An Investigation of *Coxiella burnetii* and *Coxiella*-like bacteria in the Brown Dog Tick (*Rhipicephalus sanguineus*)

This thesis is presented for the degree of Honours in Biomedical Science at

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Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

....

Telleasha Lorraine Greay

Abstract

There are a wide variety of animal reservoirs of the zoonotic bacterium *Coxiella burnetii* (*C. burnetii*), and ticks may play a significant role in the natural transmission cycle of this pathogen. Recently, domestic dogs have been implicated as reservoirs of *C. burnetii*. Dogs are the primary hosts of *Rhipicephalus sanguineus* (*R. sanguineus*), and *C. burnetii* has previously been detected in these ticks. The objectives of this study were to identify and record *R. sanguineus* ticks collected from dogs in Australia, and to investigate the prevalence of *C. burnetii* in these ticks. Subsequent to this, the bacterial microbiome of *R. sanguineus* ticks was investigated. The IS1111a transposase element gene was targeted using qPCR to detect *C. burnetii* DNA in *R. sanguineus*. The Ion Torrent™ Next-Generation Sequencing platform was used to sequence bacterial 16S rDNA in the ticks. In this study, 2,577 *R. sanguineus* ticks were morphologically identified and recorded in the Northern Territory, South Australia and Western Australia. There was no positive detection of *C. burnetii* in a subset of 31 *R. sanguineus* ticks by qPCR. Next-generation sequencing of the universal bacterial 16S rRNA gene revealed that a *Coxiella* sp. was present in 53/59 (90%) tick pools. The sequences were compared to GenBank submissions and a 100% match was obtained to a *Coxiella* sp. from *R. sanguineus* in the Philippines. A phylogenetic analysis of this *Coxiella* sp. showed that it does not group with the pathogenic *C. burnetii.* This *Coxiella* sp. may be a non-pathogenic endosymbiont of *R. sanguineus*, and future investigations could aim to assess the role of *Coxiella* endosymbionts in *R. sanguineus*, and whether this bacterium causes cross-reactivity in immunologic assays used for the diagnosis of Q fever in people.

List of Abbreviations

A	adenine or adenosine
bp	base pair
BP	before present
BSA	bovine serum albumen
C	cytosine or cytidine
C_T	cycle threshold
CVBD	canine vector-borne disease
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
et al.	and others
g	gram
G	guanine or guanosine
gDNA	genomic DNA
GIS	geographic information system
h	hour
IT	Ion Torrent
L	litre
mg	milligram
MID	multiplex identifier
min	minute
mL	millilitre
mm	millimetre
mM	millimolar
mod	modified
µ	micro
µg	microgram
µL	microlitre
NGS	next generation sequencing
No./*n*	number
NTC	no-template control
OR	odd ratio
OTU	operational taxonomic unit
PCR	polymerase chain reaction
ρM	picomolar
ρmol	picomole
qPCR	quantitative PCR
R	reverse
rDNA	ribosomal deoxyribonucleic acid
RFLP	restriction fragment length polymorphism
RH	relative humidity
RNA	ribonucleic acid
rpm	revolutions per minute

RR	risk ratio
rRNA	ribosomal ribonucleic acid
s	second
sp./spp.	species
T	thymine or thymidine
Taq	*Thermus aquaticus* DNA polymerase
U	unit
w/v	weight of solute per volume of solvent
x	times
3'	hydroxyl-terminus of DNA molecule
5'	phosphate-terminus of DNA molecule
~	approximately
&	and
$	dollars
>	greater than
<	less than
-	negative
™	trademark
®	registered trademark
%	percent
°C	degree Celsius

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CHAPTER 1: INTRODUCTION

1. Introduction

The zoonotic disease known as 'Q fever' is caused by infection with (*Coxiella burnetii*) *C. burnetii*, and is preventable by vaccination, but remains an unfortunate burden every year for hundreds of people in Australia and around the world (Gidding et al. 2009). Increased reports of human Q fever outbreaks unrelated to associations with domestic livestock emphasise the need for a better understanding of how this disease is transmitted, so that susceptible individuals can be made aware of the risks, and more effective strategies implemented to prevent Q fever outbreaks. The primary objective of this project was to detect the zoonotic pathogen *C. burnetii* in (*Rhipicephalus sanguineus*) *R. sanguineus* ticks in Australia, with the secondary aim to describe the bacterial microbiome of *R. sanguineus*.

The aim of this first chapter is to review the literature pertaining to the medical and economic impacts of Q fever, and to briefly describe the risk factors associated with the transmission of *C. burnetii* to humans, with reference to the history of its discovery and to recent outbreaks of the disease associated with domestic livestock. The detection of *C. burnetii* in wild and companion animals in Australia and overseas is discussed, with emphasis on the implication of the brown dog tick, *R. sanguineus*, as a vector of *C. burnetii*, and a potential source of zoonotic infection. New molecular tools used to investigate the presence of *C. burnetii* DNA and the broader bacterial microbiome in *R. sanguineus* are also described, with reference to recently published literature. Finally, the distribution of *R. sanguineus* in Australia is described, as well as the morphological identification of this important ectoparasite of dogs.

1.1 The medical and economic impacts of the vaccine-preventable disease Q fever

Australia is the only country that has a licensed and registered Q fever vaccine. In 1994 the Australian government-funded Q fever vaccination program included all abattoir workers, and was extended to include farmers in 2002 (Morrissey, Cotton, and Ball 2014). As a result, the

number of notifications of Q fever has reduced since 2002, yet hundreds of cases are still reported every year (Morrissey, Cotton, and Ball 2014). The number of cases of Q fever reported from January to October this year by the *National Notifiable Diseases Surveillance System* in Australia is 364 (Australia, Department of Health. 2014). In 2011 the vaccination program was extended to the families of farmers, and to others employed in the livestock-rearing industry. However, after 12 months the vaccination program for farmers ceased, and the program is now only in place for abattoir workers (reviewed in Morrissey, Cotton, and Ball 2014). Humans infected with the zoonotic disease Q fever are commonly asymptomatic, and the mild form of the disease is characterised by flu-like symptoms, fever and severe headaches (Mertens and Samuel 2007). However, the disease can be life-threatening in some patients with an acute onset of meningoencephalitis or myocarditis, or in chronically infected patients who develop endocarditis or hepatitis. People with previous cardiac valve defects, immunocompromised patients, and pregnant women are at greater risk of chronic Q fever (Maurin and Raoult 1999).

Not only can Q fever have a significant influence on the health and well-being of patients, the financial implications of the disease on national productivity are also considerable. An economic evaluation by Kermode et al. (2003) considered the financial impacts of Q fever, such as the cost to the Australian government (annual estimate of $1.3 million in 1999 for WorkCover claims), and common-law actions against employers in the meat industry (as high as $1.1 million reported for a legal settlement). The study concluded that increasing the uptake of Q-fever vaccination among meat and agricultural industry workers is a cost-effective public health strategy (Kermode et al. 2003), ushering in the vaccination policy change referred to earlier. An understanding of how human acquisition of *C. burnetii* occurs is important so that people can be made aware of the risks of exposure, and the appropriate recommendations can be made to protect against infection.

1.2 A review of the transmission of *C. burnetii* to humans

1.2.1 The association between livestock and human Q fever outbreaks led to the discovery of *C. burnetii*

The risk of exposure to *C. burnetii* has long been recognised as a risk factor for abattoir workers and people working in the agricultural sector. Less than one hundred years ago, the causative agent of Q fever was identified as *C. burnetii*, following outbreaks of the illness among workers in the meat industry. Q (query) fever was first described by Derrick (1937), who conducted an epidemiological and microbiological investigation in Queensland, Australia, which aimed to identify the causative agent of a disease outbreak among abattoir workers in 1935. Similar cases were reported as early as 1933, in Brisbane, Queensland. The prominent clinical symptoms observed in patients during that outbreak included an acute onset of fever, headache, shivers, rigor, and vomiting. The study revealed that guinea-pigs could acquire Q fever via inoculation with patient blood and urine samples, and that the guinea-pigs acquired immunity to the disease after a single exposure. However, Derrick (1937) was incorrect in assuming that the pathogen in question was a virus, after attempts to cultivate and to visualise the microorganism using microscopy from infected guinea-pig tissues failed. In 1937, Derrick sent infected guinea pig liver to Dr. F. M. Burnet in Melbourne, who transferred the infection to mice, and was successful in discovering rickettsial-like bodies in their spleens (Derrick 1937). Q fever was similarly observed in America by Cox (1941), at the Rocky Mountain Laboratory in Hamilton, Montana, USA, who was able to culture the rickettsial-like bacteria in embryonated eggs, and with his colleague Davis, managed to isolate the microorganism from ticks collected at Nine Mile Creek in Montana. Cox later showed that the bacterium responsible for causing Q fever, although morphologically similar to rickettsia, was significantly different, and suggested it should therefore be placed into a separate genus. Thus the aetiological agent of Q fever, *C. burnetii*, was named in honour of Cox and Burnet ((Philip 1948). Although Derrick was unable to identify the pathogen responsible for causing Q fever, he was the first person to describe the disease, and although a fuller knowledge exists on Q fever today, its name remains unchanged 77 years later.

1.2.2 Current understanding of how *C.* burnetii is transmitted to humans

Q fever, or coxiellosis, is a zoonosis that is considered to occur worldwide, except in New Zealand, which is believed to be *C. burnetii* free since serological surveys have produced negative results in tested cattle, sheep, dogs and humans (Hilbink et al. 1993; Kaplan and Bertagna 1955). The risk of exposure to *C. burnetii* has traditionally been considered an occupational risk for abattoir workers and farmers, as humans can acquire an infection with *C. burnetii* from contact with domestic ruminants, such as cattle and sheep, as well as from their infected products (Raoult, Marrie, and Mege 2005). Inhalation of dust particles contaminated with *C. burnetii* is thought to be the most common route of infection (Toman, Heinzen, and Samuel 2012). Due to this nature of transmission, *C. burnetii* could potentially be used as a biological weapon in aerosolised form, as a contaminant of food and water, or possibly even in mail (reviewed in Madariaga et al. 2003).

Epidemics of Q fever have usually been associated with direct or indirect contact with infected animals and their products. A recent outbreak of Q fever in the Netherlands between 2007 and 2010 is the largest reported community outbreak of Q fever (Vellema and van den Brom 2014). A total of 3,523 human cases were notified in this period of the epidemic (Roest et al. 2011), and it is thought that the outbreak in the community was associated with a high number of abortions in dairy goats and sheep in the area (Vellema and van den Brom, 2014; Roest et al. 2011). Conditions such as abortion, premature delivery, stillbirth, and weak offspring have been associated with *C. burnetii* infection of the uterus of pregnant sheep and goats (reviewed in Agerholm 2013).

Other potential sources of infection associated with infected domestic livestock include barnyards (Thomas et al. 1995), contaminated straw (van Woerden et al. 2004), and consumption of contaminated milk (Hatchette et al. 2001), due to high numbers of *C. burnetii* organisms in urine, faeces, conception products, and milk, respectively, of infected animals (reviewed in Madariaga et al. 2003). Infection with *C. burnetii* is widely recognized as an occupational hazard for people who work with or around birth products of livestock of infected animals, including farmers,

veterinarians, and zoo and slaughterhouse workers (Toman, Heinzen, and Samuel 2012). Accounts of *C. burnetii* transmission via sexual contact (Milazzo et al. 2001), vertical transmission (Raoult, Fenollar, and Stein 2002), and nosocomial acquisition (Weber and Rutala 2001) are rare. Person-to-person transmission has been reported (Mann et al. 1986), however this is considered to be unlikely as it was reported in an endemic setting (reviewed in Madariaga et al. 2003). Madariaga et al. (2003) included ticks as a rare transmission route of *Coxiella* (i.e. from tick bite to humans) although no reference was made to any studies that have reported such a finding. A later review by Thompson, Dennis and Dasch *(*2005) found that transmission of *C. burnetii* to species of mammals is well documented, however, considered the epidemiological evidence for direct transmission of *C. burnetii* to humans by ticks is lacking, based on only several anecdotal observations. Although *C. burnetii* has been detected in ticks, they are not generally considered as significant vectors of the disease to humans (Toman, Heinzen, and Samuel 2012).

1.2.3 Q fever from wildlife and companion animals – How the risks of Q fever acquisition may have been traditionally understated

As discussed in the previous sections, the main risk factor for acquiring *C. burnetii* is considered to be contact with products from infected livestock. However, wild animals and companion animals have also been associated with human Q fever outbreaks. The risk factors for human Q fever acquisition may extend to the exposure to other potential animal reservoirs of the bacterium, as *C. burnetii* has been detected in a range of different species of wildlife, domestic and companion animals.

1.2.3.1 *Coxiella burnetii* detected in Australian fauna – native and introduced species

Not only are domestic livestock, such as sheep, goats, and cattle, reservoirs of *C. burnetii*, other species of animal have also been identified as potential reservoirs of *C. burnetii.* This is not surprising given that native Australian ticks act as the invertebrate reservoir for *C. burnetii*. A number of studies in Australia have detected the presence of *C. burnetii* in native Australian wildlife, such as Western barred bandicoots (Bennett et al. 2011), kangaroos (Barralet and Parker,

2004; Banazis et al. 2010; Potter et al. 2011) common northern bandicoots, dingoes, and brushtail possums (Cooper et al. 2012). Exposure to *C. burnetii* has also been detected in introduced wild animals in Australia, such as feral cats, foxes, and feral pigs (Cooper et al. 2012).

1.2.3.2 *Coxiella burnetii* detected in companion animals overseas and in Australia

The role of companion animals, such as cats and dogs, in the transmission and maintenance of *C. burnetii* is uncertain at the present time. Following reports of human Q fever outbreaks associated with infected dogs in Nova Scotia, a retrospective investigation in this region of Canada aimed to identify the risk factors associated with human Q fever acquisition. The study found that the strongest risk factors for *C. burnetii* infection were exposure to stillborn kittens, newborn kittens, and parturient cats. Other significant risk factors identified included working on a farm, slaughtering and dressing animals, and contact with cats, cattle and sheep. Serological evidence was also presented, whereby cats' placentas were found to have high titers of antibodies to *C. burnetii* antigens (Marrie et al. 1988). More recently in Australia, Kopecny et al. (2013) evaluated *C. burnetii* seroreactivity of 27 cats living in the same breeding cattery, and investigated the *C. burnetii* infection using molecular and histological methods. The study was prompted by a Q fever outbreak in a small animal veterinary hospital in Sydney, Australia, among nine veterinary personnel and a cat owner, after a caesarean section was performed on a cat. Strong seropositivity results were obtained for this cat, and the seroprevalence of the 27 cats tested from the cattery was 26% (Kopecny et al. 2013).

A family of three contracted Q fever after exposure to a parturient dog (Buhariwalla, Cann, and Marrie 1996) and in California, a high seroprevalence rate (66%) was reported in 316 stray dogs tested for *C. burnetii* (Willeberg et al. 1980). More recently, in Australia, Cooper et al. (2011) investigated the seroprevalence of *C. burnetii* in blood samples collected from domestic dogs in veterinary clinics in Townsville, Queensland. This study established that 101 canine samples collected between 2006 and 2007 had an overall seropositivity of 21.8%. A retrospective study was also conducted on 100 samples that were collected from the same region in 1984-1985, and

these samples were found to have a seropositivity of 16.0%. The samples were either positive for phase I or phase II antigens, and small number of samples was positive to both. The dog owners participated in a questionnaire to establish risk factors, and included data such as location of dog's residence, its food sources (commercial products or home-made diets and meat), and level of exposure to ticks, domestic farm animals, and wildlife. The only factor that was found to have a positive association with seropositivity for phase II antigen was contact with wildlife, with a reported risk ratio (RR) of 2.3, and an odds ratio (OR) of 3.0. Factors that were found to have a positive association with seropositivity against phase I antigen were contact with wildlife (RR 2.4, OR 2.9), contact with ticks (RR 2.7, OR 2.9), and contact with farm animals (RR 2.2, OR 2.4). A positive association with seropositivity against either or both phase I or II antigens was obtained for contact with wildlife (RR 2.2, OR 2.8), farm animals (RR 1.9, OR 2.4), and pregnant animals (RR 1.9, OR 2.4). Importantly, this study provided evidence of *C. burnetii* infection in domestic dogs in Australia, established that seropositivity for *C. burnetii* was higher in serum samples collected from 2006-2007 than the serum samples collected in 1984-1985, and suggested that ticks may play a role in the epidemiology in this location (Cooper et al. 2011). The evidence of *C. burnetii* exposure in Australian dogs described above has prompted this present investigation, which aims to detect *C. burnetii* in *R. sanguineus* ticks on dogs. *Rhipicephalus sanguineus* may play a key role in the transmission of *C. burnetii* to dogs, together with a variety of other vertebrates that this tick may use as vertebrate sources of its blood meal.

1.3 Could ticks play a role in the transmission of *C. burnetii*?

Vectors can be defined as arthropods, or other invertebrates, that serve as a host and carrier of parasites that are physiologically dependent on the host organism for survival (obligatory parasitism) (Bogtish et al. 2013). Not only is the tick itself an obligatory parasite, dependent on a vertebrate host for survival, but it may also transmit microorganisms which depend on the tick for the completion of their life cycle (Bogtish et al. 2013). In order for the tick to be a vector that maintains a certain disease, it must transmit the causative agent vertically (transstadially or transovarially to their progeny) and horizontally (via tick bite or in faeces) to susceptible animals

(Kazar 2005). The main arthropod vectors of canine vector-borne diseases (CVBDs) are ticks, fleas, and sand flies, which can transmit a variety of pathogenic microorganisms such as viruses, bacteria, protozoa, and helminths, and these can cause significant health problems in dogs, and in humans if the pathogen is zoonotic. Some of the major bacterial CVBDs with zoonotic potential include granulocytic anaplasmosis (caused by *Anaplasma phagocytophilum*) thrombocytic anaplasmosis (*Anaplasma platys*), monocytic ehrlichiosis (*Ehrlichia canis*), borreliosis (*Borrelia burgdorferi, Borrelia garinii, and Borrelia afzelli*), and rickettsiosis (*Rickettsia rickettsii*), all of which have been reported to be zoonotic (Irwin 2014), and all can be transmitted by ticks. Tick-associated pathogens, including *C. burnetii*, can be identified using a variety of techniques, such as xenoculture (injection of infected tissue into mice) and molecular methods including conventional PCR and Sanger sequencing, and qPCR/real-time PCR. The identification of tick-associated pathogens, as well as epidemiological studies, can provide evidence to implicate ticks as vectors of suspected pathogens. However, transmission studies that demonstrate vertical and horizontal transmission of a pathogen by a tick species are required in order to confirm whether the tick is a competent vector of the pathogen of interest. The following sections will review the literature pertaining to the detection and identification of *C. burnetii* in ticks, including *R. sanguineus*¸ primarily through the use of molecular tools, and will provide an overview of the sequencing technology that can be applied to investigate the bacterial microbiome of ticks.

1.3.1 Polymerase chain reaction – A more sensitive and specific method for bacterial detection than traditional methods

Polymerase chain reaction was developed in the 1980s (Mullis and Faloona 1987), and is one of the most widely used molecular techniques to detect microorganisms in clinical specimens (Padmanabhan et al. 2013). PCR is a highly sensitive method in comparison to serological methods (Fournier, Marrie, and Raoult 1998), and has been used to identify *C. burnetii* in various types of samples, including clinical, environmental, animal faeces, and tissues of ticks (Fenollar, Fournier, and Raoult 2004). There are many different primer sequences that can be designed to target and amplify defined DNA fragments of the *C. burnetii* genome (Fenollar, Fournier, and

Raoult 2004). Insertion elements such as IS1111 and *htpAB* are most commonly used, as they occur in the genome in many copies (19 and 20, respectively), and this increases the sensitivity of the PCR assay (Klee et al. 2006). The *com*1 (CBU1910) gene, 5S and 23S rDNA genes, riboflavin synthase genes, and GDP-fucose synthase genes, are other targets that have been used to design *C. burnetii*-specific primers (Reeves et al. 2005).

1.3.1.1 The detection of *C. burnetii* in ticks

According to a review by Maurin and Raoult (1999), *C. burnetii* has been detected in more than 40 different species of tick, and the tick species that have been most frequently implicated as potential vectors of *C. burnetii* belong to the genera *Ixodes, Rhipicephalus, Amblyomma,* and *Dermacentor* (Parola and Raoult 2001). Different PCR assays, such as conventional PCR, PCR-Restriction Fragment Length Polymorphism (RFLP), and qPCR, have been used to detect *C. burnetii* DNA in ticks, and have used various primer sets in single-plex, multi-plex, and nested PCR approaches. Several studies that have detected *C. burnetii* DNA in ticks with these varied approaches are outlined in Table 1.1. The study by Spitalska and Kocianova (2002) used both traditional methods (haemocyte tests) and PCR to detect *C. burnetii* in *Ixodes ricinus, Dermacentor marginatus,* and *Haemaphysalis concinna.* This study demonstrated that haemocyte tests have lower sensitivity and specificity than PCR, as 30.64% (72/235) ticks were reported to be positive by the haemocyte tests, but only 2.55% (6/235) were positive by PCR, indicating false positivity of the haemocyte test, and false negatives from the haemocyte tests were also confirmed by PCR (Spitalska and Kocianova 2002). Spyridaki et al. (2002) also demonstrated that nested PCR was more sensitive for the detection of *C. burnetii* DNA than isolation of *C. burnetii* using a shell-vial culture system (Raoult, Torres, and Drancourt 1991), with 7/80 positives for *C. burnetii* obtained from nested PCR, as opposed to 2/80 positives for the shell-vial isolation in *R. sanguineus* ticks (Spyridaki et al. 2002). The increased sensitivity and specificity of PCR assays is a major advantage over traditional methods for bacterial detection, however, false positives can also be reported in qPCR assays, as demonstrated in the study by Sprong et al. (2011) (Table 1.1).

