenedentes	Hydrogenedentia	Hydrogenedentiales	Hydrogenedensaceae	
Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	Tangfeifania
Acidobacteria	Subgroup 18			
Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	
Planctomycetes	Phycisphaerae	Phycisphaerales	Phycisphaeraceae	Phycisphaera
Patescibacteria	Parcubacteria	Candidatus Kaiserbacteria	metagenome	metagenome
Acidobacteria	Holophagae	Subgroup 7	uncultured soil bacterium	uncultured soil bacterium
Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes vadinHA17	
Armatimonadetes				
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	
Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	
Proteobacteria	Gammaproteobacteria	Diplorickettsiales	Diplorickettsiaceae	Rickettsiella
Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	uncultured
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoracaceae	Bacteriovorax
Chloroflexi	Anaerolineae	SJA-15		
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Chiayiivirga
Proteobacteria	Gammaproteobacteria	Competibacterales	Competibacteraceae	Candidatus Competibacter
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	uncultured
Cyanobacteria	Oxyphotobacteria	Pseudanabaenales	Pseudanabaenaceae	Synechococcus PCC-7502
Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Spirosoma
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales Incertae Sedis	uncultured
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Proteocatella
Proteobacteria	Alphaproteobacteria	Rhodospirillales		

Proteobacteria	Alphaproteobacteria	uncultured	unidentified bacterioplankton marine	unidentified bacterioplankton marine
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Leptolinea
Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae	Desulfobacca
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	uncultured bacterium
Epsilonbacteraeota	Campylobacteria	Campylobacterales	Thiovulaceae	Thiovulum
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	Roseibacillus
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
Proteobacteria	Gammaproteobacteria	Cardiobacteriales	Wohlfahrtiimonadaceae	Ignatzschineria
BRC1	uncultured soil bacterium PBS-II-34	uncultured soil bacterium PBS-II-34	uncultured soil bacterium PBS-II-34	uncultured soil bacterium PBS-II-34
Bacteroidetes	Ignavibacteria	Ignavibacteriales	LD-RB-34	
Planctomycetes	Phycisphaerae	Tepidisphaerales	CPla-3 termite group	metagenome
Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Aeromonas
Proteobacteria	Alphaproteobacteria	Rhodospirillales	uncultured	unidentified bacterioplankton marine
Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	SH-PL14
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Neisseriaceae	Vitreoscilla
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-013
Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	uncultured
Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	[Cytophaga] xylanolytica group
Proteobacteria	Alphaproteobacteria	uncultured		
Bacteroidetes	Bacteroidia	Sphingobacteriales		
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	DEV007	uncultured bacterium
Proteobacteria	Deltaproteobacteria	Sva0485		
Patescibacteria	Parcubacteria	Candidatus Yanofskybacteria		
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Brevifollis
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	MWH-UniP1 aquatic group
Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	Haliangium
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 2
Bacteroidetes	Ignavibacteria	OPB56		

Bacteroidetes	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	Lentimicrobium
Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Runella
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae RC9 gut group
Cyanobacteria	Oxyphotobacteria	Nostocales	Nostocaceae	
Proteobacteria	Gammaproteobacteria	Cellvibrionales	Cellvibrionaceae	Cellvibrio
Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	
Proteobacteria	Alphaproteobacteria	SAR11 clade	Clade III	uncultured bacterium
Patescibacteria	Parcubacteria	Candidatus Nomurabacteria	Parcubacteria bacterium SCGC AAA011-A08	Parcubacteria bacterium SCGC AAA011-A08
Planctomycetes	OM190	uncultured bacterium	uncultured bacterium	uncultured bacterium
Patescibacteria	Saccharimonadia	Saccharimonadales	metagenome	metagenome
Planctomycetes	Planctomycetacia	Isosphaerales	Isosphaeraceae	
Firmicutes	Clostridia	Clostridiales		
Chloroflexi	Anaerolineae	SBR1031	A4b	
Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Tolomonas
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Comamonas
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Aquibacter
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Veillonella
Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Facklamia
Patescibacteria	Microgenomatia	Candidatus Pacebacteria	uncultured Microgenomates group bacterium	uncultured Microgenomates group bacterium
Omnitrophicaeota				
Patescibacteria	WS6 (Dojkabacteria)	uncultured bacterium	uncultured bacterium	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Sulfuricellaceae	
Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	uncultured
Epsilonbacteraeota	Campylobacteria	Campylobacterales	Helicobacteraceae	uncultured
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Dinghuibacter
Firmicutes	Clostridia	Clostridiales	Family XII	Fusibacter
Verrucomicrobia	Verrucomicrobiae	Opitutales	Opitutaceae	Opitutus
Proteobacteria	Alphaproteobacteria	Rickettsiales	SM2D12	uncultured bacterium
Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Ornithinimicrobium

Patescibacteria	Parcubacteria	uncultured Parcubacteria group bacterium	uncultured Parcubacteria group bacterium	uncultured Parcubacteria group bacterium
Patescibacteria	Microgenomatia	Candidatus Woesebacteria	uncultured bacterium	uncultured bacterium
Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	
Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	
Bacteroidetes	Bacteroidia	Sphingobacteriales	AKYH767	
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia
Bacteroidetes	Bacteroidia	Sphingobacteriales	AKYH767	uncultured bacterium
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Actibacterium
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Providencia
Acidobacteria	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	Subgroup 10
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Leptothrix
Acidobacteria	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	Subgroup 23
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 1
Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella
Bacteroidetes	Bacteroidia	Sphingobacteriales	NS11-12 marine group	uncultured Sphingobacteriia bacterium
Patescibacteria	Parcubacteria	Candidatus Nomurabacteria	Candidatus Nomurabacteria bacterium RIFCSPHIGHO2	Candidatus Nomurabacteria bacterium RIFCSPHIGHO2
Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	uncultured
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Sphaerotilus
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Neisseriaceae	Conchiformibius
Chloroflexi	Anaerolineae			
Proteobacteria	Alphaproteobacteria	Azospirillales		
Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium 1
Proteobacteria	Alphaproteobacteria	Kordiimonadales	Kordiimonadaceae	Kordiimonas
Proteobacteria	Deltaproteobacteria	Oligoflexales	Oligoflexaceae	
Planctomycetes	vadinHA49			
Patescibacteria	Parcubacteria	Candidatus Moranbacteria	uncultured soil bacterium	uncultured soil bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Zoogloea
Kiritimatiellaeota	Kiritimatiellae	WCHB1-41		

Proteobacteria	Gammaproteobacteria	Methylococcales	Methylomonaceae	Crenothrix
Nanoarchaeaeota	Woesearchaeia	uncultured archaeon	uncultured archaeon	uncultured archaeon
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Peptococcus
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	uncultured
Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Flectobacillus
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	Ellin6067
Elusimicrobia	Lineage IIb			
Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	metagenome	metagenome
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Dialister
Chlamydiae	Chlamydiae	Chlamydiales	cvE6	
Bacteroidetes	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	uncultured prokaryote
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys
Proteobacteria	Gammaproteobacteria	UBA10353 marine group		
Deinococcus- Thermus	Deinococci	Deinococcales	Deinococcaceae	Deinococcus
Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	metagenome
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Faecalibacterium
Verrucomicrobia	Verrucomicrobiae	S-BQ2-57 soil group	uncultured bacterium	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Francisellales	Francisellaceae	[Caedibacter] taeniospiralis group
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Chromobacteriaceae	
Proteobacteria	Deltaproteobacteria	Myxococcales	Blrii41	
Firmicutes				
Synergistetes	Synergistia	Synergistales	Synergistaceae	uncultured
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	
Patescibacteria	Microgenomatia	Candidatus Woesebacteria	Candidatus Woesebacteria bacterium RIFCSPHIGHO2	Candidatus Woesebacteria bacterium RIFCSPHIGHO2
Dependentiae	Babeliae	Babeliales		
Verrucomicrobia	Verrucomicrobiae	Opitutales	Opitutaceae	Lacunisphaera
Firmicutes	Bacilli	Bacillales	Planococcaceae	Planococcus
Proteobacteria	Alphaproteobacteria	Rickettsiales	Midichloriaceae	MD3-55

Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Sulfuricellaceae	Sulfuricella
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter
Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis	Unknown Family	Marinicella
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rubribacterium
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	
Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	
Deinococcus-Thermus	Deinococci	Deinococcales	Trueperaceae	Truepera
Verrucomicrobia	Verrucomicrobiae			
Proteobacteria	Alphaproteobacteria	Rickettsiales	SM1B06	uncultured bacterium
Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	Skermanella
Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	uncultured Chloroflexi bacterium
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium
Bacteroidetes	Bacteroidia	Bacteroidales	SB-5	
Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Microlunatus
Verrucomicrobia	Verrucomicrobiae	S-BQ2-57 soil group		
Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-008
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
Bacteroidetes	Bacteroidia	Cytophagales	Cytophagaceae	Cytophaga
Cyanobacteria	Melainabacteria	Obscuribacteriales	uncultured bacterium	uncultured bacterium
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Terrimonas
Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	uncultured
Patescibacteria	Parcubacteria	Candidatus Zambryskibacteria		
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus
Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Legionella
Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	[Eubacterium] coprostanoligenes group
Patescibacteria	Microgenomatia	Candidatus Pacebacteria	uncultured bacterium	uncultured bacterium
Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	SWB02

Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	
Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Solitalea
Proteobacteria	Alphaproteobacteria	SAR11 clade	Clade I	Clade Ia
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	
Patescibacteria	ABY1	Candidatus Magasanikbacteria	uncultured bacterium	uncultured bacterium
WPS-2	metagenome	metagenome	metagenome	metagenome
Patescibacteria	ABY1	Candidatus Magasanikbacteria		
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium
Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Abiotrophia
Patescibacteria	Parcubacteria	Candidatus Azambacteria	uncultured bacterium	uncultured bacterium
Chloroflexi	Anaerolineae	SBR1031	uncultured bacterium	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Psychrobacter
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelothrix
BRC1				
Proteobacteria	Deltaproteobacteria	Desulfarculales	Desulfarculaceae	Desulfatiglans
Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira
Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Blastopirellula
Patescibacteria	ABY1	Candidatus Magasanikbacteria	Candidatus Magasanikbacteria bacterium GW2011	Candidatus Magasanikbacteria bacterium GW2011
Patescibacteria	Kazania	uncultured bacterium	uncultured bacterium	uncultured bacterium
Entotheonellaeota	Entotheonellia	Entotheonellales	Entotheonellaceae	uncultured bacterium
Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	Ilumatobacter
Cyanobacteria	Oxyphotobacteria	Pseudanabaenales	Pseudanabaenaceae	Pseudanabaena PCC-7429
Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto 8
Proteobacteria	Deltaproteobacteria	Oligoflexales	0319-6G20	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	
Bacteroidetes	Bacteroidia	Bacteroidales	Marinifilaceae	Marinifilum
Firmicutes	Clostridia	Clostridiales	Defluviitaleaceae	Defluviitaleaceae UCG-011
Planctomycetes	Phycisphaerae	Tepidisphaerales	Tepidisphaeraceae	uncultured bacterium
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Insolitispirillum
Bacteroidetes	Bacteroidia	Sphingobacteriales	NS11-12 marine group	uncultured Sphingobacterium sp.

Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium
Bacteroidetes	Bacteroidia	Chitinophagales	Saprospiraceae	
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoracaceae	uncultured
Chloroflexi	Gitt-GS-136			
Chloroflexi	Ktedonobacteria	C0119	uncultured bacterium	uncultured bacterium
Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	uncultured
Actinobacteria	Actinobacteria	Frankiales		
Proteobacteria	Alphaproteobacteria	Rhizobiales	A0839	
Latescibacteria	uncultured organism	uncultured organism	uncultured organism	uncultured organism
Cyanobacteria	Oxyphotobacteria	Nostocales	Oscillatoriaceae	
Firmicutes	Clostridia	Clostridiales	Family XI	Helcococcus
Planctomycetes	Planctomycetacia			
Proteobacteria	Gammaproteobacteria	Alteromonadales	Shewanellaceae	Shewanella
Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae	Desulfomonile
Firmicutes	Bacilli	Bacillales	Planococcaceae	Sporosarcina
Patescibacteria	ABY1	Candidatus Uhrbacteria		
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
Chloroflexi	Anaerolineae	SBR1031	metagenome	metagenome
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Trichococcus
Bacteroidetes	Bacteroidia	Sphingobacteriales	NS11-12 marine group	
Nitrospirae	Thermodesulfobivibria	uncultured		
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	uncultured
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	uncultured
Patescibacteria	ABY1	Candidatus Falkowbacteria		
Proteobacteria	Gammaproteobacteria	Coxiellales	Coxiellaceae	Coxiella
Bacteroidetes	Bacteroidia	Sphingobacteriales	env.OPS 17	
Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	Desulfovirga
Proteobacteria	Gammaproteobacteria	Methylococcales	Methylomonaceae	Methyloglobulus
Proteobacteria	Deltaproteobacteria	Myxococcales	Sandaracinaceae	
Proteobacteria	Alphaproteobacteria	Caedibacterales	Caedibacteraceae	Caedibacter
Cyanobacteria	Melainabacteria	Gastranaerophilales		
Actinobacteria	Actinobacteria	Kineosporiales	Kineosporiaceae	

FBP				
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Catenibacterium
Proteobacteria	Alphaproteobacteria	Puniceispirillales	SAR116 clade	
Planctomycetes	Phycisphaerae	Tepidisphaerales	Tepidisphaeraceae	Tepidisphaera
Cyanobacteria	Melainabacteria	Vamprovibrionales	uncultured bacterium	uncultured bacterium
Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	
Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinibacillus
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Parasediminibacterium
Cyanobacteria	Oxyphotobacteria	Nostocales	Phormidiaceae	uncultured
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces
Patescibacteria	Berkelbacteria	uncultured bacterium	uncultured bacterium	uncultured bacterium
Chlamydiae	Chlamydiae	Chlamydiales		
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium
Proteobacteria	Deltaproteobacteria	Desulfuromonadales		
Proteobacteria	Gammaproteobacteria	PLTA13	uncultured bacterium	uncultured bacterium
Epsilonbacteraeota	Campylobacteria	Campylobacteriales		
Epsilonbacteraeota	Campylobacteria	Campylobacteriales	uncultured	
Chloroflexi	Dehalococcoidia	Dehalococcoidales	Dehalococcoidaceae	Dehalogenimonas
Proteobacteria	Deltaproteobacteria	Myxococcales	UASB-TL25	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Rhodoferax
Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardaceae	Pseudonocardia
Planctomycetes	Phycisphaerae	mle1-8		
Acidobacteria	Holophagae	Subgroup 7		
Actinobacteria	Actinobacteria	Micrococcales	Brevibacteriaceae	Brevibacterium
Planctomycetes	Planctomycetacia	Planctomycetales	Schlesneriaceae	Schlesneria
Planctomycetes	Pla4 lineage			
Cyanobacteria	Melainabacteria	Gastranaerophilales		
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Tyzzera 3
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerospira
Bacteroidetes	Bacteroidia	Flavobacteriales	NS9 marine group	uncultured Bacteroidetes bacterium
Actinobacteria				

Patescibacteria	Parcubacteria	Candidatus Nomurabacteria	uncultured Latescibacteria bacterium	uncultured Latescibacteria bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Ferribacterium
Firmicutes	Bacilli	Lactobacillales		
Proteobacteria	Gammaproteobacteria	Cellvibrionales		
Patescibacteria	ABY1	Candidatus Uhrbacteria	Candidatus Uhrbacteria bacterium GW2011	Candidatus Uhrbacteria bacterium GW2011
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Chitinibacteraceae	
Patescibacteria	Parcubacteria	Candidatus Zambryskibacteria	metagenome	metagenome
Bacteroidetes	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	
Proteobacteria	Deltaproteobacteria	PB19	metagenome	metagenome
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Lacibacter
Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	Rickettsia
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-009
Patescibacteria	Parcubacteria	Candidatus Yonathbacteria		
Chloroflexi	KD4-96			
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	uncultured
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Candidatus Anammoximicrobium
Patescibacteria	Parcubacteria	Candidatus Kaiserbacteria	uncultured Parcubacteria group bacterium	uncultured Parcubacteria group bacterium
Proteobacteria	Deltaproteobacteria	Myxococcales	mle1-27	
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Candidatus Planktoluna
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	
Patescibacteria	Gracilibacteria	JGI 0000069-P22		
Spirochaetes	MVP-15	metagenome	metagenome	metagenome
Bacteroidetes	Bacteroidia	Bacteroidales	Barnesiellaceae	uncultured
Actinobacteria	Coriobacteriia	Coriobacteriales	Eggerthellaceae	Senegalimassilia
Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	Fimbrioglobus
Acidobacteria	Subgroup 18			
Patescibacteria	Parcubacteria	Candidatus Zambryskibacteria	Parcubacteria bacterium C7867-006	Parcubacteria bacterium C7867-006
Proteobacteria	Gammaproteobacteria	Cellvibrionales	Halieaceae	
Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Desulfosporosinus

Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Chitinibacteraceae	Formivibrio
Actinobacteria	Actinobacteria	Frankiales	Cryptosporangiaceae	Fodinicola
Actinobacteria	Rubrobacteria	Rubrobacteriales	Rubrobacteriaceae	Rubrobacter
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Chitinibacteraceae	
Dependentiae	Babeliae	Babeliales	metagenome	metagenome
Proteobacteria	Alphaproteobacteria	Holospirales	Holospiraceae	Candidatus Bealeia
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Megasphaera
Bacteroidetes	Bacteroidia	Sphingobacteriales	NS11-12 marine group	uncultured bacterium
Tenericutes	Mollicutes	Entomoplasmatales	Entomoplasmatales Incertae Sedis	Candidatus Hepatoplasma
Proteobacteria	Deltaproteobacteria	Myxococcales	Archangiaceae	Anaeromyxobacter
Patescibacteria	Gracilibacteria			
Verrucomicrobia	Verrucomicrobiae	Chthoniobacteriales	Terrimicrobiaceae	Terrimicrobium
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-005
Proteobacteria	Alphaproteobacteria	Micavibrionales	Micavibrionaceae	uncultured
Proteobacteria	Gammaproteobacteria	211ds20		
Omnitrophicaeota	uncultured bacterium	uncultured bacterium	uncultured bacterium	uncultured bacterium
Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillales Incertae Sedis	Stella
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Pseudohongiellaceae	
Actinobacteria	Actinobacteria	Corynebacteriales		
Proteobacteria	Alphaproteobacteria	Caulobacteriales	Hyphomonadaceae	Hyphomonas
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	oc32
Patescibacteria	Parcubacteria	Candidatus Staskawiczbacteria	Parcubacteria group bacterium GW2011	Parcubacteria group bacterium GW2011
Proteobacteria	Deltaproteobacteria	Myxococcales	Phaselicystidaceae	Phaselicystis
Bacteroidetes	Bacteroidia	Flavobacteriales	Crocinitomicaceae	
Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	uncultured
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Verrucomicrobium
Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	uncultured bacterium	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Hydrogenophilaceae	Thiobacillus
Patescibacteria	Parcubacteria	Candidatus Nomurabacteria	Candidatus Nomurabacteria bacterium GW2011	Candidatus Nomurabacteria bacterium GW2011
Epsilonbacteraeota	Campylobacteria	Campylobacteriales	uncultured	uncultured bacterium