The investigation by Sprong et al. (2011) aimed to assess the prevalence of *C. burnetii* in ticks collected from vegetation and animals in Netherlands following Q fever outbreaks in previous years (Schimmer et al. 2011). Initially, multiplex qPCR assays were carried out with primer sets that targeted the multi-copy transposon element IS1111, outer membrane protein coding gene (Com1), and the isocitrate dehydrogenase gene (Icd) on questing *Ixodes ricinus* ticks. Signal was detected for the IS1111 transposon element in 20/1,891 ticks, but no signals were reported for the Com1 nor Icd targets. The qPCR products were visualised by gel electrophoresis, and the products did not correspond with the expected size of the IS element. These samples were retested with single-plex qPCR assays with only IS1111 primers, and the samples were confirmed as negative for *C. burnetii*, and the IS1111 signal in the initial multiplex qPCR assay was considered as a false-positive result. Adult ticks (genus and species not specified by the study) collected from domestic animals and livestock were also tested for *C. burnetii* DNA in single-plex qPCR assays targeting the IS1111 and Com1 genes, and five positives were obtained (one from a cat, and four from sheep) for IS1111, while there was no amplification for the Com1 gene (Sprong et al. 2011). This study demonstrates that the IS1111 target has a greater sensitivity in qPCR assays compared with the Com1 gene, as there are multiple copies of IS1111 in the *C. burnetii* genome, but also showed that false-positives can occur with this primer, and so it is important to confirm the size of the PCR product with gel electrophoresis.

Table 1.1: Detection of *C. burnetii* in ticks with PCR methodology.

PCR Assay	Reference	Tick Species	Location	Host	Gene Target of *C. burnetii*
Conventional PCR	Satta et al. (2011)	*R. sanguineus* *R. turanicus*	Sardinia, Italy	Dog Goat	superoxide dismutase gene
PCR-RFLP	Spitalska and Kocianova (2002)	*Ixodes ricinus* *Dermacentor marginatus* *Haemaphysalis concinna*	Slovakia and Hungary	Questing ticks	Com1 gene, Msp I and Sau3AI restriction sites
Single-plex real-time qPCR	Sprong et al. 2011	Adult ticks (species not specified)	Netherlands	Cat Sheep	IS1111 transposon element

Nested PCR and PCR-RFLP	Spyridaki et al. (2002)	*R. sanguineus* *Hyalomma* spp.	Cyprus	Goat Sheep	Plasmids
PCR-Reverse Line Blot (PCR-RLB)	Toledo et al. (2009)	*R. sanguineus* *H. lusitanicum* *D. marginatus* *R. pusillus* *R. sanguineus*	Central Spain	Sheep Deer Horses Foxes Questing ticks	IS1111 transposon element

1.3.1.2 The detection of *C. burnetii* in *R. sanguineus*

The studies by Satta et al. (2011), Spyridaki et al. (2002), and Toledo et al. (2009) outlined previously in Table 1.1 are examples of investigations that have detected *C. burnetii* DNA in *R. sanguineus* ticks from a variety of geographic locations. The proportion of *R. sanguineus* ticks positive for *C. burnetii* DNA reported in these studies is low. Toledo et al. (2009) reported 2/38 *R. sanguineus* collected from a variety of wild animals were positive for *C. burnetii* DNA (these *C. burnetii* positive *R. sanguineus* ticks were collected from foxes), and no positives were obtained from the 106 *R. sanguineus* ticks collected from cats and dogs. Spyridaki et al. (2002) collected 20 *R. sanguineus* from goats, and 80 from sheep, and found that overall, 7/80 from the sheep were positive for *C. burnetii* DNA. Satta et al. (2011) collected a total of 1,045 *R. sanguineus* from dogs (n = 965), sheep (n = 45), goats (n = 20), cattle (n = 10), and hedgehogs (n = 5). The DNA extractions of these ticks were mixed (pooled), and the resulting number of pools were 193 for dogs, 9 for sheep, 4 for goats, 2 for cattle, and 1 for hedgehogs. Of these *R. sanguineus* pools, 9 from the dogs, and 1 from the sheep were positive for *C. burnetii* DNA. These studies demonstrate the detection of *C. burnetii* DNA in *R. sanguineus* ticks collected from a variety of animals, including sheep, foxes, and dogs, using PCR methodology, but suggests that this bacterium is present in a small proportion of the *R. sanguineus* tick population.

1.3.1.3 The detection of *C. burnetii* in Australian ticks

In Australia, a significant amount of evidence has been obtained to support an animal-tick cycle of *C. burnetii* involving bandicoots and *Haemaphysalis humerosa* (Thompson, Dennis, and Dasch

2005). As *H. humerosa* can also feed occasionally on cattle (Derrick 1944; Smith 1940), the tick-bandicoot cycle could theoretically maintain the Q fever infection in cattle herds, but has not been proven (Thompson, Dennis, and Dasch 2005). *Coxiella burnetii* has been detected in other Australian ticks as well, by a variety of traditional and molecular techniques, and examples of such studies are outlined in Table 1.2. DNA sequencing is another molecular tool that can be used to identify pathogens and other microorganisms, and it is this technology of genome sequencing that enabled primers and probes to be developed for PCR methodology. The following section will provide a brief overview of DNA sequencing technology, and its applications for bacterial discovery, and how it has been applied in the research of bacteria associated with ticks.

Table 1.2: Detection of *C. burnetii* in ticks found in Australia

Tick species	Location	Host	Method of detection	Reference
Haemaphysalis humerosa	Queensland	Bandicoot	Guinea-pig inoculation	Smith (1940) Derrick and Smith (1940) Smith and Derrick (1940)
	Western Australia		qPCR	Bennett et al. (2011)
Ixodes holocyclus	Queensland	Bandicoot	Real-time PCR DNA sequencing	Cooper et al. (2013)
Amblyomma triguttatum	Queensland	Kangaroo	Real-time PCR DNA sequencing	Cooper et al. (2013)
		Kangaroo, goat, sheep	Isolated by mouse and guinea-pig inoculation	Pope, Scott, and Dwyer (1960)
				McDiarmid et al. (2000)
Bothriocroton auruginans	Victoria	Wombat	PCR Sequencing	Vilcins, Old, and Deane (2009)

1.3.2 First and Second Generation sequencing technologies – Tools to investigate genomes and metagenomes

DNA sequencing and genomics are relatively new scientific disciplines. Sanger sequencing was the first commercial DNA sequencing application, and was developed in the 1970s (Sanger, Nicklen, and Coulson 1977). This first generation sequencing technology by Applied Biosystems

(ABI Prism 310, later replaced ABI Prism 3700 with 96-well capillaries) was used as the main tool for the human genome project. In the 2000s, the second, or more commonly known as next generation sequencing (NGS) platforms were developed, such as the 454/Roche, Illumina/Solexa and SOLiD platforms, as well as benchtop sequencers including the Ion Torrent Personal Genome Machine (PGM) (Life Technologies), MiSeq (Illumina), GS Junior (Roche/454). The same basic principles apply across all NGS platforms, which sets them apart from first generation sequencing, in that DNA libraries are amplified *in vitro*, the DNA is sequenced by synthesis, and DNA templates are sequenced simultaneously in a massively parallel fashion (Anderson and Schrijver 2010). The main advantages of NGS platforms over traditional Sanger sequencing is the high-throughput (which refers the amount of DNA sequence that can be read with each sequencing reaction), they are more cost-effective, and are less laborious (Mardis 2011). Despite the advantages of NGS, Sanger sequencing is still used today. Sanger sequencing has been optimised to reduce the run times through the use of nucleotide-specific fluorescent dyes (Smith et al. 1985), polyacrylamide gels in capillary electrophoresis (Swerdlow and Gesteland 1990), and automatic laser fluorescence detection (Smith et al. 1986). The main limitation of NGS is that the read lengths are shorter, and the accuracy is not as high as Sanger sequencing, but will depend on the sequencing chemistry used (Morey et al. 2013). The key factors that define NGS platforms include the read length, throughput, read accuracy, read depth (number of times each base is sequenced in independent events), and cost per base (Morey et al. 2013). A discussion of the sequencing chemistries, and the advantages and disadvantages of the various NGS platforms is beyond the scope of this introduction. Next generation sequencing and Sanger sequencing has been the subject of many reviews, which can be referred to for more information (Mardis 2013; Rizzo and Buck 2012; Morey et al. 2013). Next generation sequencing technologies have a wide variety of applications including variant discovery, genome assembly, transcriptome analyses, and classification and gene discovery in metagenomic studies (Metzker 2010). In the following sections, the application of DNA sequencing to bacterial identification and characterisation of bacterial microbiomes is discussed.

1.3.2.1 Bacterial identification, characterisation and genomics

Bacterial identification was historically based on colony growth time and morphology, Gram staining, fermentation, and biochemical tests. However, most microbes are difficult, or too dangerous, to culture under laboratory conditions (Sharpton 2014). DNA sequencing has enabled the species identification and genetic diversity to be determined and characterised of previously uncultured microbial communities. Before next generation sequencing technologies were available, sequencing of prokaryotic genomes was accomplished with Sanger shotgun sequencing (Sanger and Coulson 1975; Sanger, Nicklen and Coulson 1977).

Due to the advantages of NGS over Sanger sequencing as mentioned above, NGS technologies are replacing Sanger sequencing for sequencing of small sized genomes and environmental metagenomics (reviewed in Wooley, Godzik, and Friedberg 2010). Genomics involves the analysis of genomic DNA (gDNA) from an individual organism or cell, whereas metagenomics is the analysis of gDNA from polymicrobial specimens (Padmanabhan et al. 2013). Studies of all copies of a single gene, such as 16S ribosomal DNA (rDNA), in a polymicrobial specimen have been named metagenomic studies (Hunter et al. 2012a). The 16S rRNA gene is universally distributed, and is used in NGS to study the bacterial evolution, ecology, and phylogenetic relationships between taxa, bacterial diversity in the environment, and the relative abundance of taxa of various ranks (Hugenholtz, Goebel, and Pace 1998). The classification of ribosomal RNA genes was originally proposed by Woese and Fox (1977), and the 16S rRNA gene has been used to study and characterise the bacterial community compositions in a variety of microbial samples derived from animals, and from host-free samples such as soils and ocean environments (Mizrahi-Man, Davenport, and Gilad 2013). The 16S rRNA gene is ubiquitous among prokaryotic microorganisms, and has a high degree of functional conservation, and the mutations in this gene enable the study of prokaryotic evolution (Woese 1987). The 16S rRNA gene has conserved regions that allows for primers to be designed to enable amplification of majority of bacterial taxa, but also contains nine hypervariable regions (V1-V9), which enable taxa to be distinguished (Clarridge 2004).

Sanger sequencing was used in a phylogenetic study by Weisburg et al. (1989) to obtain 16S rRNA sequences for *C. burnetii* (which was classified as a rickettsial organism at the time) and other bacterial species. A phylogenetic analysis of these sequences demonstrated that *C. burnetii* is more closely related to *Legionella* species than *Rickettsia* species, which resulted in the reclassification of *C. burnetii* (Weisburg et al. 1989). Table 1.3 below demonstrates the current classification of *C. burnetii*. Since the first publication of the complete genome sequence of the Nine Mile strain (Davis et al. 1938) in 2003 (Seshadri et al. 2003), more isolates have been published (Beare et al. 2009; Karlsson et al. 2014; Sidi-Boumedine 2014). Although *C. burnetii* was first discovered in Australia, a genome sequence for *C. burnetii* from Australia was only recently sequenced, and was identified as a novel strain (AuQ01) of *C. burnetii* (Walter et al. 2014).

Table 1.3: Classification of *C. burnetii* (adapted from Toman, Heinzen, and Samuel 2012).

PHYLUM	**PROTEOBACTERIA**
CLASS	**Gammaproteobacteria**
ORDER	**Legionellales**
FAMILY	**Coxiellaceae**
GENUS	***Coxiella***
SPECIES	***Coxiella burnetii***

1.3.2.2 The microbiome of ticks and the application of molecular tools to invertebrates

Massively parallel sequencing, or NGS, has enabled shorter regions of the 16S rRNA gene to be sequenced at a greater depth, and at a lower cost (Tringe and Hugenholtz 2008). Next generation sequencing of the 16S rRNA gene has been applied to study the bacterial microbiome of ticks, including *R. sanguineus*, and these high-throughput sequencing approaches have revealed a high amount of bacterial diversity of individual ticks (Clay et al. 2008; Heise, Elshahed, and Little 2010). A recent study conducted in the Middle East employed a 16S rRNA sequencing approach using the 454 pyrosequencing platform (Roche) to describe the bacterial community in

Rhipicephalus species. Lalzar et al. (2012) reported that the bacterial diversity of *R. sanguineus* was low, and was dominated by a *Coxiella* sp. *Rickettsia* spp. were also identified, however the prevalence was low compared to *Coxiella.* A novel *Coxiella* sp. was present, and dominant in all 187 of the ticks tested. The *Coxiella* sp. was significantly more abundant in female versus male ticks, and was stable during the questing season. The study also demonstrated that the *Coxiella* sp. could be vertically transmitted in *R. sanguineus*, as indicated by the presence of *Coxiella* bacteria in the adults, eggs, and larvae (Lalzar et al. 2012). Although there is only one species that has been formally classified in the *Coxiella* genus (*C. burnetii*), *Coxiella* sp., also described as *Coxiella* endosymbionts, have been identified in *R. sanguineus* ticks previously using PCR and direct sequencing, and although closely related to *C. burnetii*, have a homology of <98% (Bernasconi et al. 2002).

1.3.3 Transmission studies

In order for ticks to be confirmed as a vector of a pathogen, transmission studies must be conducted to ascertain whether the tick can transmit the pathogen to susceptible animals, and whether they are capable of maintaining the pathogen throughout its life cycle. A list of pathogens that have been detected in *R. sanguineus*, which may be transmitted by this tick, is provided in Table 1.4. The most important human pathogens transmitted by *R. sanguineus* are *Rickettsia conorii*, which causes Mediterranean spotted fever, and *R. rickettsii*, which causes Rocky Mountain spotted fever (Piranda et al. 2011).

Table 1.4: List of pathogens that may be transmitted by *R. sanguineus* (adapted from Dantas-Torres 2008).

Pathogen	**Associated disease**	**Reference**
Anaplasma marginale[b]	Bovine anaplasmosis	Parker and Wilson (1979)
Anaplasma platysa[a] (formerly Ehrlichia platys)	Canine cyclic thrombocytopenia	Simpson et al. (1991)
Babesia caballii[b]	Equine babesiosis	Enigk (1943)
Babesia canis	Canine babesiosis	Regendanz and Muniz (1936)
Babesia gibsoni	Canine babesiosis	Sen (1933)

Cercopithifilaria grassi (formerly Dipetalonema grassi)	Canine filariosis	Bain et al. (1982)
Coxiella burnetii	Q fever	Mantovani and Benazzi (1953)
Dipetalonema dracunculoides	Canine filariosis	Olmeda-Garcı´a et al. (1993)
Ehrlichia canis	Canine monocytic ehrlichiosis	Groves et al. (1975)
Hepatozoon canis	Canine hepatozoonosis	Nordgren and Craig (1984)
Leishmania infantum[a] (syn. Leishmania chagasi)	Canine visceral leishmaniasis	Blanc and Caminopetros (1930)
Mycoplasma haemocanis (formerly Haemobartonella canis)	Canine haemobartonellosis	Seneviratna et al. (1973)
Rangelia vitallia[a]	Nambiuvu or peste de sangue	Loretti and Barros (2005)
Rickettsia conorii	Mediterranean spotted fever	Brumpt (1932)
Rickettsia rickettsii	Rocky Mountain spotted fever	Parker et al. (1933)
Theileria equi[b] (formely Babesia equi)	Theileriosis	Enigk (1943)

[a] Despite the evidence indicating that *R. sanguineus* can be a vector of these pathogens, further research is needed to prove it; [b] *R. sanguineus* ticks seldom bite hosts other than dogs and thus its role in the transmission of these pathogens in nature is probably minor (Dantas-Torres 2008).

Only a few studies have investigated *R. sanguineus* as a potential vector of *C. burnetii*. A study by Mantovani and Benazzi (1953) conducted in Teramo, Italy, aimed to isolate *C. burnetii* from naturally infected dogs that were associated with a human Q fever outbreak on a farm. Complement-fixation tests provided positive results for *C. burnetii* in three dogs that had been collected from farms associated with human Q fever outbreaks. One of the dogs that tested positive for *C. burnetii*, and a control (negative for *C. burnetii*) dog were infested with *R. sanguineus*, which were collected from the dogs at 5-7 day intervals. The dogs' blood, material from engorged *R. sanguineus,* and *R. sanguineus* eggs were used to inoculate guinea pigs, in order to demonstrate and detect *C. burnetii* infection. Positive results for *C. burnetii* were obtained by complement-fixation tests in the guinea pigs, and were matched with pathological lesions (enlarged spleen), for the tick extract, the dog's blood extract, and the tick egg extract, from the infected dog. All results for the control were negative. The positive result of *C. burnetii* from the tick egg extract may suggest that *R. sanguineus* is capable of transovarial transmission of *C.*

burnetii (Mantovani and Benazzi 1953), however further investigations are necessary to ascertain whether the pathogen can be maintained throughout the tick's life cycle to implicate it as a vector of this disease. As one of the infected dogs in the study had been observed at the farm feeding on foetal membranes of sheep (which tested positive for *C. burnetii*) the authors suggested that infected foetal membranes from cattle and sheep may have provided the source of infection for other animals in the herd, as well as to humans and dogs (Mantovani and Benazzi 1953). A limitation of the study was that it did not demonstrate the transmission of *C. burnetii* from infected ticks to susceptible animals, therefore did not demonstrate that *R. sanguineus* is a competent vector of *C. burnetii*.

1.4 The brown dog tick (*Rhipicephalus sanguineus*)

Ticks are haematophagous ectoparasites of vertebrates, and the vertebrate host's blood is their only source of food (Sonenshine 2005). When searching for a host, unfed *R. sanguineus* can hunt, but can also use an ambush strategy, where they exhibit what is described as questing behaviour (Dantas-Torres 2010). *Rhipicephalus sanguineus*, like other ixodids (hard ticks), undergo four developmental stages in its life cycle: egg, larval stage, single nymph stage, and adult. *Rhipicephalus sanguineus* is a three-host tick, whereby each life stage feeds on a new host, and all life-stages prefer to feed on the same host species (Figure 1.1) (Dantas-Torres 2010a).

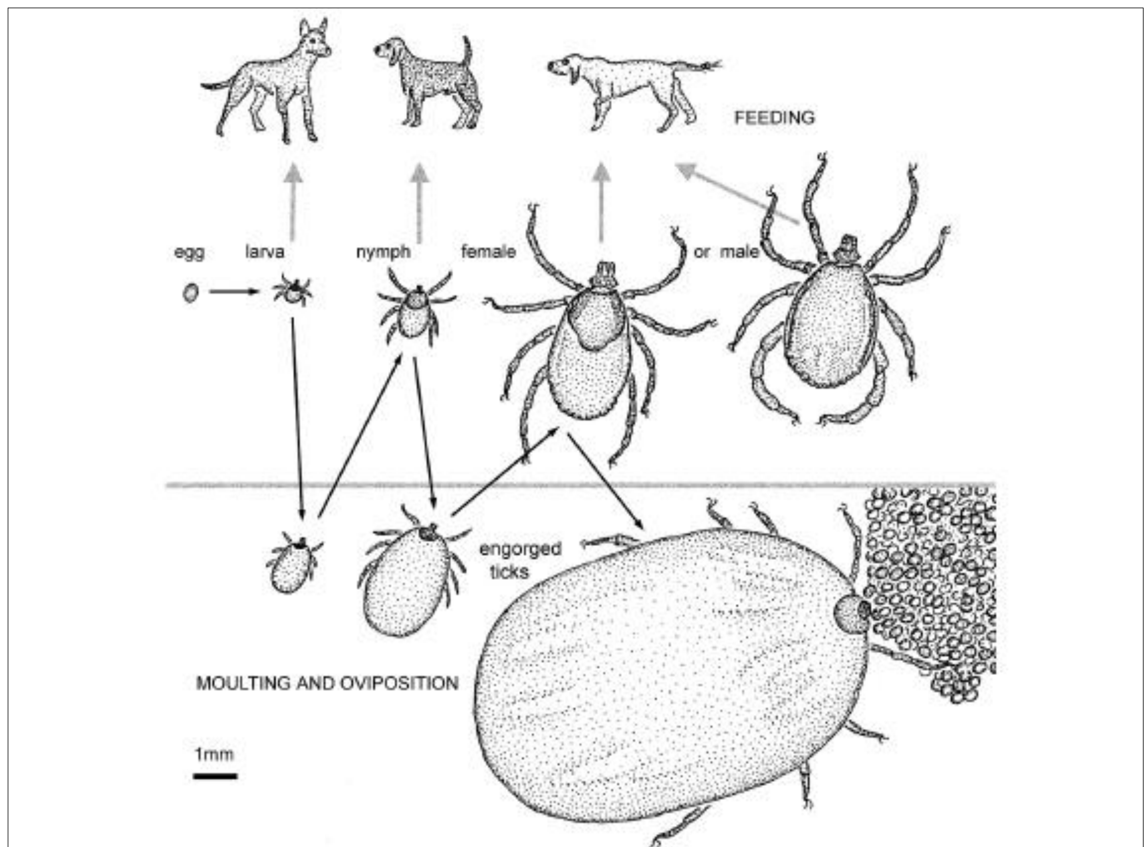

Figure 1.1: Three-host tick life-cycle. This figure demonstrates the life-cycle of *R. sanguineus* (adapted from Barker and Walker 2014).

Rhipicephalus sanguineus ticks are widely distributed throughout the world, occurring mainly within latitudes 35ºS and 50ºN (Dantas-Torres 2008). As with all ticks, the distribution of *R. sanguineus* is closely correlated with both the climate and the distribution of its primary host, in this case the domestic dog, *Canis lupus familiaris* (*C. familiaris*). *Rhipicephalus* ticks are considered to have originated in Africa 14 Mya, and subsequently dispersed into Europe and Asia (Murrell, Campbell, and Barker 2001; Otranto et al. 2014).

1.4.1 Arrival and distribution of *R. sanguineus* in Australia

It is uncertain when *R. sanguineus* was introduced into Australia. Two hypotheses considered are; (1) that the ticks were introduced on their canine hosts during European settlement (in the last 250 years) through one or more ports in Australia (Seddon 1968) or; (2) potentially with the introduction of semi-domesticated dogs (dingoes) from SE Asia across the land bridges

approximately 5,000 years BP. Interestingly however, *R. sanguineus* is rarely found on dingoes today (Oskarsson et al. 2012). It is generally recognised that *R. sanguineus* is prevalent throughout the year in tropical and sub-tropical regions, and their development is dependent on temperature and relative humidity (RH) and is most efficient at 20-35 °C and 35-96% RH (Koch and Tuck 1986). A limiting factor for the establishment of *R. sanguineus* in cold climates is exposure to temperatures less than 10 ºC (Dantas-Torres et al. 2010b), however *R. sanguineus* can also be found in Mediterranean climates (Dantas-Torres 2010a). *Rhipicephalus sanguineus* is well-adapted to live within human dwellings (Dantas-Torres 2010a), which could assist its survival in temperate zones that experience cold winter temperatures. In Australia, the distribution of *R. sanguineus* is thought to occur rarely in temperate climates, but it is reportedly abundant in areas with high levels of rainfall and humidity in northern Australia, and extending into semi-arid inland areas (Roberts, 1965).

The current geographical distribution of *R. sanguineus* in Australia is anecdotal, uncertain in its accuracy, and in need of investigation, since the most recent Australian distribution map for the brown dog tick is nearly 50 years old (Roberts 1965) and a systematic study of the geographical distribution of *R. sanguineus* in Australia has never been conducted. The distribution reported by Roberts (1965), depicted in Figure 1.2, showed *R. sanguineus* in northern regions of Australia; in the Northern Territory, Queensland, northern Western Australia, and north-eastern New South Wales (Roberts 1965).

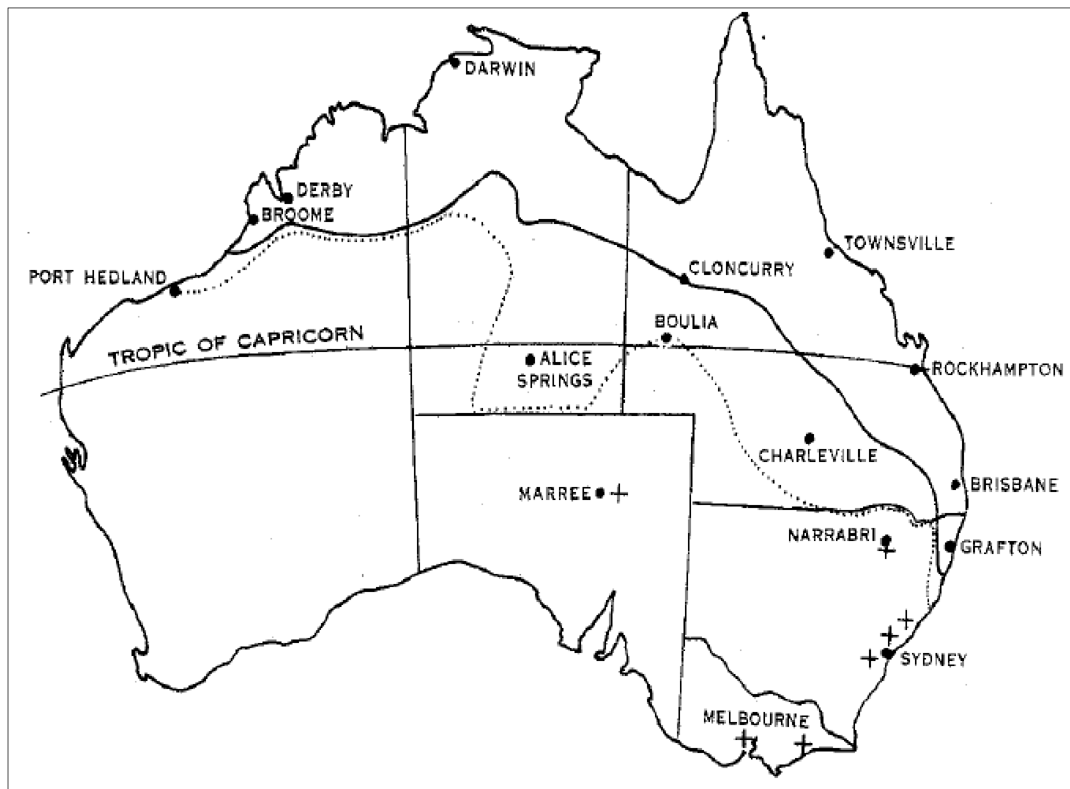

Figure 1.2: Distribution of *R. sanguineus* in Australia, indicated by the dotted line (adapted from Roberts, 1965).

1.4.2 The identification of *R. sanguineus*

There is currently no absolute consensus on the taxonomy and classification of *R. sanguineus*, largely due to the absence (presumed lost) of the type specimen, which was described by Latreille (1806). *Rhipicephalus sanguineus* is widely distributed throughout the world and there are 17 species of tick that are considered to be morphologically similar to the description of *R. sanguineus* (Gray et al. 2013). Together, these 17 *Rhipicephalus* species are referred to as the "*R. sanguineus* group," and include the following species: *R. aurantiacus*, *R. bergeoni*, *R. boueti*, *R. camicasi*, *R. guilhoni*, *R. leporis*, *R. moucheti*, *R. pumilio*, *R. pusillus*, *R. ramachandrai*, *R. rossicus*, *R. sanguineus sensu stricto* (s.s.), *R. schulzei*, *R. sulcatus*, *R. tetracornus, R. turanicus*, and *R. ziemanni* (Dantas-Torres et al. 2013).

There are two textbooks that describe the morphology of Australian ticks; by Roberts (1970), and more recently by Barker and Walker (2014). The tome *Australian Ticks* (Roberts, 1970) has been

widely used by Australian parasitologists for tick species identification for over 40 years. Barker and Walker (2014) advised that their text can be used for species identification of the 16 species (5 species of argasids or 'soft ticks,' and 11 species of ixodids) that are thought to infest Australian domestic animals and humans. This is a relatively small number of tick species compared with the 59 species of Ixodidae and Argasidae ticks described by Roberts (1970). Furthermore, in a departure from the dichotomous key used by Roberts (1970), Barker and Walker (2014) list the morphological characteristics of the tick species in a tabular format. Both books provide tick species illustrations for comparison. Barker and Walker (2014) only described adult instars (except for *Otobius megnini*), whereas Roberts (1970) often described the morphology of immature ticks (larvae and nymphs), and included some in keys. Diagrams of female and male *R. sanguineus* are provided in Figures 1.3 and 1.4, respectively, with key morphological features used for species identification labelled (Barker and Walker 2014).

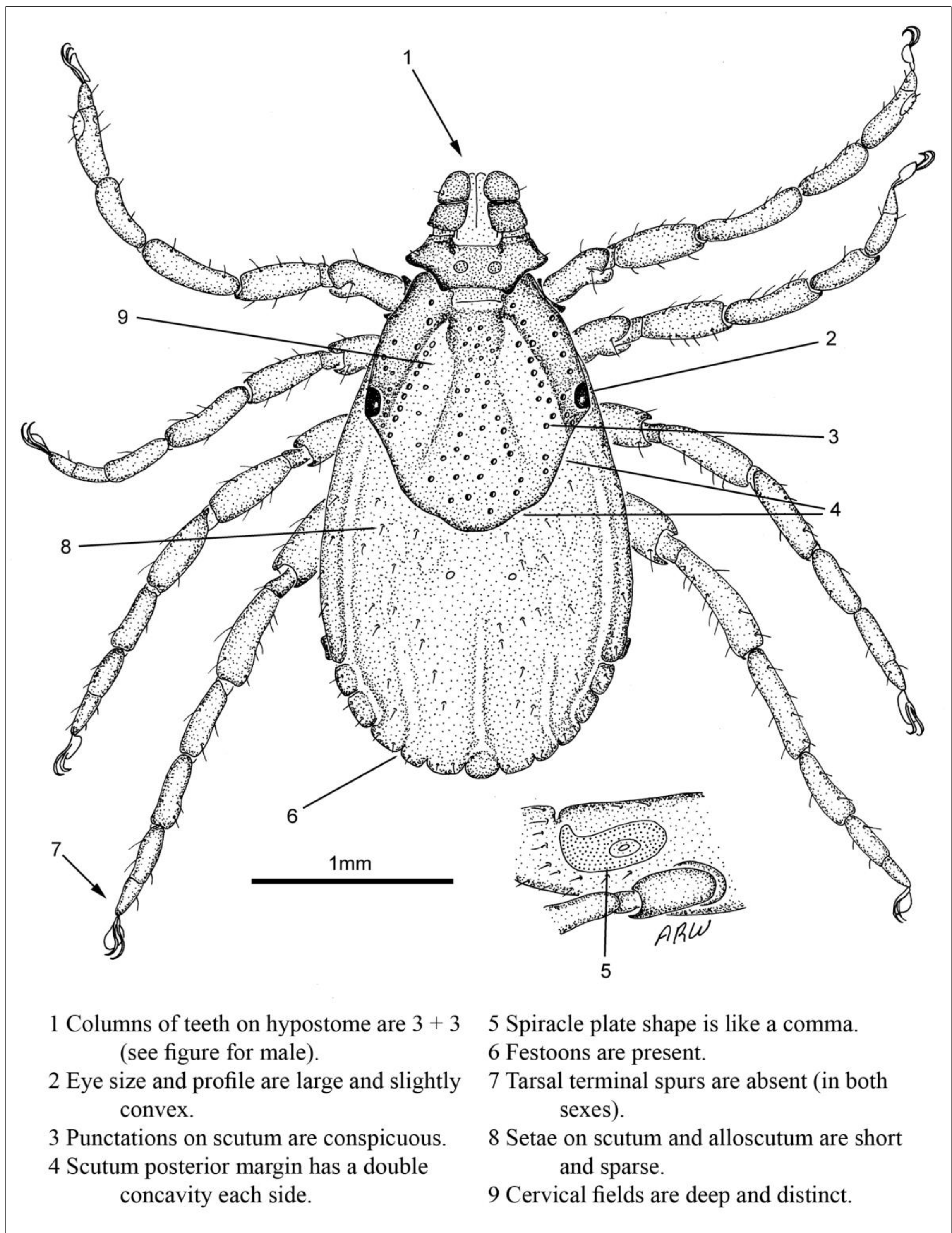

Figure 1.3: Dorsal view of female *R. sanguineus* (adapted from Barker and Walker, 2014).

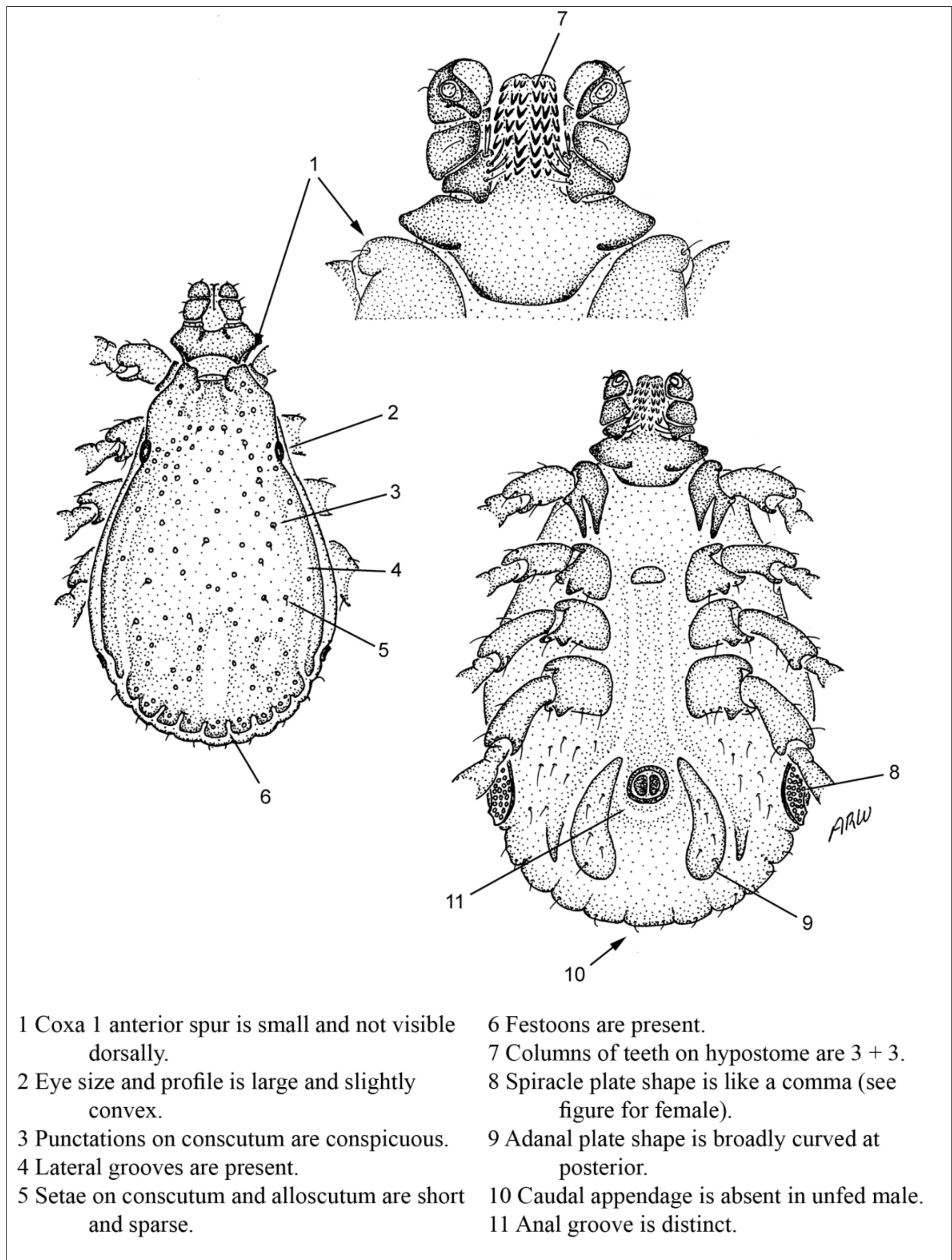

Figure 1.4: Ventral view of male *R. sanguineus* (adapted from Barker and Walker, 2014).

1.5 Detection of *C. burnetii* in *R. sanguineus* ticks

The initial and overarching aim of this project was to detect *C. burnetii* in *R. sanguineus* ticks collected from dogs in Australia. The experimental work is divided into four stages with the following aims:

1. To identify *R. sanguineus* ticks using a key of tick morphology (Roberts 1970).
2. To map the *R. sanguineus* collection locations using GIS software.
3. To detect *C. burnetii* in *R. sanguineus* ticks collected.
4. To investigate the bacterial microbiome in *R. sanguineus* collected.

The DNA sequencing will be conducted using the Ion Torrent™ sequencing platform, and *Coxiella*-specific primers will be used for the detection of *Coxiella* species, and the microbiome will be assessed using primers that target the 16S rRNA gene in bacteria.

The hypotheses that will be tested by the experimental work are as follows:

1. Ticks that feed on dogs in Australia are predominantly *R. sanguineus*.
2. *Rhipicephalus sanguineus* is mainly distributed throughout tropical and sub-tropical climates in northern Australia.
3. *Coxiella burnetii* is present in *R. sanguineus* collected from dogs.
4. *Rhipicephalus sanguineus* ticks harbour a diverse community of bacteria that makes up their microbiome.

CHAPTER 2: MATERIALS AND METHODS

2. Materials & Methods

2.1 Tick collection

In response to a nationwide advertisement requesting ticks, individual tick specimens (n = 3,276) were collected from dogs by various persons throughout Australia (see Acknowledgements) during 2012-2014, with the majority collected during the 2012/14 tick seasons. The total number of instars collected and identified from each state and territory are listed in Table 2.1. The ticks collected for this study were removed from dogs using gloves and forceps, and preserved in 70% ethanol until use. For each submission received, the source, approximate geographic location, and date of collection was recorded (Appendix A, Table A.1); additional notes on the dog breed and clinical signs were not consistently recorded. The number of submissions for each State and Territory are listed in Appendix A, Table A.2.

Table 2.1: Number of ticks collected from seven states and territories in Australia between 1996 and 2014.

Australian State/Territory	Larvae (n)	Nymphs (n)	Males (n)	Females (n)	Total
New south Wales (NSW)	2	190	74	299	565
Northern Territory (NT)	30	239	919	845	2033
Queensland (QLD)	0	29	2	29	60
South Australia (SA)	1	40	199	95	335
Tasmania (TAS)	16	5	0	36	57
Victoria (VIC)	0	0	0	5	5
Western Australia (WA)	7	25	97	91	220
Total	56	528	1,291	1,401	**3,276**

2.2 Tick identification

Ticks were removed from 70% ethanol and examined in separate petri dishes. The ticks were counted, and the developmental stages and species were identified and recorded into a meta-database (see Appendix A, Table A.1). Forceps and all other instruments used to handle the ticks were cleaned with DNA *AWAY*™ (Molecular Bio-Products Inc., San Diego, CA) between samples, and surfaces were sterilised with DNA-ExitusPlus™ (AppliChem, Darmstadt, Germany). After morphological identification, ticks were stored in 70% ethanol at 4 °C. Species identification of the ticks was based on the morphological features outlined in the Australian tick morphology key by Roberts (1970).

Photographs of the ticks were taken with an Olympus SC30 digital camera (Olympus, Center Valley, PA, United States) and analysis getIT software (Olympus, Center Valley, PA, United States) at a magnification ranging between 0.67X to 4.5X. An Olympus SZ61 stereomicroscope (Olympus, Center Valley, PA, United States) was used with a SCHOTT KL 1500 LED (SCHOTT AG Mainz, Germany) light source.

2.3 Sample mapping and selection

The collection sites of *R. sanguineus* ticks were mapped using ArcGIS (Esri, Redlands, CA, USA) using the location information provided for the samples. Where possible, the samples were mapped to a specific address, otherwise, the next most specific information was used. The geographic locations of the ticks were used as the basis for grouping (pooling) the ticks for DNA extraction. Male and female ticks for the DNA extraction were selected from three locations: Darwin (NT), Palmerston (a suburb of Darwin), and Perth (WA) (Table 2.2).

Table 2.2: Number of ticks extracted from Darwin, Palmerston and Perth

Geographic location	**Number of male *R. sanguineus* ticks extracted**	**Number of female *R. sanguineus* ticks extracted**	**Total**
Darwin	20	20	40
Palmerston	18	18	36
Perth	15	16	31
Total	53	54	107

2.4 DNA extraction

DNA was isolated from individual adult male (n = 53) and female (n = 54) ticks. Prior to DNA extraction, ticks were washed in fresh 70% ethanol, surface sterilized in 10% hypochlorite solution, and rinsed in sterile water. Ticks were first mechanically crushed, then enzymatically lysed as described below. Briefly, specimens were placed into 2 mL safelock tubes (Eppendorf, Germany), each containing a 5 mm steel bead (QIAGEN, Hilden, Germany). Female ticks were cut into segments with a sterile scalpel before added to the tube. The tubes that contained ticks and beads were flash frozen in liquid nitrogen for 1 min then mechanically crushed by shaking the tube at 40 oscillations per second for 1 min in the TissueLyser™ system (QIAGEN, Hilden, Germany). The tubes were centrifuged at 10,000 rpm for 1 min before the addition of 270 µL ATL buffer (QIAGEN, Hilden, Germany) and 30 µL Proteinase K (QIAGEN, Hilden, Germany), and incubated on an oscillating platform (400 rpm) at 50 °C for approximately 16 h (overnight).

After mechanical and enzymatic lysis, genomic DNA (gDNA) was extracted from the ticks using a QIAmp Blood and Tissue kit (QIAGEN, Hilden, Germany) using QIAGEN supplementary protocol "Purification of total DNA from insects using the DNeasy® Blood & Tissue Kit" and DNA was eluted in 65µL of buffer AE. Extraction controls were run in parallel with the DNA extractions in order to assess the amount of cross contamination and provide a background bacterial profile of the laboratory.

2.5 PCR

2.5.1 *Coxiella burnetii* qPCR assays

A *C. burnetii* specific primer set was used in this study to target *C. burnetii* DNA. The primer name, primer sequence, amplicon size, and annealing temperature is described in Table 2.3. A two-step qPCR assay for the IS1111 primer set (Banazis et al. 2010) was performed in 25 µL volumes, and consisted of 0.3 ρmol of each primer, 0.25 mM of each dNTP, 2.5 µL of 10x buffer (5 PRIME), 1 U of *Taq* DNA polymerase (5 PRIME, Hilden, Germany), 2 mM of magnesium chloride, 0.3 ρmol IS1111a probe (5'-CCCACCGCTTCGCTCGCTAA-3': 5' label 6-FAM; 3' label BHQ-1) (Banazis et al. 2010), and 5 µL of undiluted DNA extract. The qPCR assay was performed using the StepOne™ Real-Time PCR machine (version 2.1, Applied Biosystems, Foster City, CA, USA) with an initial hold cycle (50 °C, 2 min), then one cycle of denaturation (95 °C, 5 min), followed by 50 cycles of denaturation (95 °C, 20 s), and annealing and extension (60 °C, 45 s). No-template controls were included for each assay, and a positive control was included that comprised DNA extracted from the Q-Vax™ vaccine (CSL, Parkville, Australia) (Banazis et al. 2010).