Actinobacteria	Acidimicrobiia	Microtrichales		
Chloroflexi	Chloroflexia	Kallotenuales		
Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Chromobacteriaceae	Paludibacterium
Proteobacteria	Deltaproteobacteria	Sva0485		
Proteobacteria	Gammaproteobacteria	Alteromonadales		
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
Bacteroidetes	Bacteroidia	Chitinophagales	Saprospiraceae	Candidatus Aquirestis
Firmicutes	Bacilli	Bacillales	Bacillaceae	
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfomicrobiaceae	Desulfomicrobium
Proteobacteria	Gammaproteobacteria	Oceanospirillales		
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 9
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter
Actinobacteria	Thermoleophilia	Solirubrobacterales		
Proteobacteria	Alphaproteobacteria	Paracaedibacterales	Paracaedibacteraceae	uncultured
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes vadinHA17	
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Vulcaniibacterium
Proteobacteria	Alphaproteobacteria	Tistrellales	Geminicoccaceae	Candidatus Alysiosphaera
Proteobacteria	Gammaproteobacteria	Cellvibrionales	Spongiibacteraceae	
Patescibacteria	Parcubacteria	Candidatus Kaiserbacteria	groundwater metagenome	groundwater metagenome
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter
Acidobacteria	Acidobacteriia	Subgroup 2	uncultured eubacterium WD264	uncultured eubacterium WD264
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Aquimonas
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	uncultured
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Hahellaceae	Hahella
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Serratia
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Rubripirellula
Patescibacteria	Parcubacteria	Candidatus Kaiserbacteria	uncultured bacterium	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Nitrincolaceae	Corallomonas
Nitrospirae	Thermodesulfovibrionia	uncultured	uncultured prokaryote	uncultured prokaryote
Chlamydiae	Chlamydiae	Chlamydiales	cvE6	Chlamydiales bacterium CRIB 32

Chloroflexi	Anaerolineae	RBG-13-54-9	uncultured bacterium	Caldilineaceae	uncultured bacterium	Caldilineaceae
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae			
Bacteroidetes	Ignavibacteria	Kryptoniales	BSV26	Ignavibacteria	bacterium	RIFCSPLOWO2
Bacteroidetes	Ignavibacteria	Ignavibacteriales	PHOS-HE36			
Bacteroidetes	Bacteroidia	Bacteroidales	Paludibacteraceae	H1		
Proteobacteria	Alphaproteobacteria	Rickettsiales	SM2D12	unidentified bacterioplankton	marine	
Bacteroidetes	Bacteroidia	Sphingobacteriales	AKYH767			
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium		
Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis	Unknown Family	uncultured		
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	uncultured		
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus Methylophilus		
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Propionispira		
Proteobacteria	Alphaproteobacteria	Rickettsiales	Midichloriaceae	Candidatus Cyrtobacter		
Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	Candidatus Megaira		
Firmicutes	Clostridia	Clostridiales	Family XI	Peptoniphilus		
Proteobacteria	Deltaproteobacteria	Oligoflexales	Oligoflexaceae	Oligoflexus		
Acidobacteria	Blastocatellia (Subgroup 4)	DS-100				
Chloroflexi	JG30-KF-CM66	uncultured Chloroflexi bacterium	uncultured Chloroflexi bacterium	uncultured Chloroflexi bacterium		
Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae			
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Hydrogenophilaceae	Tepidiphilus		
Dependentiae	Babeliae	Babeliales	Vermiphilaceae			
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amaricoccus		
Latescibacteria						
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnobacter		
Coprothermobacteraeota	Coprothermobacteria	Coprothermobacterales	Coprothermobacteraceae	Coprothermobacter		
Armatimonadetes	Armatimonadia	Armatimonadales	Armatimonadaceae	Armatimonas		
Firmicutes	Bacilli	Bacillales	Planococcaceae	Kurthia		

Patescibacteria	Parcubacteria	Candidatus Nomurabacteria		
Patescibacteria	Parcubacteria	Candidatus Yonathbacteria	Candidatus Yonathbacteria bacterium RIFOXYC1	Candidatus Yonathbacteria bacterium RIFOXYC1
Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Marinoscillum
Patescibacteria	ABY1			
Nitrospirae	4-29-1			
Elusimicrobia	Elusimicrobia	Lineage IV	Elusimicrobia bacterium GWA2	Elusimicrobia bacterium GWA2
Planctomycetes	Planctomycetacia	Planctomycetales	Schlesneriaceae	
Bacteroidetes	Bacteroidia	Flavobacteriales	NS9 marine group	
Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae	
Firmicutes	Bacilli	Bacillales	Planococcaceae	
Proteobacteria	Alphaproteobacteria	Rickettsiales	S25-593	
Patescibacteria	Parcubacteria	Candidatus Yanofskybacteria		
Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	uncultured bacterium
Chloroflexi	TK10	metagenome	metagenome	metagenome
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Nosocomiicoccus
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rubellimicrobium
Proteobacteria	Gammaproteobacteria	Cardiobacteriales	Wohlfahrtiimonadaceae	
Cyanobacteria	Sericytochromatia	uncultured Desulfuromonadaceae bacterium	uncultured Desulfuromonadaceae bacterium	uncultured Desulfuromonadaceae bacterium
BRC1	uncultured Acidobacteria bacterium	uncultured Acidobacteria bacterium	uncultured Acidobacteria bacterium	uncultured Acidobacteria bacterium
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfocella
Patescibacteria	Parcubacteria	Candidatus Moranbacteria	uncultured bacterium	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Polaromonas
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes BD2-2	
Epsilonbacteraeota	Campylobacteria	Campylobacteriales	Thiovulaceae	Sulfuricurvum
Patescibacteria	Parcubacteria	Candidatus Adlerbacteria	uncultured bacterium	uncultured bacterium
Proteobacteria	Deltaproteobacteria	Desulfarculales	Desulfarculaceae	uncultured
Bacteroidetes	Bacteroidia	Sphingobacteriales	NS11-12 marine group	metagenome
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	

Patescibacteria	Parcubacteria	Candidatus Campbellbacteria	microbial mat metagenome	microbial mat metagenome
Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	
Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	Candidatus Planktophila
Proteobacteria	Gammaproteobacteria	CCM19a		
Lentisphaerae	Lentisphaeria	Victivallales		
Latescibacteria	uncultured Pelobacter sp.	uncultured Pelobacter sp.	uncultured Pelobacter sp.	uncultured Pelobacter sp.
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Chromobacteriaceae	Vogesella
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	TRA3-20	
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Myroides
Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	Planctomicrobium
Patescibacteria	Parcubacteria	Candidatus Jorgensenbacteria	Candidatus Adlerbacteria bacterium GW2011	Candidatus Adlerbacteria bacterium GW2011
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae NK4A214 group
Bacteroidetes	Bacteroidia	Sphingobacteriales	KD3-93	
Proteobacteria	Gammaproteobacteria	Methylococcales	Methylococcaceae	uncultured
Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Cutibacterium
Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadaceae	Candidatus Saccharimonas
Actinobacteria	Coriobacteriia	Coriobacteriales	Atopobiaceae	
Proteobacteria	Deltaproteobacteria	Oligoflexales	0319-6G20	uncultured bacterium
Rokubacteria	NC10	Rokubacteriales	uncultured bacterium	uncultured bacterium
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes vadinHA17	uncultured soil bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	mle1-7
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Novispirillum
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales		
Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	uncultured
Bacteroidetes	Bacteroidia	Cytophagales	Flammeovirgaceae	
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	
Bacteroidetes	Bacteroidia	Chitinophagales	Saprospiraceae	Phaeodactylibacter
Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	OLB12
Proteobacteria	Deltaproteobacteria	Syntrophobacteriales	Syntrophobacteraceae	uncultured
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Acetoanaerobium

Nanoarchaeaeota	Woesearchaeia	Nanoarchaeota archaeon SCGC AAA011-D5	Nanoarchaeota archaeon SCGC AAA011-D5	Nanoarchaeota archaeon SCGC AAA011-D5
Firmicutes	Bacilli	Bacillales	Listeriaceae	Listeria
Crenarchaeota	Bathyarchaeia			
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Magnetospirillaceae	
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	Rubritalea
Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	uncultured
Bacteroidetes	Bacteroidia	Sphingobacteriales	env.OPS 17	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Propionivibrio
Proteobacteria	Deltaproteobacteria	Myxococcales	Sandaracinaceae	Sandaracinus
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae UCG-006
Actinobacteria	Actinobacteria	Micrococcales	Demequinaceae	
Bacteroidetes	Bacteroidia	Chitinophagales	37-13	
Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	
Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces
Patescibacteria	WWE3	uncultured anaerobic bacterium	uncultured anaerobic bacterium	uncultured anaerobic bacterium
Elusimicrobia	Elusimicrobia	Lineage IV	Elusimicrobia bacterium CG1	Elusimicrobia bacterium CG1
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	GWD2-49-16
Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas
Planctomycetes	Phycisphaerae	CCM11a	uncultured bacterium	uncultured bacterium
Firmicutes	Clostridia	Clostridiales	Gracilibacteraceae	Lutispora
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter
Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	possible genus 04
Planctomycetes	Planctomycetacia	Isosphaerales	Isosphaeraceae	uncultured
Verrucomicrobia	Verrucomicrobiae	S-BQ2-57 soil group	uncultured Verrucomicrobiaceae bacterium	uncultured Verrucomicrobiaceae bacterium
Proteobacteria	Alphaproteobacteria	Rhodospirillales	uncultured	uncultured soil bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Thiomonas
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Derxia
Cyanobacteria	Oxyphotobacteria	Nostocales	Phormidiaceae	Planktothrix NIVA-CYA 15
Proteobacteria	Gammaproteobacteria	Steroidobacteriales	Steroidobacteraceae	Steroidobacter
Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae	uncultured

Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Defluviicoccus
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Azohydromonas
Cyanobacteria	Oxyphotobacteria	Nostocales	Phormidiaceae	Tychonema CCAP 1459-11B
Patescibacteria	Parcubacteria	Candidatus Nomurabacteria	uncultured soil bacterium	uncultured soil bacterium
Proteobacteria	Deltaproteobacteria	Syntrophobacteriales	Syntrophobacteraceae	Syntrophobacter
Actinobacteria	Acidimicrobiia	Actinomarinales	uncultured actinobacterium	uncultured actinobacterium
Elusimicrobia	Lineage IIc	uncultured bacterium	uncultured bacterium	uncultured bacterium
Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	uncultured
Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter
Elusimicrobia	Elusimicrobia	MVP-88		
Patescibacteria	Parcubacteria	Candidatus Adlerbacteria	uncultured soil bacterium	uncultured soil bacterium
Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Hathewayia
Chloroflexi	Anaerolineae	SBR1031	A4b	uncultured sludge bacterium S14
Patescibacteria	Berkelbacteria			
Patescibacteria	Parcubacteria	Candidatus Campbellbacteria	uncultured bacterium	uncultured bacterium
Actinobacteria	Acidimicrobiia	Actinomarinales	uncultured	
Bacteroidetes	Ignavibacteria	OPB56		
Patescibacteria	Parcubacteria	Candidatus Zambryskibacteria	Parcubacteria group bacterium GW2011	Parcubacteria group bacterium GW2011
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	A21b	
Proteobacteria	Alphaproteobacteria	Rhizobiales	A0839	uncultured Sphingomonadaceae bacterium
Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	uncultured Verrucomicrobiales bacterium
Bacteroidetes	Bacteroidia	Chitinophagales		
Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	uncultured
Proteobacteria	Deltaproteobacteria	Myxococcales	P3OB-42	
Proteobacteria	Alphaproteobacteria	Caulobacteriales	Hyphomonadaceae	Hirschia
Patescibacteria	Parcubacteria	Candidatus Yanofskybacteria	uncultured bacterium	uncultured bacterium
Proteobacteria	Alphaproteobacteria	Caulobacteriales	Hyphomonadaceae	UKL13-1
Patescibacteria	Microgenomatia	Candidatus Collierbacteria	uncultured bacterium	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Sutterella

Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto 13
Actinobacteria	Actinobacteria	Streptosporangiales	Nocardiopsaceae	Nocardiopsis
Chloroflexi	TK10			
Proteobacteria	Gammaproteobacteria	Acidiferrobacterales	Acidiferrobacteraceae	Sulfurifustis
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	UTCFX1
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Bordetella
Patescibacteria	ABY1	Candidatus Uhrbacteria	uncultured Parcubacteria group bacterium	uncultured Parcubacteria group bacterium
Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	GKS98 freshwater group
Fibrobacteres	Fibrobacteria	Fibrobacterales	possible family 01	
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales Incertae Sedis	
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Fastidiosipila
Elusimicrobia	Elusimicrobia	MVP-88	uncultured bacterium	uncultured bacterium
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Atopostipes
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella
Proteobacteria	Alphaproteobacteria	SAR11 clade		
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes
Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Hypnocyclicus
Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae (Subgroup 3)	Candidatus Solibacter
Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	
Proteobacteria	Gammaproteobacteria	Cellvibrionales	Porticoccaceae	Porticoccus
Dependentiae	Babeliae	Babeliales	UBA12409	uncultured bacterium
Patescibacteria	Parcubacteria	metagenome	metagenome	metagenome
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Jeotgalicoccus
Chloroflexi	TK10	Clostridiales bacterium PH28	Clostridiales bacterium PH28	Clostridiales bacterium PH28
Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Enteractinococcus
Acidobacteria	Subgroup 25			
Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Emticicia
Patescibacteria	ABY1	Candidatus Uhrbacteria		
Acidobacteria	Subgroup 17			
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloiligellaceae	

Acidobacteria	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	Thermoanaerobaculum
Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Rothia
Proteobacteria	Gammaproteobacteria	211ds20	uncultured bacterium	uncultured bacterium
Actinobacteria	Acidimicrobiia	IMCC26256		
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Pelolinea
Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	
Planctomycetes	Planctomycetacia	Planctomycetales		
Proteobacteria	Gammaproteobacteria	SZB7		
Proteobacteria	Deltaproteobacteria	Myxococcales	Nannocystaceae	uncultured
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	HIMB11
Proteobacteria	Deltaproteobacteria			
Latescibacteria	Latescibacteria	Latescibacterales	Latescibacteraceae	
Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Undibacterium
Firmicutes	Bacilli	Bacillales	Planococcaceae	Chryseomicrobium
Patescibacteria	Parcubacteria	Candidatus Nomurabacteria	uncultured Parcubacteria group bacterium	uncultured Parcubacteria group bacterium
Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae	Acholeplasma
Actinobacteria	Acidimicrobiia	Microtrichales	Microtrichaceae	Sva0996 marine group
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Acetobacteroides
Actinobacteria	Acidimicrobiia	uncultured	uncultured Acidimicrobidae bacterium	uncultured Acidimicrobidae bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Neisseriaceae	Uruburuella
Fibrobacteres	Fibrobacteria	Fibrobacterales	B18	uncultured bacterium
Proteobacteria	Deltaproteobacteria	Myxococcales	Blfdi19	uncultured prokaryote
Firmicutes	Clostridia	Clostridiales	Family XI	Finegoldia
Proteobacteria	Gammaproteobacteria	Beggiatoales	Beggiatoaceae	Beggiatoa
Proteobacteria	Gammaproteobacteria	Run-SP154		
Planctomycetes	BD7-11	uncultured marine bacterium	uncultured marine bacterium	uncultured marine bacterium
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Pseudorhodobacter
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Nitrospiraceae	Neptuniibacter
Proteobacteria	Alphaproteobacteria	SAR11 clade	Clade I	

Patescibacteria	Parcubacteria	Candidatus Vogelbacteria		
Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	
Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	Roseimarinus
Nitrospinae	P9X2b3D02			
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Paraperlucidibaca
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Quatrionicoccus
Bacteroidetes	Bacteroidia	Bacteroidales	Tannerellaceae	Parabacteroides
Proteobacteria	Gammaproteobacteria	Diplorickettsiales	Diplorickettsiaceae	uncultured
Patescibacteria	ABY1	Candidatus Falkowbacteria	Candidatus Falkowbacteria bacterium GW2011	Candidatus Falkowbacteria bacterium GW2011
Nitrospinae	P9X2b3D02	uncultured prokaryote	uncultured prokaryote	uncultured prokaryote
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Halodesulfovibrio
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas
Rokubacteria	NC10	Methyloirabilales	Methyloirabilaceae	Sh765B-TzT-35
Cyanobacteria	Melainabacteria	Obscuribacterales		
Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae (Subgroup 3)	Paludibaculum
Patescibacteria	Gracilibacteria	JGI 0000069-P22	uncultured Epsilonproteobacteria bacterium	uncultured Epsilonproteobacteria bacterium
Proteobacteria	Gammaproteobacteria	Methylococcales	Methylococcaceae	Methylocaldum
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Dokdonella
Planctomycetes	Pla4 lineage	uncultured prokaryote	uncultured prokaryote	uncultured prokaryote
Bacteroidetes	Ignavibacteria	Kryptoniales	BSV26	uncultured alpha proteobacterium
Elusimicrobia	Elusimicrobia	Lineage IV	uncultured bacterium	uncultured bacterium
Cyanobacteria	Oxyphotobacteria	Nostocales	Cyanobacteriaceae	
Proteobacteria	Gammaproteobacteria	KI89A clade		
Proteobacteria	Deltaproteobacteria	Myxococcales	Blrii41	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Neisseriaceae	Neisseria
Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae (Subgroup 1)	Granulicella
Epsilonbacteraeota	Campylobacteria	Campylobacteriales	Thiovulaceae	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	TRA3-20	uncultured beta proteobacterium
Cyanobacteria	Sericytochromatia	uncultured bacterium	uncultured bacterium	uncultured bacterium
Planctomycetes	Planctomycetacia	Isosphaerales	Isosphaeraceae	Paludisphaera

Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	Niveispirillum
Patescibacteria	ABY1	Candidatus Magasanikbacteria	Parcubacteria bacterium SG8	Parcubacteria bacterium SG8
Actinobacteria	Actinobacteria	Micrococcales	Dermabacteraceae	Brachybacterium
Bacteroidetes	Bacteroidia	Flavobacteriales	NS9 marine group	uncultured bacterium
Cyanobacteria	Oxyphotobacteria	Nostocales	Nostocaceae	Synechococcus PCC-7902
Verrucomicrobia	Verrucomicrobiae	uncultured	uncultured bacterium	uncultured bacterium
Patescibacteria	Parcubacteria	Candidatus Azambacteria	Candidatus Zambryskibacteria bacterium RIFCSPLOWO2	Candidatus Zambryskibacteria bacterium RIFCSPLOWO2
Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geothermobacter
Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Succinispira
Proteobacteria	Alphaproteobacteria	Rhodospirillales	uncultured	
Cyanobacteria	Oxyphotobacteria	Nostocales	Chroococcidiopsaceae	Gloeocapsa PCC-7428
Chloroflexi	KD4-96	uncultured bacterium	uncultured bacterium	uncultured bacterium
Patescibacteria	Parcubacteria	Candidatus Staskawiczbacteria	uncultured bacterium	uncultured bacterium
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	uncultured
Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	Chromatium
Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Empedobacter
Calditrichaeota	Calditrichia	Calditrichales	Calditrichaceae	Calorithrix
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Aurantimicrobium
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae UCG-004
Proteobacteria	Alphaproteobacteria	Reyranelles	Reyranelleaceae	Reyranelle
Proteobacteria	Alphaproteobacteria	Rickettsiales	AB1	uncultured organism
Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto 12
Acidobacteria	Subgroup 6	metagenome	metagenome	metagenome
Patescibacteria	Microgenomatia	Candidatus Collierbacteria	Candidatus Collierbacteria bacterium GW2011	Candidatus Collierbacteria bacterium GW2011
Proteobacteria	Alphaproteobacteria	Puniceispirillales	uncultured	
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Roseimicrobium
Patescibacteria	Microgenomatia	Candidatus Woesebacteria		
Proteobacteria	Alphaproteobacteria	Rickettsiales	SM2D12	uncultured soil bacterium
Proteobacteria	Alphaproteobacteria	Micavibrionales	uncultured	

Acidobacteria	Blastocatellia (Subgroup 4)	Blastocatellales	Blastocatellaceae	
Nanoarchaeaeota				
Omnitrophicaeota	Omnitrophia			
Patescibacteria	Parcubacteria	Candidatus Vogelbacteria	uncultured bacterium	uncultured bacterium
Patescibacteria	Parcubacteria	Candidatus Lloydbacteria	uncultured bacterium	uncultured bacterium
Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	uncultured verrucomicrobium DEV064
Verrucomicrobia	Verrucomicrobiae	Opitutales	Puniceicoccaceae	
Actinobacteria	Thermoleophilia			
Acidobacteria	Subgroup 22	uncultured bacterium	uncultured bacterium	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Ga0077536	uncultured bacterium	uncultured bacterium
Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Sarcina
Planctomycetes	Phycisphaerae	Tepidisphaerales	CPla-3 termite group	uncultured soil bacterium PBS-22
Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	
Chloroflexi				
Planctomycetes	Planctomycetacia	Planctomycetales	uncultured	
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Defluviimonas
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Chitinibacteraceae	Chitinibacter
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Megamonas
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Georgfuchsia
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Pelomonas
Crenarchaeota	Bathyarchaeia	uncultured methanogenic archaeon	uncultured methanogenic archaeon	uncultured methanogenic archaeon
Proteobacteria	Gammaproteobacteria	Halothiobacillales	Halothiobacillaceae	Thiovirga
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	DEV007	
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	uncultured
Epsilonbacteraeota	Campylobacteria	Campylobacteriales	Sulfurospirillaceae	Sulfurospirillum
Latescibacteria				
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Taibaiella
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Levilinea
Proteobacteria	Alphaproteobacteria	Rickettsiales	bac2nit3	

Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	
Planctomycetes	Phycisphaerae	Tepidisphaerales		
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Propionigenium
Bacteroidetes	Ignavibacteria	OPB56	uncultured sludge bacterium	uncultured sludge bacterium
Cyanobacteria	Oxyphotobacteria	Nostocales	Phormidiaceae	
Patescibacteria	ABY1	Candidatus Falkowbacteria	uncultured bacterium	uncultured bacterium
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium
Actinobacteria	Acidimicrobiia	Microtrichales	uncultured	
Acidobacteria	Subgroup 17	uncultured bacterium	uncultured bacterium	uncultured bacterium
Patescibacteria	Parcubacteria	Candidatus Moranbacteria	Candidatus Moranbacteria bacterium GW2011	Candidatus Moranbacteria bacterium GW2011
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Piscinibacter
Actinobacteria	Coriobacteriia	Coriobacteriales	Eggerthellaceae	
Proteobacteria	Gammaproteobacteria	Steroidobacteriales	Woeseiaceae	Woeseia
Bacteroidetes	Ignavibacteria	Kryptoniales	BSV26	
Proteobacteria	Deltaproteobacteria	Myxococcales	Blfdi19	uncultured bacterium
Bacteroidetes	Bacteroidia	Flavobacteriales	Crocinitomicaceae	Crocinitomix
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae UCG-010
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Subdoligranulum
Patescibacteria	Microgenomatia	Candidatus Pacebacteria		
Acidobacteria	Subgroup 22			
Proteobacteria	Gammaproteobacteria	CCD24		
Elusimicrobia	Lineage IIb	uncultured bacterium	uncultured bacterium	uncultured bacterium
Epsilonbacteraeota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 6
Firmicutes	Clostridia	Clostridiales	Christensenellaceae	uncultured
Epsilonbacteraeota	Campylobacteria	Campylobacterales	Sulfurovaceae	
Planctomycetes	Planctomycetacia	Planctomycetales	Gimesiaceae	Gimesia
Bacteroidetes	Bacteroidia	Bacteroidales	Dysgonomonadaceae	Dysgonomonas
Proteobacteria	Alphaproteobacteria	Elsterales	Elsteraceae	Elstera
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-010
FBP	uncultured bacterium	uncultured bacterium	uncultured bacterium	uncultured bacterium

Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerosinus
Firmicutes	Negativicutes	Selenomonadales		
Proteobacteria	Alphaproteobacteria	Tistrellales	Geminicoccaceae	uncultured
Lentisphaerae	Lentisphaeria			
Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	Thiothrix
Proteobacteria	Gammaproteobacteria	Francisellales	Francisellaceae	
Rokubacteria	NC10	Rokubacteriales		
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerovibrio
Latescibacteria	uncultured prokaryote	uncultured prokaryote	uncultured prokaryote	uncultured prokaryote
Acidobacteria	Subgroup 18	uncultured soil bacterium	uncultured soil bacterium	uncultured soil bacterium
Bacteroidetes	OC31	uncultured bacterium	uncultured bacterium	uncultured bacterium
Nanoarchaea	Woesearchaeia	Candidatus Pacearchaeota archaeon RBG	Candidatus Pacearchaeota archaeon RBG	Candidatus Pacearchaeota archaeon RBG
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis
Verrucomicrobia	Verrucomicrobiae			
Planctomycetes	OM190			
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Thalassospiraceae	Thalassospira
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Tabrizicola
Proteobacteria	Alphaproteobacteria	Holospirales	Holospiraceae	uncultured
Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	uncultured
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus
Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus Udaeobacter
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacter
Actinobacteria	Coriobacteriia	OPB41		
Patescibacteria	Gracilibacteria	Absconditabacteriales (SR1)	metagenome	metagenome
Actinobacteria	Thermoleophilia	Gaiellales	uncultured	
Bacteroidetes	Bacteroidia	Sphingobacteriales	NS11-12 marine group	
Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Cloacibacterium
Verrucomicrobia	Verrucomicrobiae	Opitutales	Opitutaceae	Diplosphaera

Bacteroidetes	Bacteroidia	Sphingobacteriales	LiUU-11-161	
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes vadinHA17	uncultured bacterium
Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter
Spirochaetes	MVP-15			
Proteobacteria	Deltaproteobacteria	Myxococcales	Blrii41	uncultured proteobacterium
Armatimonadetes	Armatimonadia	Armatimonadales		
Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	uncultured
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Marinilactibacillus
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	uncultured
Actinobacteria	Acidimicrobiia	Microtrichales	Iamiaceae	Iamia
Patescibacteria	Gracilbacteria	Candidatus Peregrinibacteria	uncultured bacterium	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	[Desulfobacterium] catecholicum group
Planctomycetes	Phycisphaerae	Tepidisphaerales	WD2101 soil group	uncultured soil bacterium
Proteobacteria	Deltaproteobacteria	NB1-j	hydrothermal vent metagenome	hydrothermal vent metagenome
Chloroflexi	Chloroflexia	Kallotenuales	AKIW781	uncultured bacterium
Synergistetes	Synergistia	Synergistales	Synergistaceae	JGI-0000079-D21
Proteobacteria	Alphaproteobacteria	Elsterales	uncultured	
Proteobacteria	Alphaproteobacteria	Rickettsiales	SM2D12	uncultured alpha proteobacterium
Proteobacteria	Alphaproteobacteria	Rickettsiales	SM2D12	
Thaumarchaeota	Nitrososphaeria	Nitrosopumilales	Nitrosopumilaceae	Candidatus Nitrosopumilus
Firmicutes	Clostridia	Clostridiales	Family XI	Parvimonas
Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae (Subgroup 3)	Bryobacter
Actinobacteria	Acidimicrobiia	Microtrichales	Microtrichaceae	IMCC26207
Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Roseomonas
Actinobacteria	Actinobacteria	Streptosporangiales	Streptosporangiaceae	
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	
Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	possible genus 06
Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Catonella
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium

Bacteroidetes	Rhodothermia	Rhodothermales	Rhodothermaceae	uncultured
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylocystis
Cyanobacteria	Oxyphotobacteria	Nostocales	Microcystaceae	Microcystis PCC-7914
Acidobacteria	Blastocatellia (Subgroup 4)	DS-100		
Patescibacteria	Gracilibacteria	Candidatus Peribacteria		
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Sva0081 sediment group
Bacteroidetes	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae	Ignavibacterium
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales Incertae Sedis	Phreatobacter
Bacteroidetes	Bacteroidia	Bacteroidales	p-251-o5	uncultured Bacteroidales bacterium
Patescibacteria	Gracilibacteria	Candidatus Peregrinibacteria		
Kiritimatiellaeota	Kiritimatiellae	Kiritimatiellales	Kiritimatiellaceae	MSBL3
Proteobacteria	Deltaproteobacteria	Myxococcales	uncultured	uncultured bacterium
Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae	Syntrophus
Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured
Epsilonbacteraeota	Campylobacteria	Campylobacterales	Sulfurovaceae	Sulfurovum
Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	Roseiflexus
Verrucomicrobia	Verrucomicrobiae	S-BQ2-57 soil group		
Patescibacteria	Saccharimonadia	Saccharimonadales	uncultured Candidatus Saccharibacteria bacterium	uncultured Candidatus Saccharibacteria bacterium
Proteobacteria	Alphaproteobacteria	Rhizobiales	Pleomorphomonadaceae	
Patescibacteria	ABY1	Candidatus Buchananbacteria	Candidatus Buchananbacteria bacterium RIFCSPLOWO2	Candidatus Buchananbacteria bacterium RIFCSPLOWO2
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Albimonas
Proteobacteria	Deltaproteobacteria	Myxococcales	Blfdi19	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Variovorax
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Candidatus Aquiluna
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Gallionellaceae	uncultured
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Cetobacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Chitinibacteraceae	Iodobacter
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Selenomonas

Patescibacteria	ABY1	Candidatus Falkowbacteria	Candidatus Falkowbacteria bacterium GW2011	Candidatus Falkowbacteria bacterium GW2011
Patescibacteria	Saccharimonadia	Saccharimonadales	unidentified	unidentified
Acidobacteria	Aminicenantia	Aminicenantales		
Chloroflexi	Dehalococcoidia			
Acidobacteria	Subgroup 5			
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	RBG-16-58-14
Chloroflexi	Anaerolineae	C10-SB1A		
Patescibacteria	Saccharimonadia	Saccharimonadales	uncultured soil bacterium	uncultured soil bacterium
Patescibacteria	Saccharimonadia	Saccharimonadales	uncultured bacterium	uncultured bacterium
Spirochaetes	Leptospirae	Leptospirales	Leptospiraceae	Leptospira
Proteobacteria	Alphaproteobacteria	Micropepsales	Micropepsaceae	uncultured
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes BD2-2	
Proteobacteria	Deltaproteobacteria	Myxococcales	KD3-10	metagenome
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Bythopirellula
Dependentiae	Babeliae	Babeliales	UBA12409	metagenome
Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	uncultured verrucomicrobium DEV114
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Alloprevotella
Patescibacteria	Parcubacteria	Candidatus Moranbacteria	uncultured anaerobic bacterium	uncultured anaerobic bacterium
Bacteroidetes	Bacteroidia	Bacteroidales	Dysgonomonadaceae	
Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured organism	uncultured organism
Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Turicella
Firmicutes	Clostridia	Clostridiales	Family XIII	Anaerovorax
Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Anaerobiospirillum
Proteobacteria	Alphaproteobacteria	Micavibrionales		
Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema 2
Firmicutes	Clostridia	Clostridiales	TC1	uncultured bacterium
Cyanobacteria	Melainabacteria	Caenarcniphilales	uncultured bacterium	uncultured bacterium
Proteobacteria	Deltaproteobacteria	Myxococcales	bacteriap25	
Chloroflexi	Chloroflexia	Thermomicrobiales	AKYG1722	uncultured alpha proteobacterium
Patescibacteria	Parcubacteria	Candidatus Terrybacteria	uncultured deep-sea bacterium	uncultured deep-sea bacterium

Fibrobacteres	Chitinivibrionia	Chitinivibrionales	Chitinivibrionaceae	Chitinivibrio
Firmicutes	Clostridia	Clostridiales	Family XIII	Family XIII AD3011 group
Verrucomicrobia	Verrucomicrobiae	Opitutales	Puniceicoccaceae	Puniceicoccus
Actinobacteria	Actinobacteria	Corynebacteriales	Dietziaceae	Dietzia
Actinobacteria	Acidimicrobiia	Microtrichales	Microtrichaceae	Candidatus Microthrix
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NS4 marine group
Chloroflexi	Ktedonobacteria	B12-WMSP1		
Proteobacteria	Deltaproteobacteria	Myxococcales	mle1-27	
Patescibacteria	Gracilibacteria	uncultured bacterium	uncultured bacterium	uncultured bacterium
WS2	uncultured Firmicutes bacterium	uncultured Firmicutes bacterium	uncultured Firmicutes bacterium	uncultured Firmicutes bacterium
Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae	
Actinobacteria	Actinobacteria	Actinomycetales		
Planctomycetes				
Proteobacteria	Deltaproteobacteria	NB1-j	uncultured soil bacterium	uncultured soil bacterium
Cyanobacteria	Oxyphotobacteria	Nostocales	Chroococcidiopsaceae	uncultured bacterium
Bacteroidetes	Bacteroidia	Sphingobacteriales	env.OPS 17	uncultured Bacteroidetes bacterium
WS2	uncultured bacterium	uncultured bacterium	uncultured bacterium	uncultured bacterium
Chloroflexi	Anaerolineae	SBR1031	A4b	uncultured organism
Verrucomicrobia	Verrucomicrobiae	Opitutales		
Bacteroidetes	Ignavibacteria	Kryptoniales	MSB-3C8	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	966-1
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-002
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae UCG-003
Patescibacteria	Parcubacteria	Candidatus Lloydbacteria		
Rokubacteria	NC10	Rokubacteriales		
Actinobacteria	Actinobacteria	Frankiales	Nakamurellaceae	Nakamurella
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ruegeria
Bacteroidetes	Ignavibacteria	OPB56	Ignavibacteria bacterium RIFOXYA2	Ignavibacteria bacterium RIFOXYA2
Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	metagenome

Bacteroidetes	Bacteroidia	Flavobacteriales	NS9 marine group	metagenome
Proteobacteria	Alphaproteobacteria	Caulobacteriales	Hyphomonadaceae	Hellea
Elusimicrobia	Elusimicrobia	MVP-88	metagenome	metagenome
Patescibacteria	Saccharimonadia	Saccharimonadales	uncultured candidate division WS5 bacterium	uncultured candidate division WS5 bacterium
Planctomycetes	Phycisphaerae	Tepidisphaerales	WD2101 soil group	
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	Haloferula
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Falsirhodobacter
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	DSSF69
Proteobacteria	Gammaproteobacteria	Steroidobacteriales	Steroidobacteraceae	
Verrucomicrobia	Verrucomicrobiae	Chthoniobacteriales		
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sandarakinorhabdus
Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardaceae	Saccharomonospora
Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Acetobacterium
Cyanobacteria	Oxyphotobacteria	Nostocales	Phormidiaceae	Phormidium IAM M-71
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	uncultured
Proteobacteria	Gammaproteobacteria	Methylococcales	Methylococcaceae	Methyloparacoccus
Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium
Planctomycetes	Phycisphaerae	Tepidisphaerales	WD2101 soil group	uncultured planctomycete
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Peptostreptococcus
Proteobacteria	Alphaproteobacteria	Micavibrionales	uncultured	uncultured bacterium
Planctomycetes	Planctomycetacia	Isosphaerales	Isosphaeraceae	Singulisphaera
Proteobacteria	Deltaproteobacteria	Myxococcales	P3OB-42	uncultured bacterium
Patescibacteria	ABY1	Candidatus Uhrbacteria	metagenome	metagenome
Firmicutes	Clostridia	Clostridiales	JTB215	
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus
Chlamydiae	Chlamydiae	Chlamydiales	Parachlamydiaceae	Candidatus Rubidus
Chlamydiae	Chlamydiae	Chlamydiales	Simkaniaceae	
Firmicutes	Bacilli			
Cyanobacteria	Oxyphotobacteria	Leptolyngbyales	Leptolyngbyaceae	
Patescibacteria	Parcubacteria	Candidatus Kaiserbacteria	Candidatus Kaiserbacteria bacterium RIFOXYB1	Candidatus Kaiserbacteria bacterium RIFOXYB1