2.5.2 Universal bacterial 16S qPCR assays

Extracted DNA from male and female *R. sanguineus* ticks from Darwin (n = 40), Palmerston (n = 36), and Perth (n = 31) were screened by real-time qPCR for bacterial DNA. Bacterial DNA was amplified using the universal bacterial 16S primers 27F-mod and 338R (Turner et al. 1999) (Table 2.3). No-template controls (NTCs) were included for each qPCR assay. The qPCR reactions were carried out in 25 µL volumes, consisting of 2.5 µL of 10x buffer (5 PRIME), 2 mM magnesium chloride (5 PRIME), 0.25 mM of each deoxyribonucleoside triphosphate (dNTP) (FisherBiotec, Wembley, WA, Australia), 1 µL of 1 mg/mL Bovine Serum Albumen (BSA), 0.6 µl of 5x SYBR green dye (Life Technologies, Carlsbad, CA, USA), 0.4 µM of each primer (Integrated DNA Technologies, Coralville, IA, USA), 0.625 U of *Taq* DNA polymerase (5 PRIME, Hilden, Germany), and 2 µL gDNA. Quantitative PCR reactions were performed using

the StepOne™ Real-Time PCR machine (version 2.1, Applied Biosystems, Foster City, CA, USA) with one cycle of denaturation (95 °C, 5 min), followed by 35 cycles of denaturation (95 °C, 30 s), annealing (55 °C, 30 s), and extension (72 °C, 45 s).

Table 2.3: Primer sets and PCR conditions for DNA amplification

Gene target	**Primer name and sequence (5'-3' orientation)**	**Amplicon size (bases)**	**Annealing Temperat-ure**	**Referen-ce**
16S rRNA gene (V1 and V2 hyper-variable regions)	Universal 27F mod* = AGAGTTTGATCCTGGCTYAG Universal 338R = TGCTGCCTCCCGTAGGAGT	~312 (see results)	55 °C	*This study
C. burnetii IS1111a transposase gene	IS1111aF = GTTTCATCCGCGGTGTTAAT IS1111aR = TGCAAGAATACGGACTCACG	498	60 ° C	Banazis et al. 2010. GenBank accession no. M80806.
Ion Torrent ™ A/P1 sequences	IT A primer = CCATCTCATCCCTGCGTGTCT CCGACTCAG IT P1 primer = CCTCTCTATGGGCAGTCGGTG AT	>400	55 °C	Ion Torrent ™

2.6 Gel electrophoresis

PCR products were run on 2% (*w/v*) agarose gel electrophoresis. The agarose was dissolved in 1x TAE buffer and stained with 1x Gel Red (FisherBiotech, Wembley, WA, Australia). A 100 bp molecular weight ladder (Promega, Madison, WI, USA) was used to determine the size of all PCR products. The DNA was visualised using Ultra-Violet transillumination and an AlphaDigiDoc transillumination system (BioRad, Hercules, CA, USA).

2.7 Tick pooling for Ion Torrent sequencing

Ticks from the same submissions were pooled in 5 µL volumes for each DNA extraction, which resulted in 59 tick pools overall (see Table 2.4).

Table 2.4: Tick pools for sequencing

Geographic location	**Male tick pools (n)**	**Female tick pools (n)**	**Total**
Darwin	9	9	18
Palmerston	11	11	22
Perth	6	13	19
Total	26	33	59

2.8 Ion Torrent sequencing

A Life Technologies® Ion Torrent semiconductor sequencing platform was used to sequence the hyper-variable regions V1 and V2 in the bacterial 16S rDNA gene from tick pools (n = 59). The Ion Torrent equipment and reagents were supplied by Life Technologies (Foster City, CA), and were used per manufacturer's instructions. Fusion-tag primers consisted of universal bacterial 16S 27F mod and 338R primer sequences, a unique 6-8 bp-long multiplex identifier (MID) tag specific for each sample in both the forward and reverse primers (see Appendix B, Table B.1), and the Ion Torrent sequencing adapters (ITA/P1, Table 2.3). The fusion-tag primer architecture is outlined in Figure 2.1.

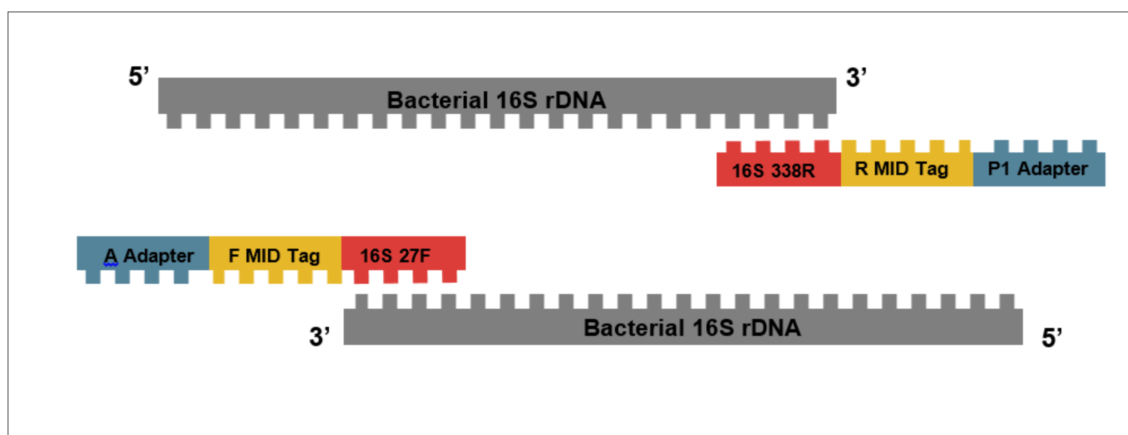

Figure 2.1: Fusion-tag primer architecture. 16S 27F: universal bacterial 16S forward primer; 16S 338R: universal bacterial 16S reverse primer; F MID tag: forward primer MID tag; R MID tag: reverse primer MID tag; A Adapter: sequencing adapter for forward primer; P1 adapter: sequencing adapter for reverse primer.

2.8.1 Fusion-tag qPCR

The fusion-tag qPCR was setup in a DNA-free lab (Cooper and Poinar 2000). Each reaction mixture was a 25 µL volume, and consisted of 2.5 µL of 10x buffer (5 PRIME, Hilden, Germany), 0.5 µL of 25 mM magnesium chloride, 0.04 µg/µL BSA, 0.25 µM of dNTPs, 0.12x of SYBR green (Life Technologies), 0.625 U *Taq* DNA polymerase (5 PRIME, Hilden, Germany), 1 µL for each forward and reverse primer, and sterile water was added to make the volume up to 25 µL. The forward and reverse primers were added in a particular combination so as to generate a unique sample-specific tag to enable the pooled sequences to be matched back to the individual sample for the analysis. Undiluted DNA for all samples was added in 2 µL volumes to the reaction. Two NTCs were included, one from the fusion-tag PCR setup lab, and one for the DNA setup hood. Thermal cycling equipment and conditions used for the fusion-tag qPCR were as for the 16S qPCR (see Section 2.5.2, Table 2.3).

2.8.2 PCR purification

Based on cycle threshold (C_T) values and slopes of the curves that resulted from the fusion-tag qPCR assays, tick pools were pooled into equimolar ratios. The pools that resulted (n = 9) were

purified with the Agencourt® Ampure XP Bead DNA Purification protocol (Beckman Coulter Genomics, USA) with the modification that a 1.2 ratio was used over the standard 1.8. This was done in order to remove large primer dimer sequences over 100 bp in length. A 2% agarose gel was run for 1 h at 76 volts for the pre- and post-Ampure products, and a with 50 bp molecular weight ladder (Promega, Madison, WI, USA) was included, to ensure that primer dimer was removed, and that the product was retained.

2.8.3 Relative PCR quantification

The nine fusion-tag pools were diluted to 1/1000 and a qPCR was carried with the IT A/P1 primers listed in Table 2.3, Section 2.5.2. The qPCR reactions were performed in 25 µL volumes, which consisted of 0.4 µM of each primer, 12.5 µL of 2x PowerSYBR (Life Technologies, Carlsbad, CA, USA), and was made up to 23 µL with sterile water, and 2 µL of the 1/1000 dilution was added. The qPCR was performed with the StepOne™ Real-Time PCR machine (version 2.1, Applied Biosystems, Foster City, CA, USA) with one cycle of denaturation (95 °C, 5 min), followed by 35 cycles of denaturation (95 °C, 30 s), and annealing and extension (60 °C, 45 s).

2.8.4 Absolute quantification

The nine fusion-tag pools were pooled into one final mixture in equimolar ratios based on the C_T values from the relative quantification PCR assay (see Appendix B, Tables B.2 - B.4). The following dilutions were made for the sample: 1/100; 1/1000; 1/2000; 1/4000; 1/8000; 1/16000; 1/32000; and 1/64000. Serial dilutions of a 152 bp synthetic oligonucleotide were also included in the assay as follows: 10^3; 10^4; 10^5; 10^6; 10^7; 10^8; and 10^9. The sample dilutions and the standards were run under the same conditions as per section 2.8.3. The C_T values that resulted from the assay were used to plot a standard curve (see Appendix B, Table B.5 and Figure B) so that the copy number of the sample could be estimated.

2.8.5 OT2

Emulsion PCR was conducted on the Ion OneTouch™ ES (enrichment system) instrument (Life Technologies, Carlsbad, CA, USA) with the Ion PGM™ Template OT2 400 kit (Life Technologies, Carlsbad, CA, USA). The reaction was carried out using 100 ρM of library DNA and the protocol was followed as per manufacturer's instructions.

2.8.6 Ion Torrent PGM

The DNA fusion-tag library was sequenced using an Ion™ PGM instrument (Life Technologies, Carlsbad, CA, USA) with an Ion™ PGM 400 sequencing kit (Life Technologies, Carlsbad, CA, USA), and a 316 v2 sequencing chip, following the manufacturer's protocol 'Ion PGM™ Sequencing 400 Kit - For use with the Ion Personal Genome Machine® (PGM™) System and the Ion 314™ Chip v2, Ion 316™ Chip v2, and Ion 318™ Chip v2.'

2.9 Sequence analysis

2.9.1 Sequence deconvolution and quality filtering

The Ion Torrent reads were imported into the Geneious software package version 7.1.7 (Biomatters Ltd., NZ) in a FASTQ file format, and deconvoluted. Sequences with 100% matched primers were identified, annotated, and extracted. Sequences less than 100 bp and greater than 419 bp in length were excluded and sequences with mismatches were excluded from the analysis. The MID tags were identified and the corresponding sample number was assigned. The primers were removed (trimmed), and the sequences were renamed for UPARSE (Edgar 2013). A FASTQ file from Geneious was imported into the program UPARSE, which was used to discard low quality reads, chimeric sequences, and less than five sequences per unique read were removed.

2.9.2 QIIME

The open source software 'Quantitative Insights Into Microbial Ecology' (QIIME) (Caporaso et al. 2010) was used to assign operational taxonomic units (OTUs) to the unique sequences for each

tick sample ID using the curated database GreenGenes (available at http://greengenes.lbl.gov/). The percent composition of each taxa and bar graphs were generated in QIIME.

2.9.3 MEGAN

The annotated and filtered sequences in Geneious were also imported as a FASTA file into YABI (Hunter, Macgregor, et al. 2012), where the sequence similarity was compared to the non-curated NCBI GenBank nucleotide submissions. These BLAST files were imported into MEtaGenome ANalyzer (MEGAN) version 5.6.0 (Huson et al. 2007) to visualise the closest species match for the sequences.

CHAPTER 3: RESULTS

3. Results

3.1 Morphological identification of Ixodidae removed from dogs.

Four genera (*Amblyomma*, *Haemaphysalis*, *Ixodes*, and *Rhipicephalus*) of ixodids were identified from n = 3,276 ticks collected from dogs. One species of *Amblyomma* (*A. triguttatum*), two species of *Haemaphysalis* (*H. bancrofti* and *H. longicornis*), three species of *Ixodes* (*I. cornuatus*, *I. holocyclus*, and *I. tasmani*), and one species of *Rhipicephalus* (*R. sanguineus*) ticks matched the descriptions by Roberts (1970), and were recorded (Appendix A, Table A.1), and photographs of specimens from each species are presented in Figures 3.1 – 3.7. The number of instars recorded for each species are reported in Table 3.1. As noted by others previously, *Ixodes holocyclus* and *I. cornuatus* were difficult to distinguish based on the whether the cornua were well-defined and blunt vs. mildly rounded, and therefore identification was primarily based on the leg colour observed in the ticks. *Ixodes holocyclus* were observed to have light yellow 2nd and 3rd pairs of legs, and dark brown 1st and 4th pairs of legs (see Figure 3.4), whereas all four pairs of legs in *I. cornuatus* were brown (see Figure 3.5). This morphological feature has recently been supported by Barker and Walker (2014) as an additional feature that can assist morphological identification of *I. holocyclus* and *I. cornuatus*.

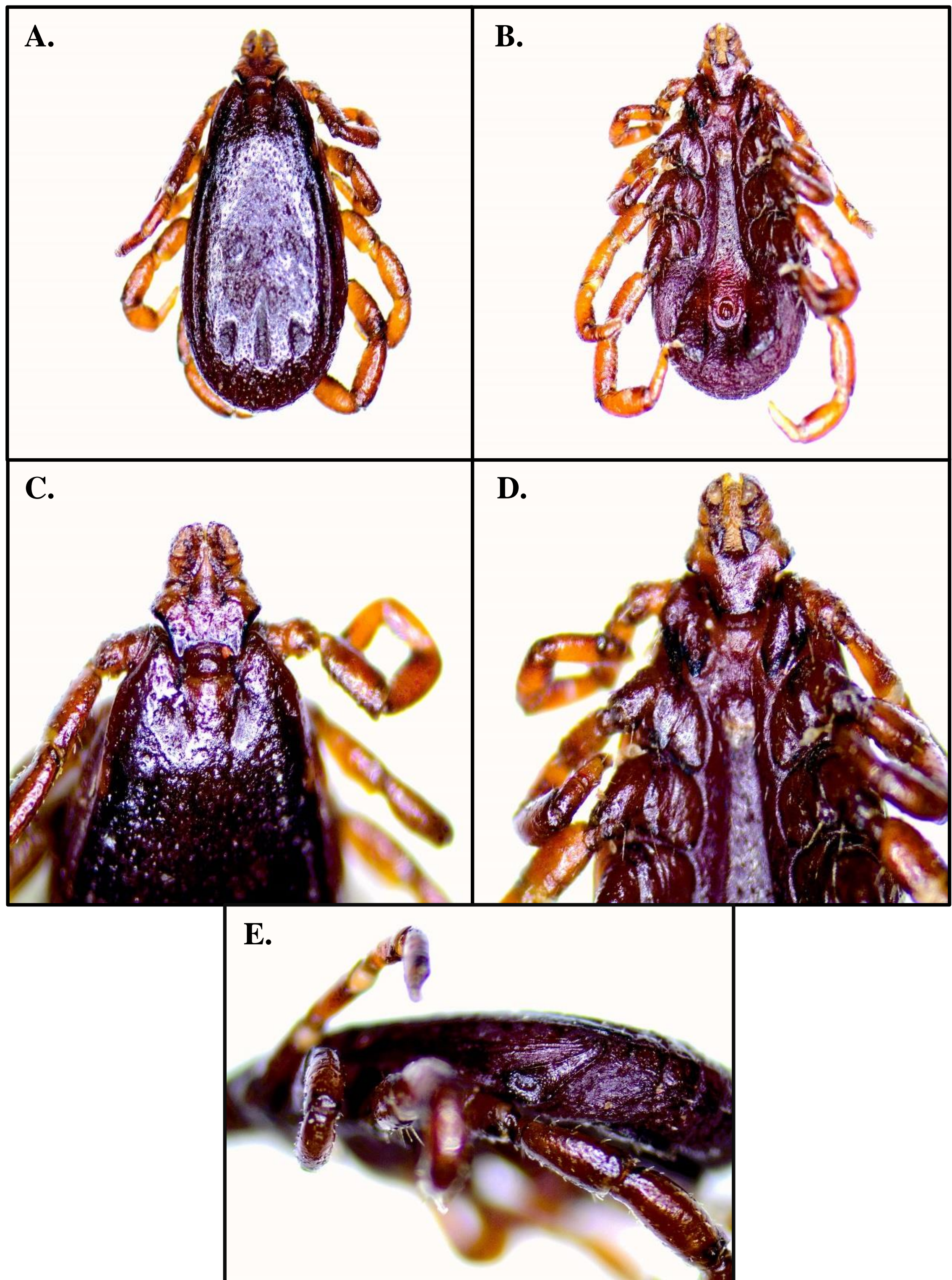

Figure 3.1: Morphological features used to identify male *R. sanguineus* ticks. A and C. dorsal view; B and D. ventral view; E. lateral view.

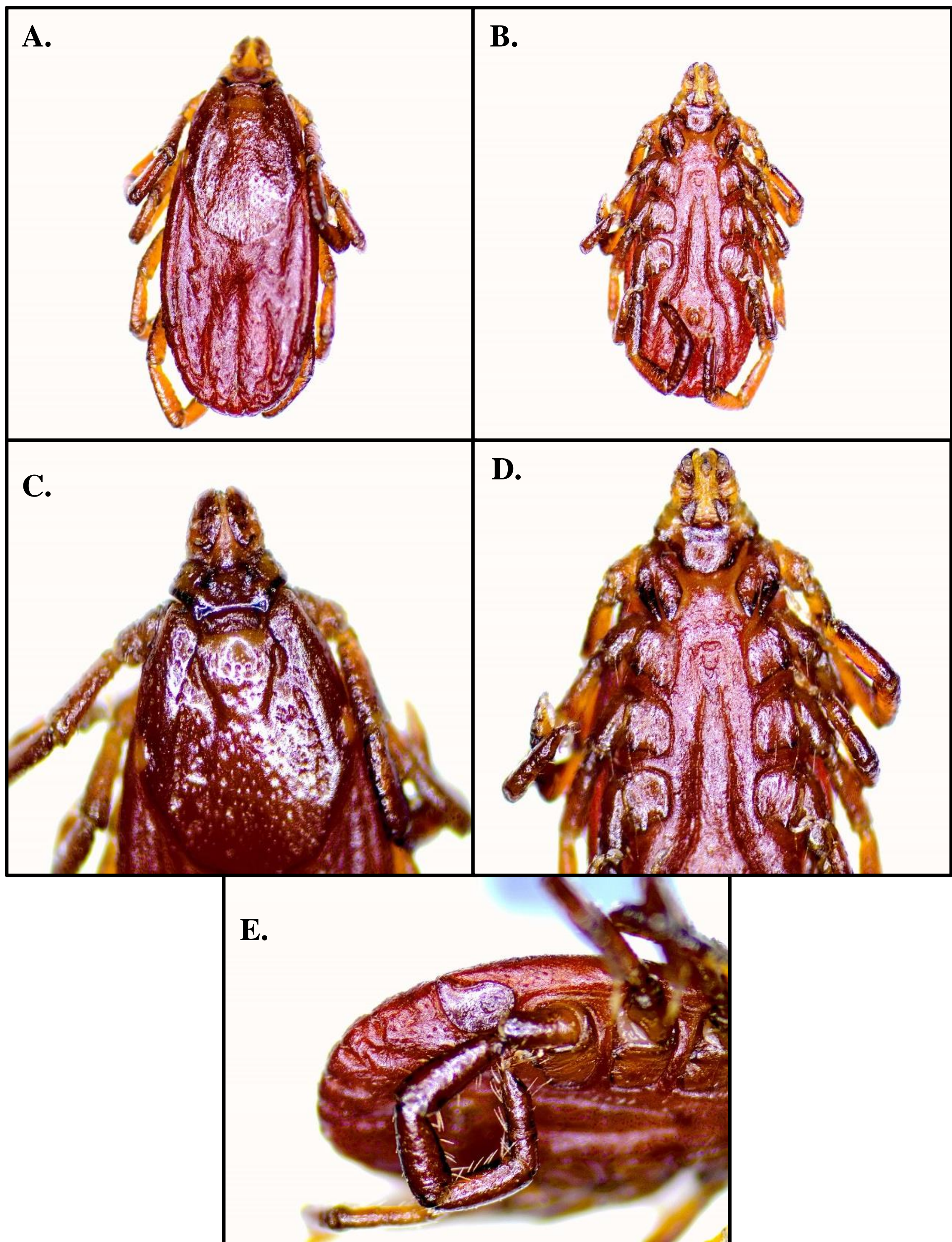

Figure 3.2: Morphological features used to identify female *R. sanguineus* ticks. A and C. dorsal view; B and D. ventral view; E. lateral view.

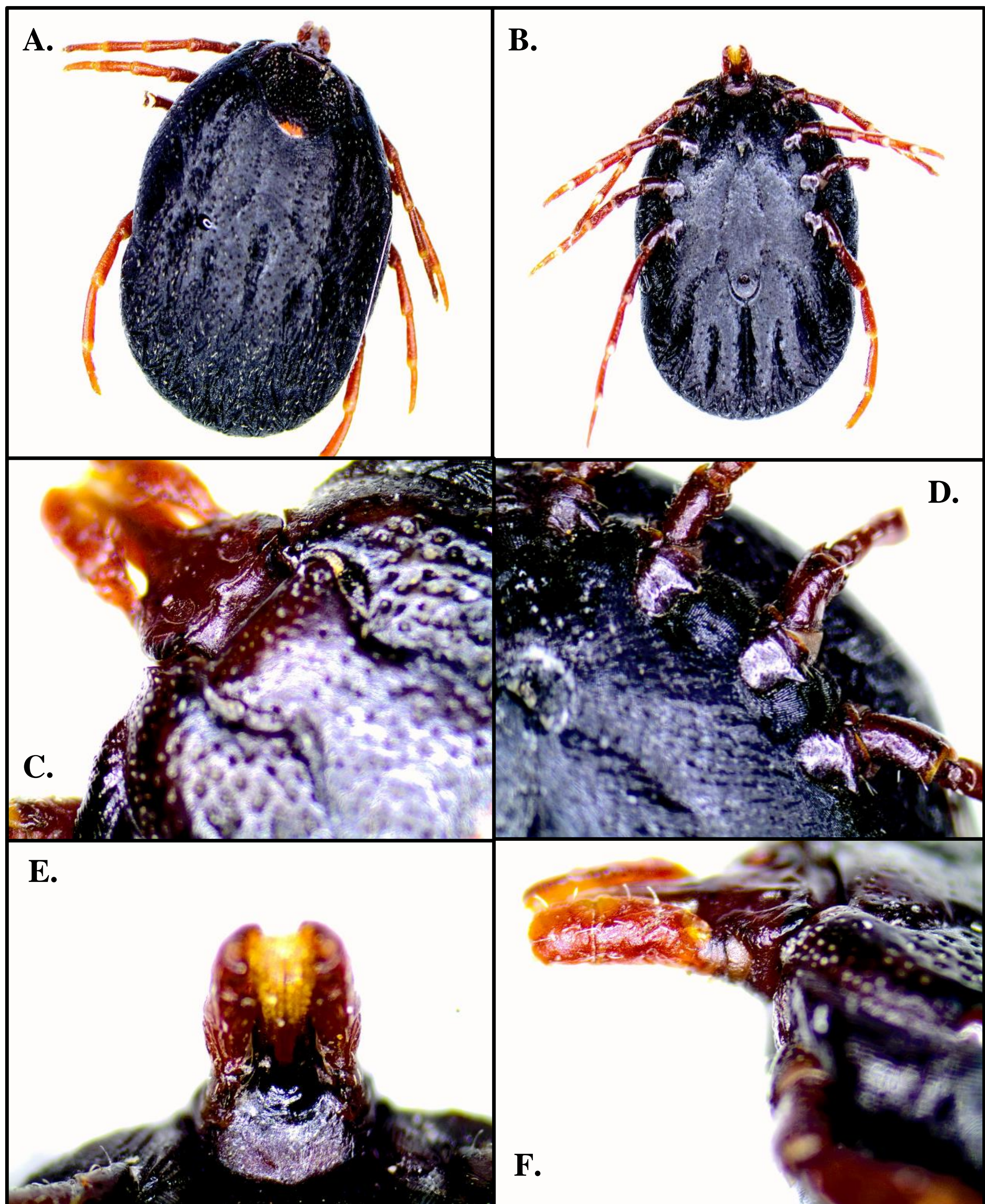

Figure 3.3: Morphological features used to identify female *A. t. triguttatum* ticks. A and C. dorsal view; B, D and E. ventral view; E. lateral view.

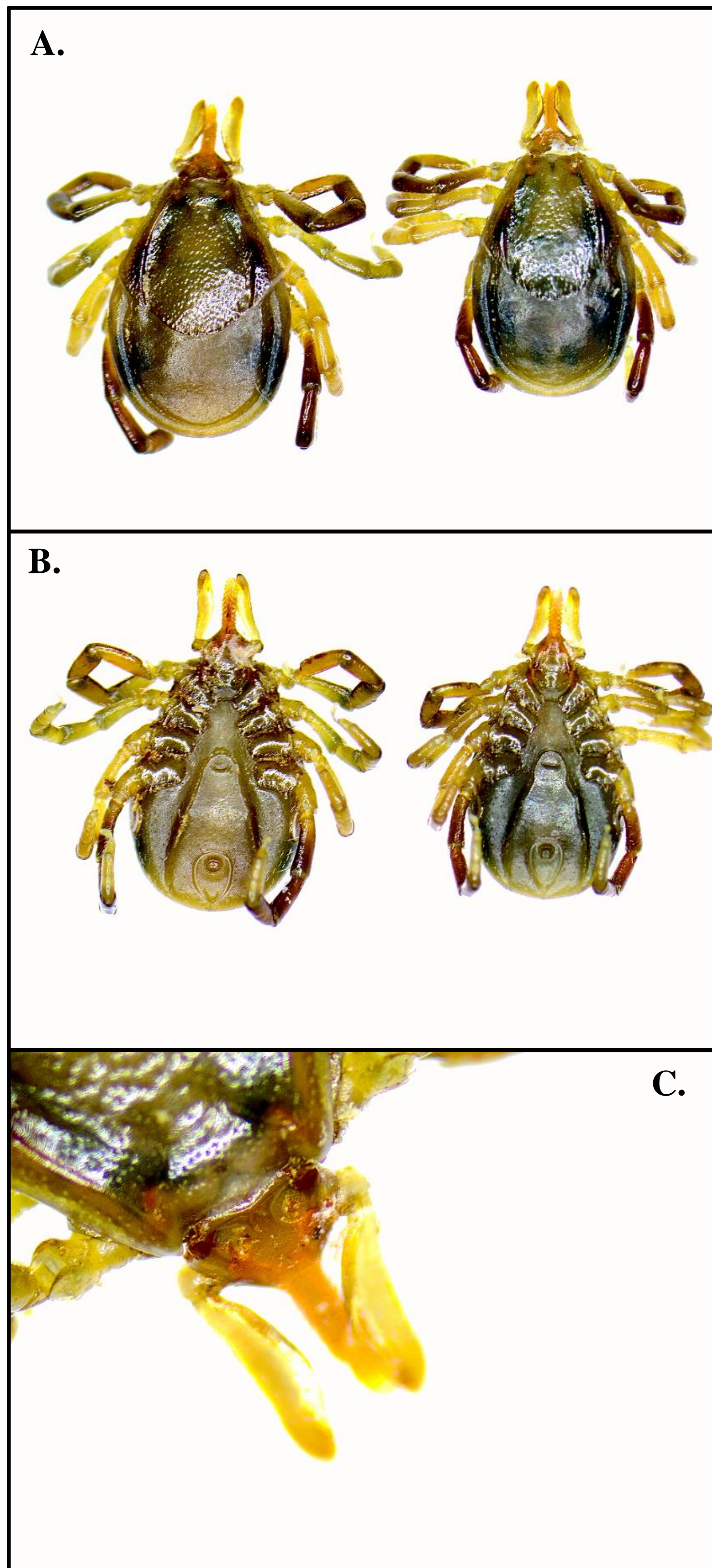

Figure 3.4: Morphological features used to identify female *I. holocyclus* ticks. A and C. dorsal view; B. ventral view.

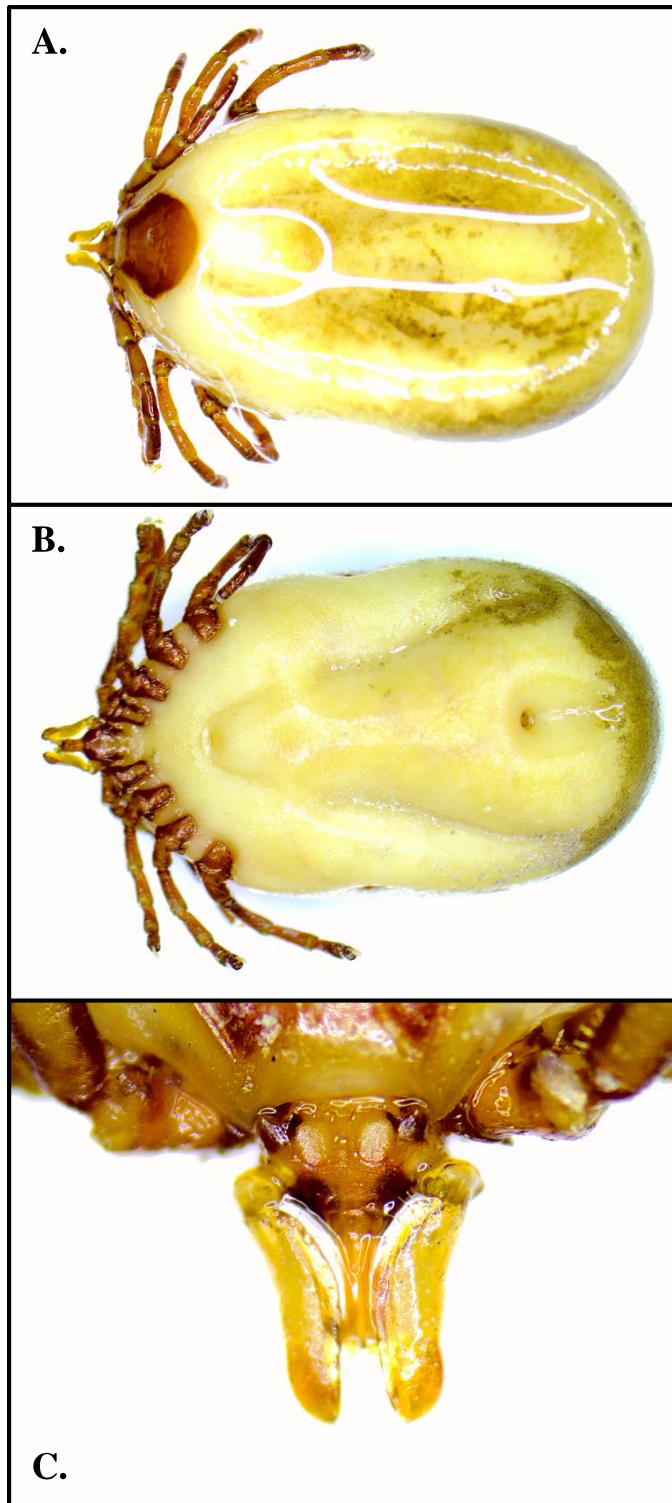

Figure 3.5: Morphological features used to identify female *I. cornuatus* ticks. A and C. dorsal view; B. ventral view.

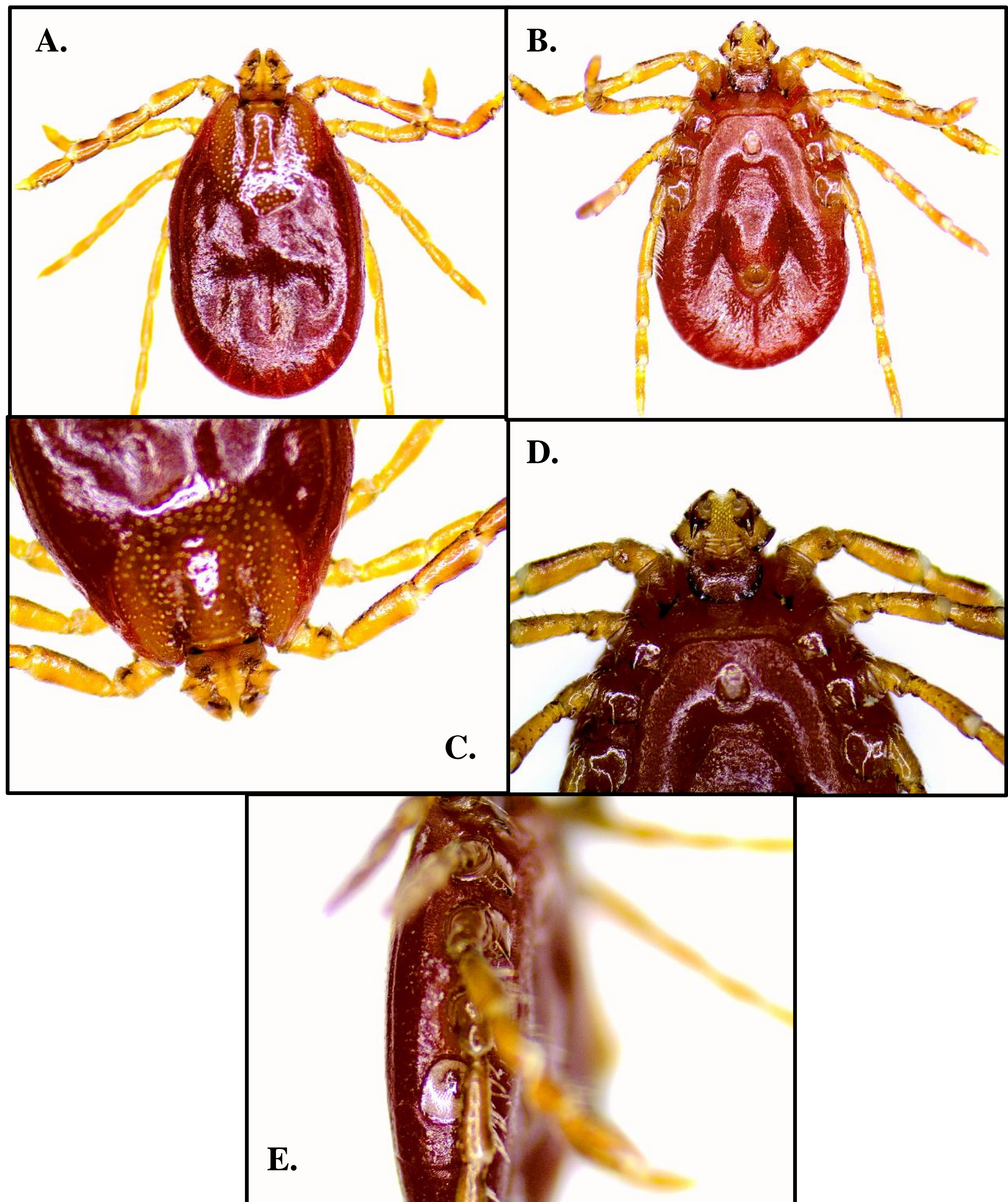

Figure 3.6: Morphological features used to identify female *H. longicornis* ticks. A and C. dorsal view; B and D. ventral view; E. lateral view.

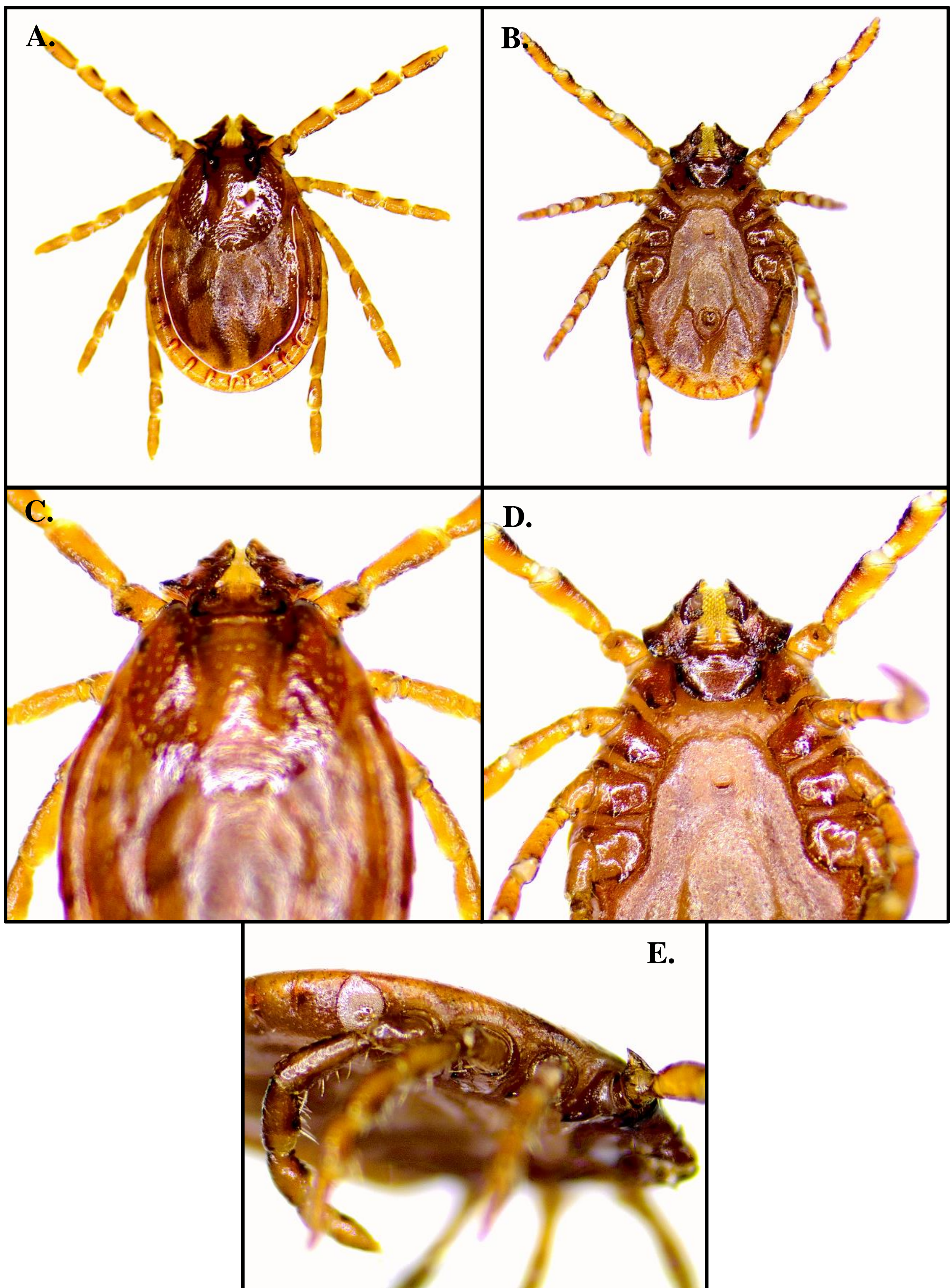

Figure 3.7: Morphological features used to identify female *H. bancrofti* ticks. A and C. dorsal view; B and D. ventral view; E. lateral view.

Table 3.1: Number of instars for seven tick species identified.

Tick species	Larvae (n)	Nymphs (n)	Males (n)	Females (n)
Amblyomma triguttatum (triguttatum)	0	5	0	4
Haemaphysalis bancrofti	0	0	0	1
Haemaphysalis longicornis	2	150	0	60
Ixodes cornuatus	0	0	0	4
Ixodes holocyclus	0	72	76	279
Ixodes tasmani	16	2	0	27
Rhipicephalus sanguineus	38	299	1,215	1,025
Total	**56**	**528**	**1,291**	**1,401**
Net Total				**3,276**

3.2 Distribution of *R. sanguineus* in Australia

The focus for this study was to detect *C. burnetii* in *R. sanguineus*, therefore only the *R. sanguineus* (*n* = 2,577) recordings were mapped (Figure 3.8). The majority of these ticks were collected from NT (*n* = 2,033), and the collection sites mapped to ten geographic locations. Fewer were collected from SA (*n* = 335) and WA (*n* = 210) at two and five geographic locations, respectively (Table 3.2). A large number of ticks were collected from Indigenous communities in all three states (Appendix A, Table A.1). The collection sites from WA were mainly distributed along the western coastline of the State. Overall, the latitude ranged between 12° 37’ S and 32° 22’ S, and the longitude ranged between 122° 20’ E and 137° 86’ E for the *R. sanguineus* ticks recorded and mapped in this study.

Figure 3.8: Locations of *R. sanguineus* specimens in this study. Collection sites are indicated by the red points.

Table 3.2: Number of *R. sanguineus* recorded for 17 geographical locations from NT, SA, and WA, with latitude and longitude reported.

State	Location	Number of *R. sanguineus* recordings	Latitude and longitude (decimal value)*
Northern Territory	Darwin	489	Min: (-12.440034, 130.856539); Max: (-12.379233, 130.8708950)
	Katherine	132	(-14.470843, 132.283503)
	Knuckey Lagoon	7	(-12.426825, 130.934141)
	Lake Nash	50	(-20.981094, 137.861604)
	Mutitjulu	429	Min: (-25.3523, 131.0667) Max: (-25.351457, 131.063954)
	Nyirripi	67	(-22.6475681, 130.54944939)

	Palmerston	756	Min: (-12.509025, 130.994619) Max: (-12.480066, 130.984006)
	Tennant Creek	14	(-19.648306, 134.186642)
	Yuelamu	6	(-22.257958, 132.204607)
	Yuendumu	83	(-22.253296, 131.795945)
	NT Total	**2033**	
South Australia	Coober Pedy	28	Min: (-29.037845, 134.723814) – Max: (-29.037845, 134.723814)
	Oodnadatta	307	(-27.546529, 135.447026)
	SA Total	**335**	
Western Australia	Cable Beach	26	(-17.950181, 122.196423)
	Carnarvon	42	(-24.871625, 113.675619)
	Karratha	6	(-20.736709, 116.846295)
	Kurnangki & Minardi	70	(-18.194272, 125.568678)
	Perth	66	Min: (-32.221725, 116.0072) Max: (-31.7848, 115.7678)
	WA Total	**210**	

*** Minimum and maximum latitude (in decimal form) is reported for geographic locations where >1 submissions were received.**

3.3 *Coxiella burnetii* qPCR assays

A highly specific hydrolysis probe qPCR assay was used to determine the presence or absence of *C. burnetii* DNA in *R. sanguineus* samples from Darwin (n = 40), Palmerston (n = 36), and Perth (n = 31). Amplification was observed in both of the 10^2 and 10^5 copy number positive controls for the Perth samples, and all *R. sanguineus* samples, including the NTC and extraction control, from Perth failed to amplify (see Figure 3.9). These results indicated that failure of amplification in *R. sanguineus* samples was due to the absence of detectable *C. burnetii* DNA, as the amplification observed in the positive controls ruled out PCR failure as the cause of no amplification. The qPCR products were run on a 2% agarose gel to confirm the absence of

amplified DNA. Consequently, no *C. burnetii* DNA was detected in *R. sanguineus* samples tested from Perth (0/31). The amplification plot in Figure 3.10 for the samples from Darwin shows that amplification was observed in only the 10^5 *C. burnetii* positive control, and fluorescence was not observed in the samples, NTC or extraction control, or the 10^2 *C. burnetii* positive control. Likewise, amplification was observed for the 10^5 *C. burnetii* positive control in the Palmerston qPCR assay (Figure 3.11), and no amplification was detected in the 10^2 positive control. A small amount of fluorescence was detected for 7 samples from Palmerston.