Chloroflexi	Dehalococcoidia	RBG-13-46-9	uncultured bacterium	uncultured bacterium
Patescibacteria	ABY1	Candidatus Kerfeldbacteria	Parcubacteria group bacterium ADurb.Bin326	Parcubacteria group bacterium ADurb.Bin326
Proteobacteria	Alphaproteobacteria	Rickettsiales	Anaplasmataceae	Anaplasma
Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Terrimicrobiaceae	FukuN18 freshwater group
Firmicutes	Clostridia	Clostridiales	Family XIII	[Eubacterium] saphenum group
Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	uncultured
Proteobacteria	Gammaproteobacteria	211ds20	uncultured beta proteobacterium	uncultured beta proteobacterium
Patescibacteria	Microgenomatia	Candidatus Pacebacteria	uncultured anaerobic bacterium	uncultured anaerobic bacterium
Patescibacteria	Gracilibacteria	uncultured Candidatus Gracilibacteria bacterium	uncultured Candidatus Gracilibacteria bacterium	uncultured Candidatus Gracilibacteria bacterium
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Spongiimicrobium
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoracaceae	
Patescibacteria	ABY1	Candidatus Komeilibacteria	Candidatus Komeilibacteria bacterium RIFCSPHIGHO2	Candidatus Komeilibacteria bacterium RIFCSPHIGHO2
Proteobacteria	Alphaproteobacteria	Rhodospirillales	uncultured	uncultured Rhodospirillaceae bacterium
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Subsaxibacter
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	dgA-11 gut group
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	UTBCD1
Proteobacteria	Gammaproteobacteria	CCM19a	uncultured soil bacterium	uncultured soil bacterium
Cyanobacteria	Sericytochromatia			
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales Incertae Sedis	Alsobacter
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Pir2 lineage
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium
Proteobacteria	Alphaproteobacteria	Paracaedibacterales	Paracaedibacteraceae	
Acidobacteria	Blastocatellia (Subgroup 4)	Elev-16S-573	uncultured soil bacterium	uncultured soil bacterium
Patescibacteria	Parcubacteria	Candidatus Vogelbacteria	uncultured Parcubacteria group bacterium	uncultured Parcubacteria group bacterium
Firmicutes	BRH-c20a			
Omnitrophicaeota	Omnitrophica WOR	Omnitrophica WOR	Omnitrophica WOR	Omnitrophica WOR
Bacteroidetes	Rhodothermia	Balneolales	Balneolaceae	

Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera
Proteobacteria	Alphaproteobacteria	Rickettsiales	Midichloriaceae	
Actinobacteria	Actinobacteria	Kineosporiales	Kineosporiaceae	Kineosporia
Proteobacteria	Alphaproteobacteria	Tistrellales	Geminicoccaceae	Geminicoccus
Bacteroidetes	Bacteroidia	Sphingobacteriales	ST-12K33	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Ottowia
Dadabacteria	Dadabacteriia	Dadabacteriales		
Patescibacteria	ABY1	Candidatus Magasanikbacteria	uncultured Parcubacteria group bacterium	uncultured Parcubacteria group bacterium
FBP	uncultured Carnobacterium sp.	uncultured Carnobacterium sp.	uncultured Carnobacterium sp.	uncultured Carnobacterium sp.
FCPU426	metagenome	metagenome	metagenome	metagenome
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Roseospora
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales Incertae Sedis	Nordella
Proteobacteria	Gammaproteobacteria	SZB30		
Proteobacteria	Gammaproteobacteria	Alteromonadales	uncultured	
Chloroflexi	Anaerolineae	RBG-13-54-9		
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Brachymonas
Patescibacteria	Saccharimonadia	Saccharimonadales	uncultured bacterium SBR1071	uncultured bacterium SBR1071
Patescibacteria	Parcubacteria	wastewater metagenome	wastewater metagenome	wastewater metagenome
Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Bergeyella
Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadaceae	uncultured bacterium
Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Mariniradius
Armatimonadetes	Fimbriimonadia	Fimbriimonadales	Fimbriimonadaceae	uncultured Tetrasphaera sp.
Cyanobacteria	Oxyphotobacteria	Nostocales	Oscillatoriaceae	Planktothricoides SR001
Bacteroidetes	Bacteroidia	Sphingobacteriales	LiUU-11-161	metagenome
Chloroflexi	Chloroflexia	Chloroflexales		
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Burkholderia-Caballeronia- Paraburkholderia
Patescibacteria	Parcubacteria	Candidatus Kaiserbacteria		
Chloroflexi	Dehalococcoidia	GIF3		
Chloroflexi	JG30-KF-CM66			

Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis	Unknown Family	Candidatus Ovatusbacter
Cyanobacteria	Oxyphotobacteria	Nostocales	Xenococcaceae	Pleurocapsa PCC-7319
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Proteus
Firmicutes	Bacilli	Bacillales	Planococcaceae	Domibacillus
Elusimicrobia	Elusimicrobia			
Patescibacteria	Gracilibacteria	Candidatus Peribacteria	uncultured bacterium	uncultured bacterium
Verrucomicrobia	Verrucomicrobiae	Opitutales	Opitutaceae	uncultured
Chloroflexi	Anaerolineae	Ardenticatenales	uncultured	metagenome
Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	uncultured soil bacterium
Bacteroidetes	Bacteroidia	Cytophagales	Amoebophilaceae	uncultured
Fusobacteria	Fusobacteriia	Fusobacteriales		
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Macrococcus
Nitrospinae	P9X2b3D02			
Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Acidiphilium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	uncultured bacterium
Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Collinsella
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	uncultured bacterium
Patescibacteria	Parcubacteria	Candidatus Kaiserbacteria	Parcubacteria group bacterium CG1	Parcubacteria group bacterium CG1
Latescibacteria	Latescibacteria	Latescibacterales	Latescibacteraceae	uncultured bacterium
Proteobacteria	Alphaproteobacteria	Thalassobaculales	Oceanibaculaceae	Oceanibaculum
Patescibacteria	Parcubacteria	Candidatus Colwellbacteria		
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	IS-44
Dependentiae	Babeliae	Babeliales	Babeliaceae	
Latescibacteria	uncultured Gemmatimonadetes bacterium	uncultured Gemmatimonadetes bacterium	uncultured Gemmatimonadetes bacterium	uncultured Gemmatimonadetes bacterium
Planctomycetes	OM190	uncultured Planctomycetaceae bacterium	uncultured Planctomycetaceae bacterium	uncultured Planctomycetaceae bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Gallionellaceae	Gallionella

Proteobacteria	Deltaproteobacteria	Myxococcales	Nannocystaceae	Nannocystis
Proteobacteria	Deltaproteobacteria	Sva0485	uncultured soil bacterium	uncultured soil bacterium
Chloroflexi	Chloroflexia	Chloroflexales	Chloroflexaceae	FFCH7168
Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured bacterium	uncultured bacterium
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga
Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Polycyclovorans
Chloroflexi	Chloroflexia	Thermomicrobiales	AKYG1722	
Cyanobacteria	Oxyphotobacteria	Nostocales	Nostocaceae	Ancylothrix 8PC
Bacteroidetes	Bacteroidia	Chitinophagales	uncultured	
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Dubosiella
Euryarchaeota	Methanomicrobia	Methanosarcinales		
Planctomycetes	Phycisphaerae	Phycisphaerales	Phycisphaeraceae	Urania-1B-19 marine sediment group
Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Succiniclasticum
Proteobacteria	Gammaproteobacteria	R7C24		
Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Desulfurivibrio
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia-Shigella
BRC1	uncultured soil bacterium	uncultured soil bacterium	uncultured soil bacterium	uncultured soil bacterium
Patescibacteria	ABY1	Candidatus Kerfeldbacteria	metagenome	metagenome
Patescibacteria	ABY1	Candidatus Falkowbacteria		
Bacteroidetes	Bacteroidia	Sphingobacteriales	Z4MB62	uncultured bacterium
Euryarchaeota	Thermoplasmata	SG8-5	uncultured archaeon	uncultured archaeon
Bacteroidetes	Ignavibacteria	SJA-28	uncultured bacterium	uncultured bacterium
Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Xiphinematobacteraceae	Candidatus Xiphinematobacter
Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Fonticella
Proteobacteria	Gammaproteobacteria	JTB23		
Calditrichaeota	Calditrichia	Calditrichales	Calditrichaceae	uncultured prokaryote
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Pseudohongiellaceae	Pseudohongiella
Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosaetaceae	Methanosaeta
Chloroflexi	Chloroflexia	Chloroflexales	Chloroflexaceae	Candidatus Chloroploca

Acidobacteria	Subgroup 25			
Proteobacteria	Deltaproteobacteria	SAR324 clade(Marine group B)	uncultured prokaryote	uncultured prokaryote
Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae	uncultured
Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Acidocella
Patescibacteria	ABY1	Candidatus Kerfeldbacteria		
Chloroflexi	Gitt-GS-136			
Proteobacteria	Gammaproteobacteria	Cellvibrionales	Spongiibacteraceae	BD1-7 clade
Proteobacteria	Gammaproteobacteria	Francisellales	Francisellaceae	Francisella
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	uncultured
Patescibacteria	Gracilibacteria	Candidatus Abawacabacteria	uncultured Microgenomates group bacterium	uncultured Microgenomates group bacterium
Planctomycetes	Planctomycetacia	Planctomycetales	Gimesiaceae	uncultured
Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis	Unknown Family	Candidatus Berkiella
Actinobacteria	Actinobacteria	Streptosporangiales	Thermomonosporaceae	Actinocorallia
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	GOUTA6
Bacteroidetes	Bacteroidia	Chitinophagales	37-13	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Ramlibacter
Bacteroidetes	Bacteroidia	Bacteroidales	Dysgonomonadaceae	Proteiniphilum
Fibrobacteres	Chitinivibrionia	Chitinivibrionales	Chitinivibrionaceae	possible genus 03
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	uncultured
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Neisseriaceae	Simonsiella
Bacteroidetes	Bacteroidia	Bacteroidetes VC2.1 Bac22		
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Cruella
Bacteroidetes	Bacteroidia	Flavobacteriales	Cryomorphaceae	uncultured
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	
Chlamydiae	Chlamydiae	Chlamydiales	Simkaniaceae	Candidatus Renichlamydia
Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanosarcina
Actinobacteria	Nitriliruptoria	Euzebyales	Euzebyaceae	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Caenimonas

Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	uncultured soil bacterium	uncultured soil bacterium
Tenericutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae	Candidatus Bacilloplasma
Proteobacteria	Alphaproteobacteria	Rickettsiales	SM2D12	metagenome
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacterium
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Alishewanella
Lentisphaerae	Oligosphaeria	Oligosphaerales	Lenti-02	
FBP				
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Plesiomonas
Patescibacteria	Berkelbacteria	Candidatus Berkelbacteria bacterium RIFOXYA2	Candidatus Berkelbacteria bacterium RIFOXYA2	Candidatus Berkelbacteria bacterium RIFOXYA2
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Tropicibacter
Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Adhaeribacter
Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	uncultured
Dependentiae	Babeliae	Babeliales	Vermiphilaceae	uncultured bacterium
Entotheonellaeota	Entotheonellia	Entotheonellales	Entotheonellaceae	
Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	Desulforhabdus
Chloroflexi	Anaerolineae	SBR1031	wastewater metagenome	wastewater metagenome
Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	
Proteobacteria	Gammaproteobacteria	Diplorickettsiales	Diplorickettsiaceae	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Lautropia
Patescibacteria	WS6 (Dojkabacteria)	metagenome	metagenome	metagenome
Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	
Elusimicrobia	Lineage IIb	uncultured soil bacterium	uncultured soil bacterium	uncultured soil bacterium
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	
Patescibacteria	WWE3	candidate division WWE3 bacterium RIFCSPHIGHO2	candidate division WWE3 bacterium RIFCSPHIGHO2	candidate division WWE3 bacterium RIFCSPHIGHO2
Cyanobacteria	Oxyphotobacteria	Oxyphotobacteria Incertae Sedis	Unknown Family	Leptolyngbya ANT.L52.2
Bacteroidetes	Ignavibacteria	Ignavibacteriales		
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Desulfoprimum
Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Dyadobacter
Bacteroidetes	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	uncultured Sphingobacteriales bacterium

Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Methylobacillus
Proteobacteria	Deltaproteobacteria	NB1-j		
Proteobacteria	Deltaproteobacteria	Myxococcales	Blfdi19	
Acidobacteria	Blastocatellia (Subgroup 4)	Blastocatellales	Blastocatellaceae	Blastocatella
Cyanobacteria	Melainabacteria	Caenarcaniphilales	microbial mat metagenome	microbial mat metagenome
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Bosea
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	B1-7BS	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Paenalcaligenes
Bacteroidetes	Bacteroidia	Chitinophagales	Saprospiraceae	Haliscomenobacter
Firmicutes	Bacilli	Bacillales	Bacillaceae	Anoxybacillus
Proteobacteria	Alphaproteobacteria	Parvibaculales	Parvibaculaceae	Mf105b01
Actinobacteria	Actinobacteria	Frankiales	uncultured	
Patescibacteria	Gracilibacteria	Gracilibacteria bacterium canine oral taxon 394	Gracilibacteria bacterium canine oral taxon 394	Gracilibacteria bacterium canine oral taxon 394
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Spongiibacterium
Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	bacterium enrichment culture clone auto10
Bacteroidetes	Bacteroidia	Bacteroidales	Marinifilaceae	uncultured bacterium
Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Streptobacillus
Proteobacteria	Deltaproteobacteria	PB19	uncultured bacterium	uncultured bacterium
Kiritimatiellaeota	Kiritimatiellae	metagenome	metagenome	metagenome
Patescibacteria	Parcubacteria	Candidatus Yonathbacteria	uncultured bacterium	uncultured bacterium
Proteobacteria	Deltaproteobacteria	SAR324 clade(Marine group B)		
Chloroflexi	Chloroflexia	Chloroflexales	Herpetosiphonaceae	Herpetosiphon
Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium PCC-6307
Bacteroidetes	Bacteroidia	Sphingobacteriales	S15A-MN91	uncultured bacterium
Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae (Subgroup 3)	PAUC26f
Patescibacteria	WWE3	candidate division WWE3 bacterium RIFOXYC2	candidate division WWE3 bacterium RIFOXYC2	candidate division WWE3 bacterium RIFOXYC2
Patescibacteria	ABY1	Candidatus Magasanikbacteria	Candidatus Falkowbacteria bacterium RIFOXYD2	Candidatus Falkowbacteria bacterium RIFOXYD2

Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Pedomicrobium
Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	
Patescibacteria	WWE3			
Bacteroidetes	Ignavibacteria	Ignavibacteriales	PHOS-HE36	uncultured soil bacterium
Proteobacteria	Deltaproteobacteria	Bradymonadales		
Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	LD29
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	A7P-90m
Planctomycetes	Phycisphaerae	Phycisphaerales	AKAU3564 sediment group	
Proteobacteria	Alphaproteobacteria	Rickettsiales	Anaplasmataceae	Ehrlichia
Chlamydiae	Chlamydiae	Chlamydiales	Parachlamydiaceae	
Proteobacteria	Alphaproteobacteria	Elsterales	Elsteraceae	uncultured
Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Sva1033	
Proteobacteria	Gammaproteobacteria	Immundisolibacterales	Immundisolibacteraceae	Immundisolibacter
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Dickeya
Proteobacteria	Gammaproteobacteria	CHAB-XI-27	uncultured bacterium	uncultured bacterium
Chloroflexi	Anaerolineae	1-20	uncultured bacterium	uncultured bacterium
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Winogradskyella
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Aquimarina
Entotheonellaeota	Entotheonellia	Entotheonellales	Entotheonellaceae	
Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Aeromicrobium
Proteobacteria	Deltaproteobacteria	Myxococcales	P3OB-42	uncultured soil bacterium
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	uncultured
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Saccharospirillaceae	Thalassolituus
Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Patulibacter
Proteobacteria	Alphaproteobacteria	Rhizobiales	Stappiaceae	Labrenzia
Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Parapedobacter
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Marispirillum
Spirochaetes	Leptospirae	Leptospirales	Leptospiraceae	RBG-16-49-21
Proteobacteria	Alphaproteobacteria	Holosporales	Holosporaceae	
Planctomycetes	OM190	uncultured Planctomyces sp.	uncultured Planctomyces sp.	uncultured Planctomyces sp.
Patescibacteria	Microgenomatia	Candidatus Collierbacteria	uncultured Anaerolineales bacterium	uncultured Anaerolineales bacterium

Proteobacteria	Alphaproteobacteria	Paracaedibacterales	Paracaedibacteraceae	Candidatus Finniella
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Butyricicoccus
Chloroflexi	Dehalococcoidia	S085		
Verrucomicrobia	Verrucomicrobiae	LD1-PB3		
Chloroflexi	Chloroflexia	Kallotenuales	AKIW781	
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Candidimonas
Bacteroidetes	Bacteroidia	Chitinophagales	uncultured	uncultured soil bacterium
Patescibacteria	Parcubacteria	Candidatus Azambacteria		
Dependentiae	Babeliae	Babeliales	uncultured bacterium SBRH58	uncultured bacterium SBRH58
Patescibacteria	Parcubacteria	Candidatus Nomurabacteria	Parcubacteria bacterium RAAC4	Parcubacteria bacterium RAAC4
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Rahnella
Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Sporomusa
Patescibacteria	Parcubacteria	Candidatus Lloydbacteria	Candidatus Lloydbacteria bacterium RIFOXYC12	Candidatus Lloydbacteria bacterium RIFOXYC12
Chloroflexi	Anaerolineae	RBG-13-54-9	uncultured bacterium	uncultured bacterium
Planctomycetes	Phycisphaerae	Pla1 lineage	uncultured bacterium	uncultured bacterium
Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto 11
Proteobacteria	Deltaproteobacteria	Sva0485	uncultured prokaryote	uncultured prokaryote
Firmicutes	Negativicutes	Selenomonadales	uncultured	
Armatimonadetes	uncultured	metagenome	metagenome	metagenome
Firmicutes	Bacilli	Bacillales	Listeriaceae	Brochothrix
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	
Patescibacteria	Parcubacteria	Candidatus Wolfebacteria		
Chloroflexi	Dehalococcoidia	GIF9	uncultured bacterium	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Tepidimonas
Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto 15
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Caldimonas
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	[Aquaspirillum] arcticum group
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Alkanindiges
Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	uncultured actinobacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	A21b	uncultured Rhodocyclaceae bacterium

Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Niastella
Margulisbacteria	uncultured bacterium	uncultured bacterium	uncultured bacterium	uncultured bacterium
Proteobacteria	Deltaproteobacteria	Sva0485	uncultured bacterium Spirochaetales	uncultured bacterium Spirochaetales
Chlamydiae	Chlamydiae	Chlamydiales	Simkaniaceae	Candidatus Rhabdochlamydia
Proteobacteria	Deltaproteobacteria	Myxococcales	Blrii41	uncultured bacterium
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Massilia
Chloroflexi	Anaerolineae	SBR1031	A4b	uncultured bacterium
Proteobacteria	Deltaproteobacteria	Myxococcales	Eel-36e1D6	uncultured bacterium Polyangiaceae
Acidobacteria	Acidobacteriia	Subgroup 13		
Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Nitrincolaceae	uncultured
Proteobacteria	Deltaproteobacteria	Syntrophobacteriales	Syntrophaceae	Smithella
Lentisphaerae	Lentisphaeria	Victivallales	Victivallaceae	
Gemmatimonadetes	S0134 terrestrial group	uncultured Gemmatimonadetes bacterium	uncultured Gemmatimonadetes bacterium	uncultured Gemmatimonadetes bacterium
Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Chitinophaga
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Turcibacter
Lentisphaerae	Lentisphaeria	Victivallales	GWF2-44-16	uncultured bacterium
Proteobacteria	Deltaproteobacteria	Syntrophobacteriales	Syntrophaceae	uncultured
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Chitinimonadaceae	Chitinimonas
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Roseobacter clade CHAB-I-5 lineage