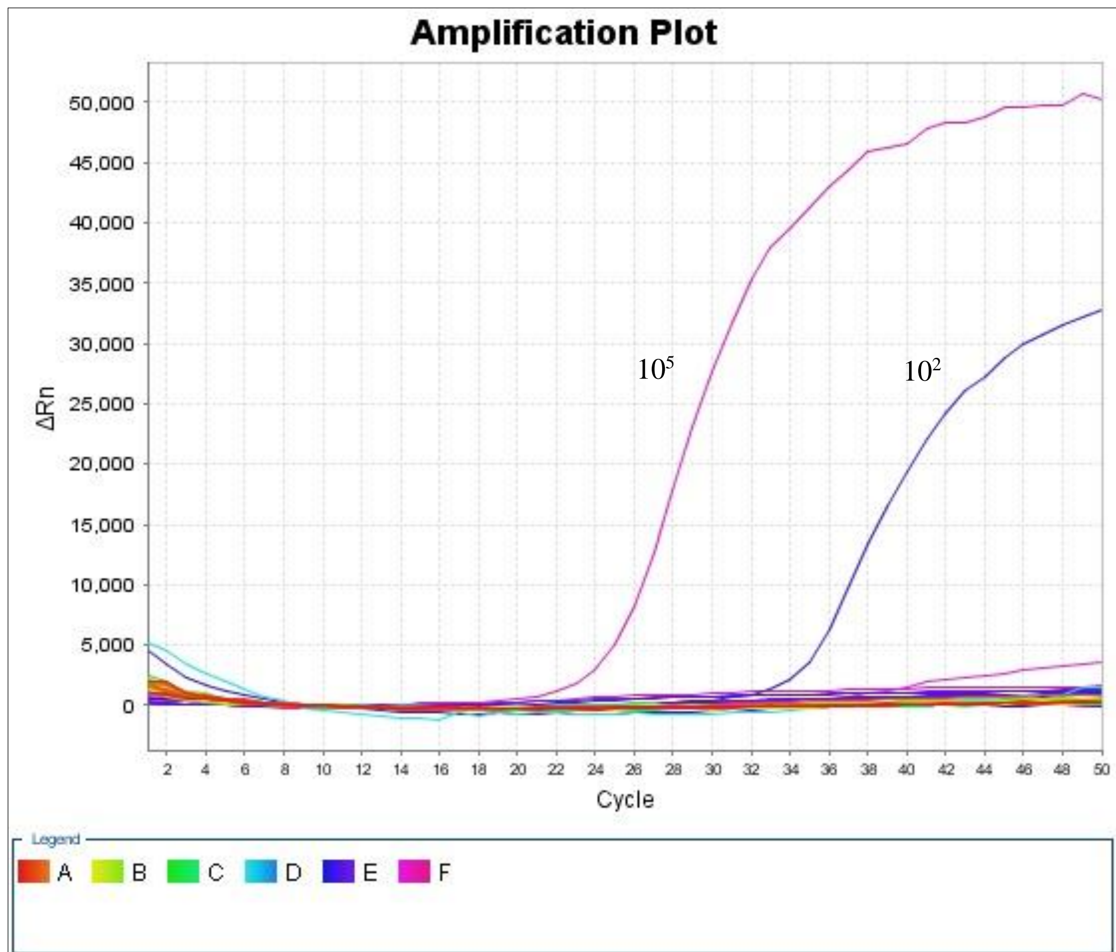

Figure 3.9: The amplification plot obtained for the *Coxiella burnetii* qPCR assay for *R. sanguineus* samples from Perth. Amplification curves for the positive controls are labelled for 10^5 and 10^2 copy numbers.

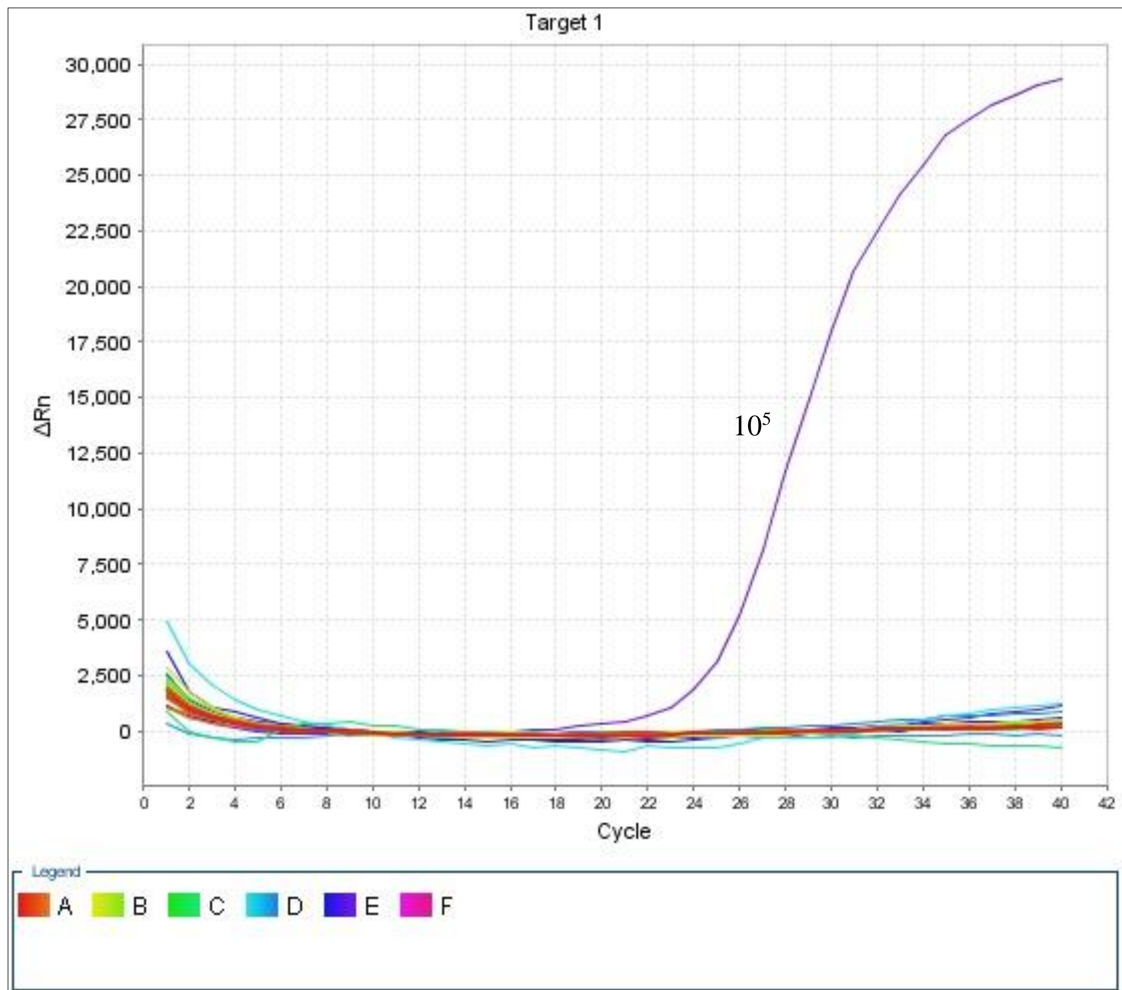

Figure 3.10: The amplification plot obtained for the *C. burnetii* qPCR assay for *R. sanguineus* samples from Darwin. Amplification curve for the positive control is labelled for 10^5 copy numbers.

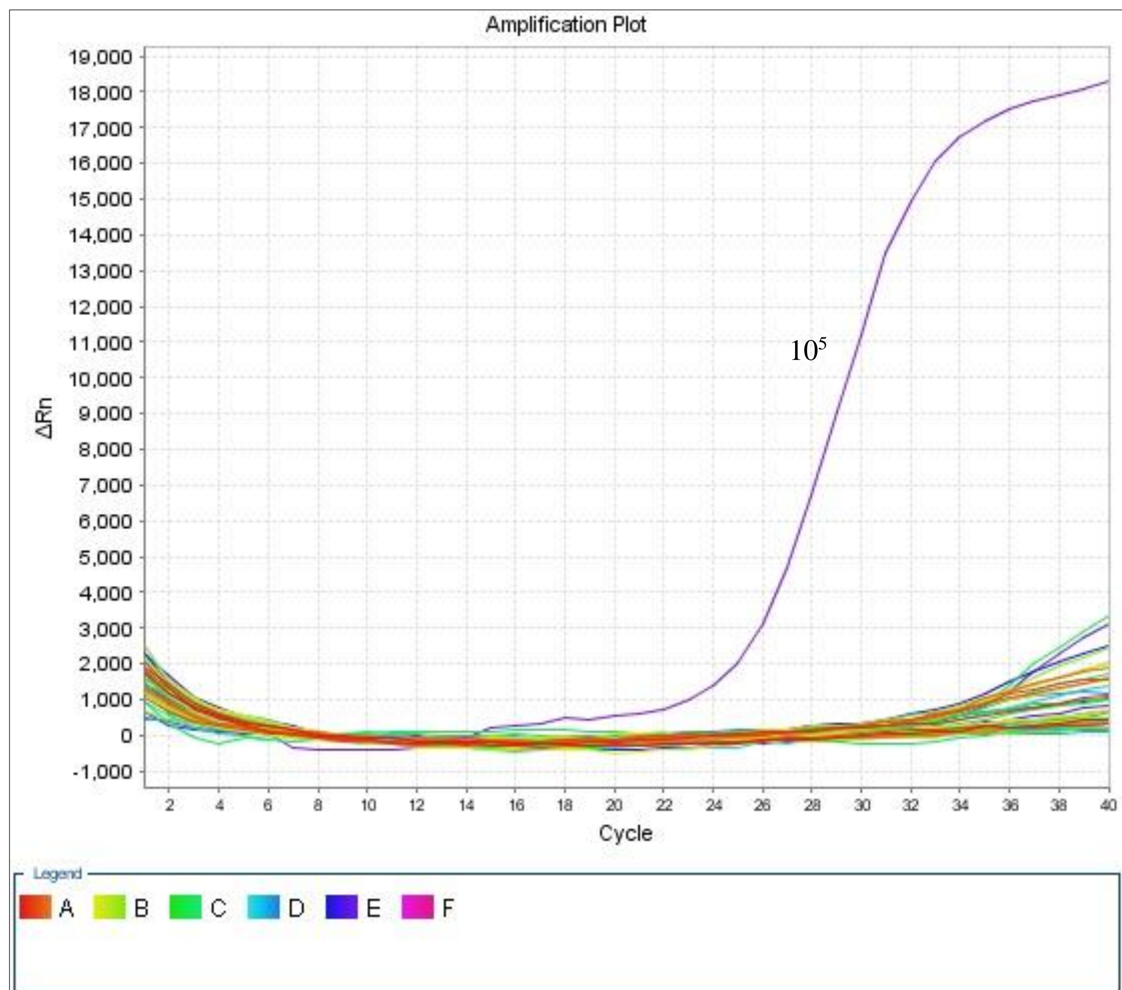

Figure 3.11: The amplification plot obtained for the *C. burnetii* qPCR assay for *R. sanguineus* samples from Palmerston. Amplification curve for the positive control is labelled for 10^5 copy numbers.

3.4 Next Generation sequencing

3.4.1 Universal bacterial 16S qPCR assays

Initial assays of universal bacterial 16S qPCR that were performed with the Universal 27F mod* and Universal 338R primers on the DNA extracted from the sub-set of *R. sanguineus* from Darwin (n = 40), Palmerston (n = 36), and Perth (n = 31) were used to screen samples for bacterial DNA before sequencing library preparation. Amplification was observed in all undiluted DNA extracts (see Appendix C, Figures C.1 - C.3). Nineteen samples from Darwin and 2 samples from Perth had suboptimal PCR efficiency indicating the presence of PCR inhibitors in the sample. However,

serial dilutions (1:10 and 1:100) of the samples that were carried out to dilute potential PCR inhibitors did not improve PCR efficiency, and so undiluted samples were used for sequencing library preparation. Amplification was also observed in no-template and extraction controls, however this is likely due to the presence of ubiquitous environmental bacteria.

3.4.2 PCR purification

Purification of the fusion-tag qPCR products successfully reduced the amount of primer dimer and short sequences in the samples, as shown in Figure 3.12. Fluorescent bands between 300-350 bases indicates the presence of fusion-tag qPCR products, which is most apparent in lanes 2-6 and 11-15, and the florescent bands smaller than this indicates the presence of primer dimer and short sequences. Primer dimer appears to be absent in the post-Ampure products (lanes 11-19).

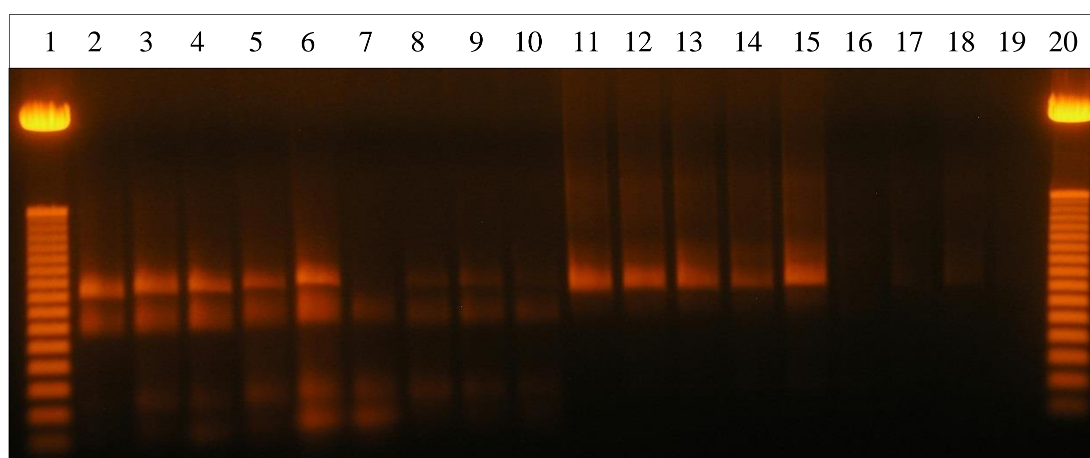

Figure 3.12: Gel electrophoresis (2%) image of pre- and post-Ampure 16S rRNA qPCR products. Lanes 2-10 contain the pre-Ampure PCR products; lanes 11-19 contain the post-Ampure products; Lanes 1 and 10 contain a 50 bp ladder.

3.4.3 Ion Torrent sequencing results

The loading density (number of wells containing live ion sphere particles) for the 316 chip was 85 % (see Figure 3.13). This resulted in 5,393,643 reads with 100% enrichment, of which 20% were polyclonal. Following the removal of polyclonal reads and low quality reads, the total

number of usable reads was 4,268,908. The mean read length was 234 bp, while the median and mode read lengths were 302 bp and 371 bp respectively, as depicted in Figure 3.14. The majority of reads obtained above 300 bp indicated that the fusion-tagged products had been sequenced, with some short reads obtained that were less than 100 bp, which were potentially primer dimer and reads that did not sequence the entire length.

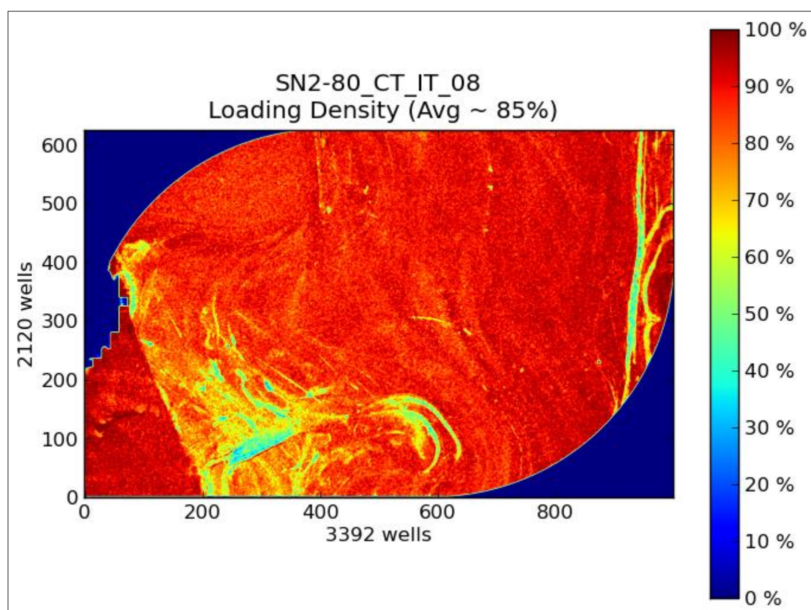

Figure 3.13: Bead loading density on Ion Torrent 316 chip using 400bp V2 chemistry

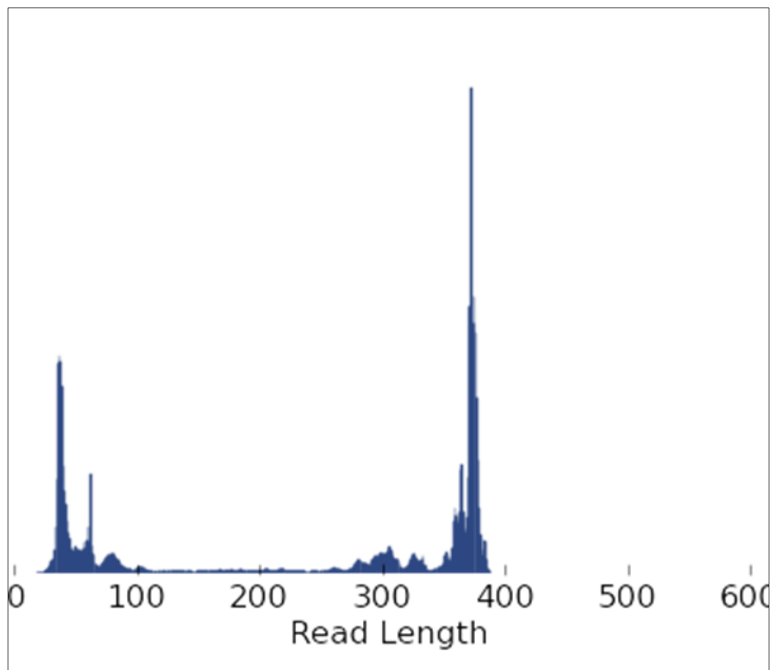

Figure 3.14: Read length histogram obtained from the Ion Torrent server.

3.4.4 Sequencing results

The 4,268,908 reads were imported into Geneious (version 7.1.7), where the Universal 16S 27F mod and 338R primer pairs and MID tags were identified, annotated, and trimmed. Of these reads, the Universal 27F mod primer was identified in 3,572,585 of the reads, and both universal bacterial 27F mod and 338R were found in 1,238,135 reads. The reads with a 100% match for the forward and reverse primer were extracted, trimmed, and assigned sample IDs based on the matching MID tags in Geneious. Reads less than 100 and greater than 419 bases in length were excluded, resulting in 1,048,827 reads that were imported and quality filtered in UPARSE. After < 5 sequences per unique read and chimeras were removed, 387,193 reads were assigned OTUs in UPARSE at a 97% level. Final filtered sequences had an average nucleotide length of 312 bases (Table 3.3).

Table 3.3: Sequencing statistics for bacterial 16S rDNA reads.

Statistics on sequences	**No. of reads**
Initial sequences	4,268,908
Barcode & size filtering	1,048,827
Unique reads	311,862
Unique reads >5 (singleton + chimera removal)	13,835
OTUs	1,194
Min seq length nt	100
Average seq legnth nt	312
Max seq length nt	419

The taxonomy was assigned to the OTUs in QIIME, and it was found that *Coxiella* DNA sequences were present in 16/18 (89%) tick pools from Darwin, 18/22 (82%) tick pools from Palmerston, and 19/19 (100%) tick pools from Perth (Table 3.4). All *Coxiella* DNA sequences grouped to one OTU (OTU #3). The average percentage of *Coxiella* DNA reads was highest for Perth (55.62%), followed by Darwin (18.27%), and Palmerston had the lowest average (17.34%). The percent of *Coxiella* reads for each tick pool, extraction control, and NTC are included in Appendix D, Table D.1. The NTCs for the fusion-tag setup lab and for the DNA extraction hood had 0% *Coxiella* DNA reads. The Darwin extraction control also had 0% *Coxiella* DNA reads, however the Palmerston extraction control and Perth extraction control had *Coxiella* DNA reads detected in 0.1% and 0.2% of the total bacterial DNA reads, respectively. The top ten most abundant bacterial phyla reads from Darwin, Palmerston, and Perth samples are reported in Table 3.5. Figure 3.15 displays the bacterial composition in male and female ticks from the three localities sampled, and the NTC and extraction controls.

Table 3.4: The number of *Coxiella* DNA sequences for the *Coxiella* OTU (UPARSE), and the presence or absence of three unique *Coxiella* sequences (Geneious) indicated, for all samples and negative controls.

Sample ID	*Coxiella* OTU_3	*R. sanguineus* Unique Sequence 1	*R. sanguineus* Unique Sequence 2	*R. sanguineus* Unique Sequence 3
136RSM	1438	✓	✓	✓
198RSF	520	✓	✓	✓
259RSF	292	✓	✓	✓
259RSM	3	✓	✗	✗
260RSF	259	✓	✓	✓
260RSM	120	✓	✗	✓
261RSF	**0**	✓	✓	✗
261RSM	13	✓	✓	✗
262RSF	15	✓	✓	✓
262RSM	1	✗	✗	✗
263RSM	137	✓	✓	✗
264RSF	5	✗	✗	✗
264RSM	1114	✓	✓	✓
265RSF	737	✓	✓	✓
265RSM	1185	✓	✓	✓
266RSF	1821	✓	✓	✓
266RSM	313	✓	✓	✓
267RSF	116	✓	✓	✓
267RSM	910	✓	✓	✓
282RSF	6287	✓	✓	✓
285RSF	8560	✓	✓	✓
286RSM	407	✓	✓	✓
637RSF	**0**	✗	✗	✓
640RSF	65	✓	✓	✓
640RSM	57	✓	✓	✓
641RSF	371	✗	✓	✓
641RSM	78	✗	✓	✓
644RSF	**0**	✗	✗	✗
644RSM	1	✓	✗	✗
646RSF	9	✓	✗	✗
646RSM	460	✓	✓	✓
647RSF	401	✓	✓	✓
649RSF	46	✓	✓	✗
649RSM	12	✓	✓	✗
650RSF	14	✗	✗	✗
650RSM	657	✓	✓	✓

651RSM	1	✗	✗	✗
652RSF	1	✗	✗	✗
652RSM	16	✓	✓	✗
653RSF	31	✓	✓	✗
653RSM	2	✓	✗	✗
654RSF	1	✓	✗	✗
654RSM	5	✓	✗	✗
655RSF	104	✓	✓	✓
656RSM	22	✓	✓	✗
695RSM	1693	✓	✓	✓
696RSF	947	✓	✓	✓
697RSF	973	✓	✓	✓
697RSM	1285	✓	✓	✓
698RSF	560	✓	✓	✓
699RSF	432	✓	✓	✓
699RSM	316	✓	✓	✓
76RSF	1364	✓	✓	✓
770RSF	2008	✓	✓	✓
770RSM	836	✓	✓	✓
879RSF	702	✓	✓	✓
880RSF	1443	✓	✓	✓
881RSF	683	✓	✓	✓
882RSF	3126	✓	✓	✓
Clean Lab NTC	**0**	✗	✗	✗
Cryptick Lab NTC	**0**	✗	✗	✗
Darwin Extraction Control	**0**	✗	✗	✗
Palmerston Extraction Control	3	✓	✗	✗
Perth Extraction Control	3	✓	✗	✗

Check represents presence; cross represents absence.

Table 3.4: Percent of *Coxiella* reads, range and number of positive samples for *R. sanguineus* from Darwin, Palmerston, and Perth.

Location and Sex	**Average % of Coxiella Reads (%)**	**Range (%)**	**Number of samples positive for Coxiella**
Darwin Males	16.22	0.00 – 48.00	8/9
Darwin Females	20.31	0.00 – 91.40	8/9
Palmerston Males	12.78	0.00 – 64.90	9/11
Palmerston Females	21.9	0.00 – 86.40	9/11
Perth Males	47.77	11.80 – 81.40	6/6
Perth Females	59.25	7.90 – 99.20	13/13

Table 3.5: Top ten phyla associated with *R. sanguineus* from Darwin, Palmerston and Perth.

Darwin	**%**	**Palmerston**	**%**	**Perth**	**%**
Staphylococcus	35.91	Coxiella	17.34	Coxiella	55.62
Coxiella	18.27	Staphylococcus	14.23	Clostridiaceae	4.56
Streptococcus	5.71	Corynebacterium	8.90	Weeksellaceae	4.25
Xanthomonadaceae	5.09	Variovorax	8.10	Propionibacterium	2.83
Veillonella	4.15	Propionibacterium	5.66	Xanthomonadaceae	2.78
Corynebacterium	3.22	Herbaspirillum	3.13	Staphylococcus	2.70
Variovorax	2.92	Enterobacteriaceae	2.39	Variovorax	1.46
Propionibacterium	1.49	Chryseobacterium	2.23	Ralstonia	1.18
Streptophyta	1.41	Veillonella	1.99	Bacillus	1.06
Pelomonas	1.37	Pelomonas	1.96	Planococcaceae	0.99

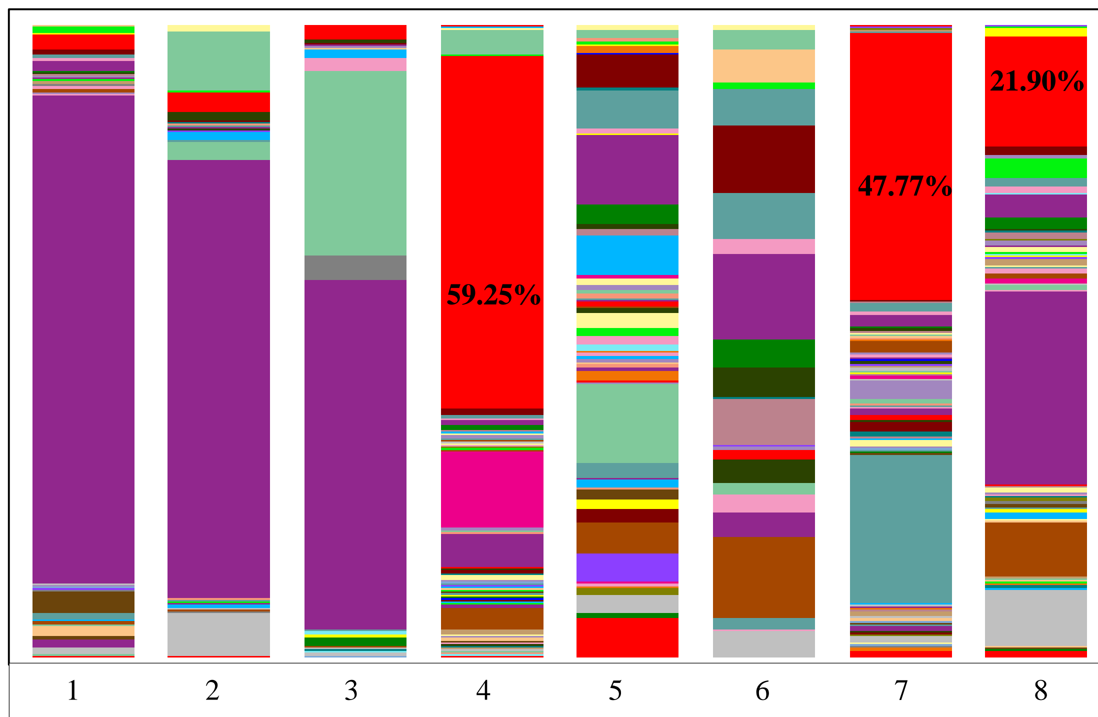

Figure 3.15: Bacterial diversity for male and female tick pools from Darwin, Palmerston, and Perth, generated in QIIME. Column 1 = Palmerston male; column 2 = Darwin male; column 3 = Darwin female; column 4 = Perth female; column 5 = NTC; column 6 = extraction controls; column 7 = Perth male; column 8 = Palmerston female. Red: *Coxiella* sp. The legend for other bacterial phyla is included in Appendix D, Table D.2.

In order to identify and further characterise the *Coxiella* sp. present in the tick pools, the most abundant unique sequences were queried against GenBank in Geneious (version 7.1.7), and a 100% match was obtained for the *Coxiella* sp. DNA present in *R. sanguineus* ticks to an uncultured *Coxiella* sp. (accession number JX185722) (Table 3.6). Three unique *Coxiella* sp. sequences were obtained in the most abundant reads (denoted as *R. sanguineus* sequence 1, 2, and 3). Two of the unique *Coxiella* sequences '*R. sanguineus* sequence 1' and *R. sanguineus* sequence 3,' and an *R. sanguineus Coxiella* sp. sequence (accession number D84559), and a *C. burnetii* sequence (accession number AY342037) were aligned against '*R. sanguineus* sequence 2' (Figure 3.17). One single nucleotide polymorphism (SNP) was identified in *'R. sanguineus* sequence 1,' and two SNPs were identified in '*R. sanguineus* 3.' The most variability observed in the *R. sanguineus Coxiella* sp. and *C. burnetii* sequence occurred between bases 111-122.

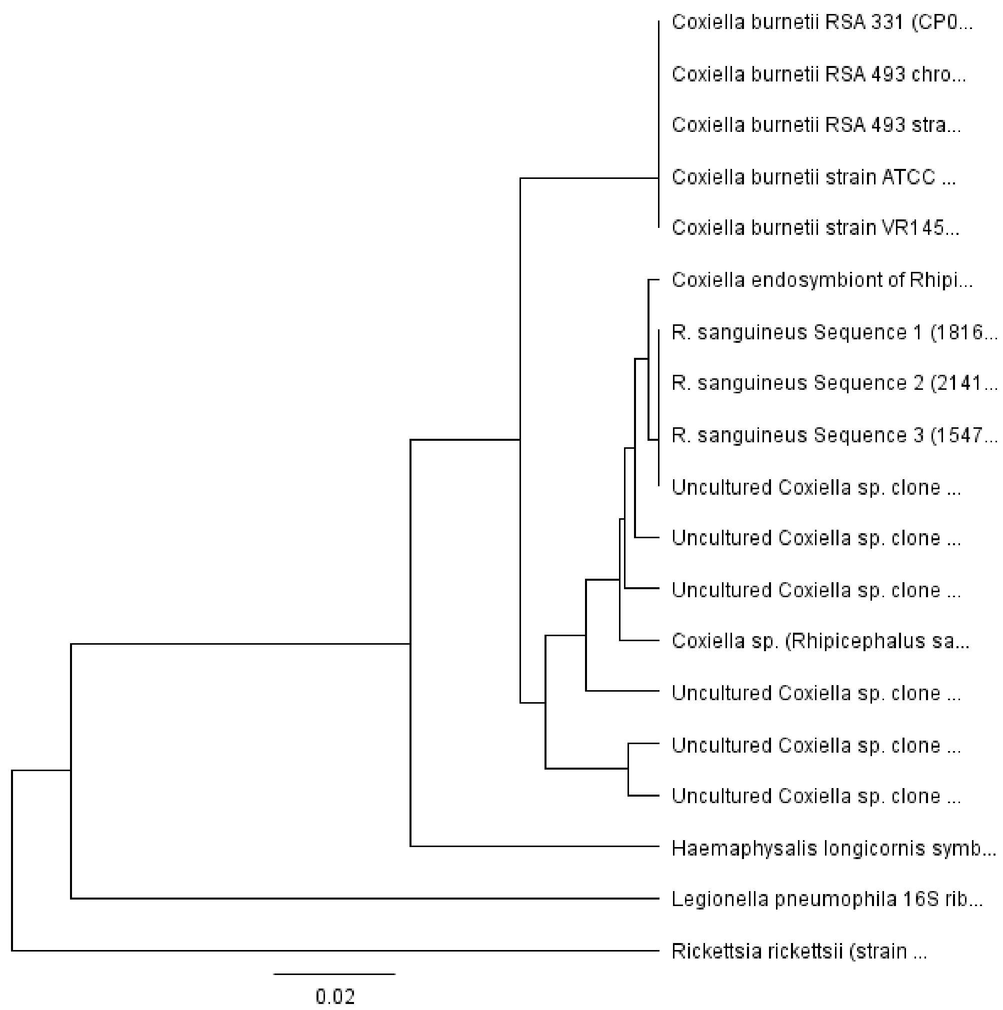

Figure 3.16: Phylogenetic relationships between *Coxiella* 16S rRNA sequences obtained from *R. sanguineus*. Built in Geneious version 7.1.7.

Table 3.6: Top ten matches from NCBI for *R. sanguineus* sequence 3

Sequence description	Accession number	Percent match
Uncultured Coxiella sp. clone D23C 16S ribosomal RNA gene, partial sequence	JX185722	100.0
Uncultured Coxiella sp. clone D23E 16S ribosomal RNA gene, partial sequence	JX185723	99.8
Coxiella endosymbiont of Rhipicephalus turanicus isolate DGGE gel band 11.3 16S ribosomal RNA gene, partial sequence	JQ480822	99.7
Uncultured Coxiella sp. clone D25B 16S ribosomal RNA gene, partial sequence	JX185724	99.4
Uncultured Coxiella sp. clone D25C 16S ribosomal RNA gene, partial sequence	JX185725	98.8
Coxiella sp. (*Rhipicephalus sanguineus* symbiont) gene for 16S rRNA, partial sequence	D84559	98.6
Uncultured Coxiella sp. clone 1357 16S ribosomal RNA gene, partial sequence	EU143670	96.9
Uncultured Coxiella sp. clone 1358 16S ribosomal RNA gene, partial sequence	EU143669	96.7
Coxiella burnetii strain ATCC VR-615 16S ribosomal RNA gene, partial sequence >gi|296245391|gb|HM208383.1| *Coxiella burnetii* culture-collection ATCC:VR-615 16S ribosomal RNA gene, partial sequence	NR_104916	96.2
Coxiella burnetii RSA 493 strain RSA 493 16S ribosomal RNA, complete sequence	NR_074154	96.2

```
                        1          11         21         31         41         51         61         71         81         91
                        |          |          |          |          |          |          |          |          |          |
R. sanguineus Seq 2 ATTGAACGCT AGCGGCATGC CTAACACATG CAAGTCGAAC GGCAGCGGGN GGGAGCTTGC TCCCTGACGG CGAGTGGCGG ACGGGTGAGT AACACGTAGG
R. sanguineus Seq 1 .......... .......... .......... .......... .........N .......... .......... .......... .......... ..........
R. sanguineus Seq 3 .......... .......... .......... .......... .........G .......... .......... .......... .......... ..........
Coxiella sp. (RS)   .......... .......... .......... .......... .........N .......... .......... .......... .......... ..........
C. burnetii (VR145) .......... .......... T......... .......... .......C.N .......... ......G... .......... .......... ..TG......

                    101        111        121        131        141        151        161        171        181        191
                    |          |          |          |          |          |          |          |          |          |
R. sanguineus Seq 2 AATCTACCTT AATTATAATA GTTAGTGGGG GATAACCCGG GGAAACTCGG GCTAATACCG CATAATCTCT TCAAAGCAAA GCGGGGGATC TTCGGACCTC
R. sanguineus Seq 1 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
R. sanguineus Seq 3 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Coxiella sp. (RS)   .........C .T.ANNNNNN NN........ .......... .......... .......... .......... .......... .......... ..........
C. burnetii (VR145) .......... NNNNNNNNNN NG........ .......T.. .......... .......... .......... .......... .......... ..........

                   201        211        221        231        241        251        261        271        281        291
                    |          |          |          |          |          |          |          |          |          |
R. sanguineus Seq 2 GTGCTATGAG ATGAGCCTGC GTCGGATTAG CTAGTTGGTA GGGTAATGGC CTACCAAGGC GAGCGATCCG TAGCTGGTCT GAGAGGACGA TCAGCCACAC
R. sanguineus Seq 1 .......... .......... .......... .......... .......... .......... ..N....... .......... .......... ..........
R. sanguineus Seq 3 .......... .......... .......... .......... .......... .......... ..N....... .......... .......... ..........
Coxiella sp. (RS)   .......... .......... .......... .......... .......... .......... ..N....... .......... .......... ..........
C. burnetii (VR145) .......A.. ........A. .......... ..T......G .......... .......... ..N....... .......... .......... ..........

                    301        311        321
                    |          |          |
R. sanguineus Seq 2 TGGGACTGAG ACACGGCCCA G
R. sanguineus Seq 1 .......... .......... .
R. sanguineus Seq 3 .......... .......... .
Coxiella sp. (RS)   .......... .......... .
C. burnetii (VR145) .......... .......... .
```

Figure 3.17: An alignment of three unique *R. sanguineus Coxiella* sequences with a *Coxiella* sp. endosymbiont and *C. burnetii*. *R. sanguineus* Seq 1: 18,164 sequences, 319 bp; *R. sanguineus* Seq 2: 2,141 sequences, 320 bp; *R. sanguineus* Seq 3: 1,547 sequences, 320 bp; *Coxiella* sp. (RS): *Coxiella* sp. *Rhipicephalus sanguineus* symbiont, Accession No. D84559; *C. burnetii* (VR145): *Coxiella burnetii* strain VR145, Accession No. AY342037; [.] denotes a conserved nucleotide; and [N] denotes a deletion.

CHAPTER 4: DISCUSSION AND CONCLUSIONS

4. Discussion

4.1 *Rhipicephalus sanguineus* identification

The first aim of the experimental work for this study was to identify the tick species removed from dogs. All 3,276 ticks collected from dogs were morphologically identified using the Australian tick identification key outlined by Roberts (1970). Of the 3,276 ticks that were examined, it is not surprising that the majority (79%) were *R. sanguineus*, as dogs are the known primary hosts of these ticks. Although dogs are not the preferred host of the other six tick species identified (*A. t. triguttatum*, *H. bancrofti*, *H. longicornis, I. cornuatus, I. holocyclus,* and *I. tasmani*), these ticks have been previously recorded as using dogs as their mammalian hosts in Australia (Roberts 1970).

Unlike most genera in the key presented by Roberts (1970), which are arranged in a dichotomous form, only a description of the morphology for *R. sanguineus* is provided for the genus *Rhipicephalus*, as this was considered to be the only *Rhipicephalus* sp. present in Australia at the time Roberts authored the key. However, the nomenclature of the genus *Boophilus* (Curtice 1981) has since changed to *Rhipicephalus* (*Boophilus*), supported by recent molecular evidence that has demonstrated these two genera as paraphyletic (Murrell and Barker 2003). Although the nomenclature of *Boophilus microplus* (Canestrini 1887) described in Roberts (1970) has now changed to *Rhipicephalus australis* (Estrada-Pena et al. 2012), this did not impose a limitation to the identification of *R. sanguineus* in this study as *R. sanguineus* was morphologically distinguished from *R. australis* due to the presence of festoons.

Given that the ticks examined in this present study were collected in Australia, it seemed appropriate to use the Australian tick key by Roberts (1970) to morphologically identify these ticks. Therefore, the species identification of *R. sanguineus* in this study is limited to an Australian context, as the morphological features were observed to match the descriptions provided by Roberts (1970) who described the morphology of Australian tick species. Even though *R. sanguineus* and *R. australis* are considered to be the only *Rhipicephalus* spp. present in Australia

at the current time (Barker and Walker 2014), it is possible that other tick species could be inadvertently introduced into this country as a result of international movements of animals and humans.

On a global scale, there are considered to be 17 species of *Rhipicephalus* in the so-called "*R. sanguineus* group" that share similar morphological features, and can be difficult to differentiate (Gray et al. 2013). Several of these species require a more detailed examination of finer anatomical features than are outlined by Roberts (1970) for species differentiation. A more recent and comprehensive description of *Rhipicephalus* spp. has been provided by Walker, Keirans, and Horak (2000), and the morphology of the specimens were illustrated with scanning electron micrographs (SEMs), and the finer morphological features of the ticks are described. Walker, Keirans, and Horak (2000) noted the similarity between *R. sanguineus*, *R. sulcatus*, and *R. turanicus*, and these species require a detailed morphological comparison of the scutal punctation patterns, genital aperture shape, adanal plate shape, and spiracular plate shape to be differential.

Despite efforts to classify and identify tick species based solely on morphology, the recent use of molecular tools to investigate tick taxonomy and phylogeny has provided rapid insight into the classification and evolutionary relationships between taxa. A recent study by Dantas-Torres et al. (2013) has provided evidence that there may be more than one species, or sub-species, of *R. sanguineus* and *R. turanicus* that may not be identified through the use of the currently published tick morphology keys. Inaccuracies in morphological tick species identification can compromise the validity of the results obtained, and inferences made, in research investigations. Thus, accurate species identification is important in order to gain a high level of certainty in studies that report tick host recordings, distribution patterns, and species-specific pathogens. This emphasises the need for future studies that use both a morphological and molecular approach to investigate the taxonomy of *R. sanguineus* ticks and other *Rhipicephalus* spp., and this approach would improve the accuracy of tick species identification.

4.2 Distribution of *R. sanguineus* ticks in Australia.

The second aim of this project was to record the distribution of *R. sanguineus* ticks collected in this study, and this was achieved for the substantial number of *R. sanguineus* ticks that were obtained (n = 2,577) (see Figure 3.8 in Section 3.2). The majority of the *R. sanguineus* ticks that were recorded matched the distribution range for *R. sanguineus* developed nearly 50 years ago (see Figure 1.2 in Section 1.4.1). However, 20% of the *R. sanguineus* ticks recorded in this study occurred south of the 1965 distribution range. Although there have been many anecdotal observations in the last 50 years of *R. sanguineus* occurring south of the range described by the 1965 distribution map (Roberts, 1965), there have been no recent publications of the distribution of *R. sanguineus* in Australia, and this study has provided a substantial number of recordings for *R. sanguineus* that challenge the distribution range. The specimens recorded for SA and WA outside the distribution range in this study suggest that the distribution of *R. sanguineus* in Australia has extended further south of this limit and it seems probable that this tick species is well-established throughout a much more extensive range. *Rhipicephalus sanguineus* ticks are prevalent throughout the year in tropical and subtropical areas, but in temperate climates are less active during cooler climates in winter (Papadopoulos et al. 1996), and this may explain why *R. sanguineus* ticks have more often been observed and recorded in the tropical and sub-tropical areas of Australia.

Another possible explanation of the *R. sanguineus* ticks recorded outside the Roberts (1965) distribution range in this study is that there may be different species of *R. sanguineus* in Australia. Returning to the study by Dantas-Torres et al. (2013), two paraphyletic lineages of *R. sanguineus* were identified: the tropical (northern) lineage, which were identified as *R. sanguineus* sensu lato (Walker, Keirans, and Horak 2000); and the temperate (southern) lineage, which were similar, but not identical, in morphology to *R. sanguineus*. Previous molecular studies of *R. sanguineus* have also identified these two divergent lineages (Burlini et al. 2010; Levin et al. 2012), and these lineages have previously been suggested to represent two different species (Nava et al. 2012). The southern lineage OTU, as well as three other OTUs, were identified by Dantas-Torres et al.

(2013), who hypothesised that these are undescribed species under the names *R. sanguineus*, and *R. turanicus*. Furthermore, the 50 *R. sanguineus* ticks included in the study by Dantas-Torres et al. (2013) from NSW in Australia were morphologically and genetically identified as *R. sanguineus* s.l., and corresponded with the tropical lineage. Interestingly, the *R. sanguineus* (*Rhipicephalus* sp. II) OTU identified that corresponded with the temperate lineage were collected from Spain, Portugal, and Italy, all of which experience Mediterranean climates. Future morphological and molecular investigations could be conducted on the *R. sanguineus* ticks recorded in Perth, south-west Western Australia, which has a Mediterranean climate, to elucidate whether these ticks belong to the southern lineage, and whether there is more than one species of *R. sanguineus* in Australia.

4.3 The detection of *C. burnetii* in *R. sanguineus*

The third aim of the project was to detect *C. burnetii* in *R. sanguineus* ticks collected from dogs, and there was no *C. burnetii* DNA detected in the *R. sanguineus* extractions from Perth (0/31). Although qPCRs were also done on a subset of ticks from Darwin (n = 40) and Palmerston (n = 36) with *C. burnetii*-specific primers, the qPCR assay from Palmerston exhibited a small increase in fluorescence signal between cycles 34-40 above the threshold level. Fluorescence was also detected in the NTC, therefore the fluorescence observed in the samples from Palmerston due to amplification of *C. burnetii* DNA is unlikely. However, further gel electrophoresis assays should be conducted to confirm whether this is amplification of specific products.

In order to amplify only *C. burnetii* DNA in *R. sanguineus* ticks, the primers that were used in the qPCR assays in this study targeted the IS1111a transposase gene, and this IS element gene target in *C. burnetii* has been shown to be specific for *C. burnetii* amplification via standard PCR assays (Reeves et al. 2005), and highly specific hydrolysis probe qPCR assays (Banazis et al. 2010). The failure to detect *C. burnetii* in *R. sanguineus* ticks in this study only applies to the subset of ticks tested, and is not an assessment of the overall prevalence of *C. burnetii* in the *R. sanguineus* population from the localities selected. Furthermore, the latter inference cannot be

made in this study as the ticks that were collected were not random samples. Previous studies that have reported *C. burnetii* in *R. sanguineus* ticks have reported only a small percentage of *C. burnetii* positive ticks tested in the studies (Satta et al. 2011; Spyridaki et al. 2002; Toledo et al. 2009), therefore a larger sample size of ticks tested may be required to increase the chance of detecting *C. burnetii* DNA in *R. sanguineus* ticks, if it is indeed present in *R. sanguineus* in Australia. Future studies could aim to assess the overall prevalence of *C. burnetii* in *R. sanguineus*, as these ticks may transmit this pathogenic bacterium among animal reservoirs, however, transmission studies are also required to confirm this. Although dogs have been implicated as reservoirs of *C. burnetii* (Buhariwalla, Cann, and Marri 1996; Cooper et al. 2011), further studies should be conducted to confirm the prevalence of *C. burnetii* in dogs.

4.4 Identification of *Coxiella* sp. in the microbiome of *R. sanguineus*

Finally, the fourth aim of the project was to assess the bacterial microbiome of *R. sanguineus*. This aim was achieved by sequencing the 16S rRNA gene (V1 and V2 hypervariable regions), and the results are presented in Tables 3.4 – 3.6 and Figures 3.15 – 3.17. An interesting finding was that a *Coxiella* sp. (but importantly not *C. burnetii*) was present in a high proportion of the tick pools overall (53/59), and this is the first report of a *Coxiella* sp. detected in *R. sanguineus* in Australia. *Coxiella* spp. that are distinct from *C. burnetii* have been previously detected in *Rhipicephalus* spp. (Bernasconi et al., 2002; Noda, Munderloh, and Kurtti 1997). Furthermore, *Coxiella* endosymbionts that have been identified previously in *R. sanguineus* ticks found elsewhere by 16S rDNA sequencing (Bernasconi et al., 2002; Noda, Munderloh, and Kurtti 1997), and were detected in a smaller proportion (3/24) (Bernasconi et al. 2002) of *R. sanguineus* ticks collected from dogs compared with the findings of the present study.

The three unique *Coxiella* sequences obtained in this study are likely to have been obtained from a single *Coxiella* sp., as they differed only by 1-2 SNPs in the 16S rDNA sequences, which could represent errors introduced by the Ion Torrent™ PGM. Ion Torrent™ PGM insertion and deletion errors can occur due to inaccurate flow-calls, which have been reported to occur at a rate of 2.84%

(Bragg et al. 2013). The *Coxiella* sp. sequence (*R. sanguineus* sequence 3) obtained in this present study matched 100% to a *Coxiella* sp. sequence submitted to GenBank (accession number JX185722) by Ybañez (2013), and these sequences were obtained from *R. sanguineus* ticks in Cebu, Philippines. The >99% matches of '*R. sanguineus* sequence 3' to other *Coxiella* sp. sequences (accession numbers JX185723, JQ480822, and JX185724, see Table 3.6) were also submitted by Ybañez (2013). As noted in the literature review, *Rhipicephalus sanguineus* ticks were introduced into Australia only relatively recently (likely within the last 250 years during European settlement), but the lineage of these ticks is has not been confirmed by any current study. The 100% match between the *Coxiella* sp. obtained in this study and the *Coxiella* sp. from *R. sanguineus* in the Philippines may be a reflection of the origin of this *Coxiella* endosymbiont, and hence *R. sanguineus* in Australia. The origin of this tick species would be better inferred by a phylogenetic study of these ticks from different geographic regions.

Staphylococcus spp. were the most abundant genera of the total bacterial reads obtained for Darwin ticks (35.91%), second-most for Palmerston (14.23%), but only 2.7% of the bacterial reads were *Staphylococcus* spp. for Perth ticks. *Staphylococcus* spp. are commonly found on human and animal skin, including dogs (Stepanovic et al. 2001), so the detection of *Staphylococcus* spp. in *R. sanguineus* ticks could be due to contact of these ticks with the skin microbiota of dogs, or contamination in the laboratory from humans, as the NTCs and extraction controls were also found to contain *Staphylococcus* spp. reads. *Staphylococcus pseudintermedius* has been found to constitute about 90% of staphylococci isolated from canine healthy carriers and of dogs with underlying skin disease (Griffeth et al. 2008; Fazakerley et al. 2009). If the *Staphylococcus* spp. detected in *R. sanguineus* in this study is due to contact with dog microbiota, then the higher proportion of ticks that were found to contain *Staphylococcus* in the tropics (Darwin and Palmerston) may reflect the epidemiology of this bacterium, and could be investigated in the future. Other animal and human skin and/or gastrointestinal bacterial species identified in the most abundant reads of the ticks were *Streptococcus, Propionibacterium*, and *Veillonella*, and these bacteria were also found in the NTCs and extraction controls. Therefore,

these may also be acquired from contact with dog skin, or represent contamination in the laboratory, and may not be representative of the microbiome of the ticks.

Ubiquitous environmental bacteria were also present in the most abundant reads of the tick samples, NTCs, and extraction controls, and included *Xanthomonadaceae, Variovorax, Clostridiaceae, Herbaspirillum, Streptophyta, Pelomonas, Planococcaceae, Chryseobacterium,* and *Clostridiaceae*. These environmental bacterial reads in the ticks may represent bacteria within the tick's microbiome that have been acquired from the environment, or may be present due to laboratory contamination. If the environmental bacteria had been acquired by the ticks from the original collection environment, the bacteria could be specific to that particular environment, however, a recent study by Hawlena et al. (2013) that quantified the effects of vertebrate host-related, arthropod-related, and environmental factors on the bacterial community composition of ticks and fleas found that environmental factors did not impact the bacterial community composition. The ticks and fleas in their study were collected over a range of conditions and sites, but only arthropod-related variables, such as the species and life stage, were significant variables that accounted for the variation in bacterial composition. However, geographic differences have been detected in other tick-associated bacterial communities (Wielinga et al., 2006; Clay et al., 2008). Hawlena et al. (2013) observed minor effects of host traits on the microbial community composition, and hypothesised that this may be due to dominant endosymbionts in microbiome of ticks that are vertically transmitted.

Another possible explanation of the presence of environmental DNA in the tick pools, extraction controls, and NTCs in this study is that this may also be due to contamination of DNA extraction kits, which have been recently shown to contain contaminating environmental DNA (Salter et al. 2014). Other sequencing studies of bacteria in ticks (Carpi et al. 2011; Vayssier-Taussat et al. 2013; Egyed and Makrai 2014), and other arthropods such as mosquitoes (Valiente Moro et al. 2013) have demonstrated the presence of environmental and skin bacteria, but whether this is due

to laboratory contamination, bacteria acquired by the ticks from the environment, or vertical transmission to their progeny, is currently unknown.

Rhipicephalus sanguineus ticks are of veterinary and medical significance as they have been reported to transmit pathogens, such as *Ehrlichia canis* (Groves et al. 1975) and *Hepatozoon canis* (Nordgren and Craig 1984) to dogs, and *Rickettsia conorii* (Brumpt 1932) and *Rickettsia rickettsii* (Parker et al. 1933) to humans. There were no sequences detected in this current study that belong to the order Rickettsiales. A small number of *Rickettsiella* sequences were obtained for one tick pool and an extraction control (sample ID 699RSM, 2 sequences, 0.1% of reads; Perth extraction control, 11 sequences, 0.6% of reads). The detection of a higher number of *Rickettsiella* sp. in the Perth extraction control compared to the one tick pool may represent contamination of the lab with this bacterium, as no other tick pools were found to have *Rickettsiella*. Rickettsiella are closely related to *Coxiella*, and have been re-classified in the family *Coxiellaceae* (Leclerque and Kleespies 2008). The genus *Rickettsiella* contains three recognised arthropod pathogenic species *Rickettsiella popilliae*, *R. grylli*, and *R. chironomid* (Cordaux et al. 2007), and *Rickettsiella* spp. have been previously detected in *Ixodes* spp. (Anstead and Chilton 2014; Leclerque and Kleespies 2012) . In Australia, Vilcins et al. (2009) identified *Rickettsiella* in *I. tasmani* ticks collected from Koalas on Phillip Island, and the closest sequence matches (>99%) were obtained for *R. melolonthae* and *R. Myrmeleo* spp. to the 16S *Rickettsiella* sequences. *Rickettsiella* spp. are under study as potential insect control agents (Leclerque and Kleespies 2012), but it has not been established whether ticks are capable of transmitting this bacterium to its vertebrate hosts, or whether it can cause disease in vertebrates. Given that *Rickettsiella* is an arthropod pathogen, the detection of this bacterium within a tick pool from Perth could be indicative of its presence in the tick's microbiome, however, as sequences of this bacterium were also identified in an extraction control, the possibility of laboratory contamination cannot be ruled out.

4.5. The study's limitations

An early aim of this project was to determine the prevalence of *C. burnetii* in ticks removed from dogs. As noted previously, a larger number of ticks collected randomly would have to be analysed in order to provide robust data of prevalence. The ticks were collected from domestic dogs and submitted on a voluntary basis. This sampling method is classified as a convenience sampling, or non-probability sampling. Therefore, probability based inferential statistics could not be applied in this study as the assumption of random independent samples that are representative of the study population of interest is violated. Thus, no relationships between the ticks' bacterial composition and variables such as life-stage or geographical location can be inferred with any certainty using statistical methods, and inferences with regard to the overall prevalence of *C. burnetii* in the *R. sanguineus* population could not be made. The original aim was therefore amended to the 'detection' of *Coxiella* in ticks.

A second limitation pertains to the morphological identification of ticks. If other *Rhipicephalus* spp. in the *R. sanguineus* group have been introduced into Australia, and were present in the ticks collected from dogs in this study, the possibility of misidentification of some of these ticks cannot be excluded. A detailed morphological examination of the scutal punctuation patterns, genital aperture shape, adanal plate shape, and spiracular plate shape based on the description by Walker, Keirans, and Horak (2000) was not applied in this study. However, even if such morphological examinations had been carried out in this study, the recent evidence (Dantas-Torres et al., 2013) that there is potentially more than one species most similar to *R. sanguineus* and *R. turanicus*, which did not match the morphology outlined by Walker, Keirans, and Horak (2000), uncertainty would still remain of the species identification based only on morphology. Thus, the species identification of ticks in this study is limited to the descriptions by Roberts (1970). This highlights the need for a combined morphological and molecular approach for tick identification.

A limitation with regard to bacterial identification is that OTUs assigned at a 97% similarity level did not resolve the species of most bacteria obtained in the sequencing dataset. Therefore, only a

broad assessment of bacterial genera present in the ticks was gained. Although the NGS approach using the universal bacterial 16S rRNA gene target has provided a large dataset of bacterial genera present in the ticks, it cannot determine whether the abundant skin and environmental bacterial reads were representative of the ticks' microbiome, or whether these reads that were obtained are due to laboratory contamination, as they were also found in the negative controls.

4.6 Future research directions

Although Roberts' tick key has been the gold standard for the morphological identification of ticks in Australia for many years, molecular tools can and should now be used to aid tick identification. Molecular tools are providing new insights into the identity of tick taxa, and the evolutionary relationships among tick species, and should be used to strengthen standard tick morphology keys, as novel species are identified and ticks are reclassified. It is clear from recent studies overseas that the taxonomy of *R. sanguineus* requires future morphological and molecular clarification, and such investigations could be conducted on *R. sanguineus* found in Australia. Phylogenetic analyses could provide insight into the possible origin of these introduced ticks in Australia. For example, one approach for a molecular investigation of the *R. sanguineus* taxonomy and phylogeny could be to target the mitochondrial genetic markers such as the 12S mitochondrial rDNA or cytochrome c oxidase subunit 1 (cox1) genes in *Rhipicephalus* spp. DNA for species-specific identification (Szabo et al. 2005; Burlini et al. 2010; Levin et al. 2012; Nava et al. 2012; Dantas-Torres et al. 2013).

The *R. sanguineus* ticks recorded in this study south of the range of the Roberts (1965) *R. sanguineus* distribution map suggest that an update of the *R. sanguineus* distribution in Australia is required. Future distribution modelling is required for *R. sanguineus* recordings in order to establish the current distribution of these ticks. However, the taxonomic issues referred to above should first be investigated and resolved to enable more accurate recordings and reliable distribution models to be generated.

Determination of prevalence requires the population to be randomly sampled. Collection of random samples of ticks from domestic animals in a study population is difficult: it would require that every individual in the study population of interest has an equal chance of being selected. A method for obtaining random samples of ticks can be obtained for questing ticks in the environment by flagging (Carpi et al. 2011), and such studies are able to draw statistical inferences regarding average bacterial diversity of instars in a study population. Future studies could aim to assess the overall prevalence of *C. burnetii* in *R. sanguineus*, and other bacterial species associated with *R. sanguineus* based on random samples, in order to gain an overall consensus of the bacterial profile of these ticks from different geographic regions. Such studies would be important to better understand the epidemiology of tick-borne pathogens, and could enable risk assessments for tick-associated pathogens in geographic areas to be made.

The finding of *Coxiella* DNA closely related to, yet distinct from, *C. burnetii* in *R. sanguineus* ticks is an interesting finding. Little is known about the functions of symbiotic *Coxiella*-like bacteria in ticks, but it has been proposed that they may synthesise nutrients that can be used by arthropod hosts (Jasinskas, Zhong, and Barbour 2007), and may be beneficial to the tick host (Wu et al. 2006). Previous studies have shown that *Coxiella* endosymbionts can be transmitted transovarially (Reeves 2005; Clay 2008), which indicates that ticks may act as reservoirs of *Coxiella* endosymbionts, as they are capable of maintaining the bacteria in their population in nature. It is also uncertain whether *Coxiella* sp. bacterium is pathogenic, and few studies have implied that these bacteria have a role in disease in animals (Shivaprasad et al. 2008). It is unclear whether *R. sanguineus* are reservoirs of *C. burnetii*, and future studies could aim to investigate the roles of *Coxiella* symbionts in the life strategies and life-cycle of *R. sanguineus*, and their pathogenicity. Transovarial and transtadial transmission studies could be conducted to investigate the maintenance of this bacterium in the *R. sanguineus* life-cycle.

4.7 Conclusion

The primary aim of this project was to investigate the zoonotic pathogen *C. burnetii* in *R. sanguineus* ticks collected from dogs in Australia, and this was achieved for a subset of the overall *R. sanguineus* ticks collected in the study. The bacterial microbiome of these ticks was assessed, and a *Coxiella* endosymbiont was identified as a dominant bacterium in majority of the samples; this may be the first report of this *Coxiella* sp. in *R. sanguineus* in Australia. Given that this *Coxiella* sp. was found to be a dominant bacterium in the microbiome of *R. sanguineus* ticks tested in this study, future research could aim to investigate whether this bacterium is prevalent throughout the *R. sanguineus* population, and investigations should also determine whether this bacterium causes cross-reactivity in immunologic assays for *C. burnetii*. Future research should aim to investigate the role of this *Coxiella* sp. in *R. sanguineus*, to assess whether this bacterium is a non-pathogenic endosymbiont of *R. sanguineus*, and to determine whether this bacterium is harmful to dogs or other animals. *Rhipicephalus sanguineus* was found to be the predominant tick species collected from dogs, and a large number of recordings for this tick was obtained in this study. The *R. sanguineus* ticks were recorded mostly within northern and central Australia, in areas that experience mostly tropical and sub-tropical climates, however 20% of the overall *R. sanguineus* ticks were recorded in the southern parts of Australia. This finding suggests that the distribution of *R. sanguineus* is more extensive than has previously been considered.

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APPENDICES

Appendix A.

Table A.1: Metadata spreadsheet.

Samp-le ID#	Tube ID#	Genus	Species	#L	# N	# M	# F	State	Postcode	City/Town	Local Info	Latitude	Longitude	Location	Collector
PI **048**	48IHF	Ixodes	holocyclus	0	0	0	5	VIC							ANIC, CSIRO
PI **076**	76RSF	Rhipicephalus	sanguineus	0	0	0	1	WA	6162	Beaconsfield	N/A	-32.067527	115.764181	Perth	Member of public
PI 136	136RSM	Rhipicephalus	sanguineus	0	0	5	0	WA	6150	Murdoch	N/A	-32.062085	115.832701	Perth	Murdoch University Veterinary Hospital
PI 136	136RSN	Rhipicephalus	sanguineus	0	1	0	0	WA	6151	Murdoch	N/A	-32.062085	115.832701	Perth	Murdoch University Veterinary Hospital
PI 151	151IHF	Ixodes	holocyclus	0	0	0	1	NSW							Turramurra Vet Hospital
PI 154	154IHF	Ixodes	holocyclus	0	0	0	3	NSW							Turramurra Vet Hospital
PI 154	154IHN	Ixodes	holocyclus	0	1	0	0	NSW							Turramurra Vet Hospital
PI 155	155IHF	Ixodes	holocyclus	0	0	0	1	NSW							Turramurra Vet Hospital
PI 156	156IHF	Ixodes	holocyclus	0	0	0	1	NSW							Turramurra Vet Hospital
PI 158	158IHN	Ixodes	holocyclus	0	1	0	0	NSW							Turramurra Vet Hospital
PI 167	167RSF	Rhipicephalus	sanguineus	0	0	0	5	WA	6765	N/A	Kurnangki	-18.194272	125.568678	Kurnangki & Minadri	Aboriginal Reserve
PI 168	168RSF	Rhipicephalus	sanguineus	0	0	0	3	WA	6765	N/A	Kurnangki	-18.194272	125.568678	Kurnangki & Minadri	Aboriginal Reserve
PI 169	169RSF	Rhipicephalus	sanguineus	0	0	0	8	WA	6765	N/A	Kurnangki	-18.194272	125.568678	Kurnangki & Minadri	Aboriginal Reserve

PI 169	169RSM	Rhipicephalus	sanguineus	0	0	10	0	WA	6765	N/A	Kurnangki	-18.194272	125.568678	Kurnangki & Minadri	Aboriginal Reserve
PI 170	170RSF	Rhipicephalus	sanguineus	0	0	0	5	WA	6765	N/A	Kurnangki	-18.194272	125.568678	Kurnangki & Minadri	Aboriginal Reserve
PI 170	170RSM	Rhipicephalus	sanguineus	0	0	13	0	WA	6765	N/A	Kurnangki	-18.194272	125.568678	Kurnangki & Minadri	Aboriginal Reserve
PI 172	172RSF	Rhipicephalus	sanguineus	0	0	0	4	WA	6765	N/A	Minardi	-18.194272	125.568678	Kurnangki & Minadri	Aboriginal Reserve
PI 172	172RSM	Rhipicephalus	sanguineus	0	0	4	0	WA	6765	N/A	Minardi	-18.194272	125.568678	Kurnangki & Minadri	Aboriginal Reserve
PI 173	173RSF	Rhipicephalus	sanguineus	0	0	0	3	WA	6765	N/A	Minardi	-18.194272	125.568678	Kurnangki & Minadri	Aboriginal Reserve
PI 173	173RSM	Rhipicephalus	sanguineus	0	0	2	0	WA	6765	N/A	Minardi	-18.194272	125.568678	Kurnangki & Minadri	Aboriginal Reserve
PI 174	174RSL	Rhipicephalus	sanguineus	7	0	0	0	WA	6765	N/A	Minardi	-18.194272	125.568678	Kurnangki & Minadri	Aboriginal Reserve
PI 175	175RSF	Rhipicephalus	sanguineus	0	0	0	2	WA	6765	N/A	Minardi	-18.194272	125.568678	Kurnangki & Minadri	Aboriginal Reserve
PI 175	175RSM	Rhipicephalus	sanguineus	0	0	4	0	WA	6765	N/A	Minardi	-18.194272	125.568678	Kurnangki & Minadri	Aboriginal Reserve
PI 176	176IHF	Ixodes	holocyclus	0	0	0	1	TAS							Forbes St Vet Clinic
PI 177	177ITF	Ixodes	tasmani	0	0	0	3	TAS							Forbes St Vet Clinic
PI 178	178ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 179	179ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 180	180ICF	Ixodes	cornuatus	0	0	0	1	TAS							Forbes St Vet Clinic
PI 181	181(ORIG)	Ixodes	cornuatus + holocyclus	0	0	0	2	TAS							Forbes St Vet Clinic
PI 182	182ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 184	184ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 185	185(ORIG)	Ixodes	cornuatus	0	0	0	1	TAS							Forbes St Vet Clinic
PI 186	186ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 187	187(ORIG)	Ixodes	cornuatus	0	0	0	1	TAS							Forbes St Vet Clinic
PI 198	198RSF	Rhipicephalus	sanguineus	0	0	0	1	WA	6150	Murdoch	N/A	-32.062085	115.832701	Perth	Murdoch University

															Veterinary Hospital
PI 203	203IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 209	209IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 211	211IHF	Ixodes	holocyclus	0	0	0	4	NSW							Member of public
PI 211	211HLF	Haemaphysalis	longicornis	0	0	0	1	NSW							Member of public
PI 211	211IHM	Ixodes	holocyclus	0	0	2	0	NSW							Member of public
PI 211	211HLN	Haemaphysalis	longicornis	0	4	0	0	NSW							Member of public
PI 212	212IHF	Ixodes	holocyclus	0	0	0	8	NSW							Member of public
PI 212	212HLF	Haemaphysalis	longicornis	0	0	0	2	NSW							Member of public
PI 212	212IHM	Ixodes	holocyclus	0	0	7	0	NSW							Member of public
PI 212	212HLN	Haemaphysalis	longicornis	0	3	0	0	NSW							Member of public
PI 213	213IHF	Ixodes	holocyclus	0	0	0	2	NSW							Member of public
PI 213	213HLF	Haemaphysalis	longicornis	0	0	0	2	NSW							Member of public
PI 213	213HLN	Haemaphysalis	longicornis	0	8	0	0	NSW							Member of public
PI 214	214IHF	Ixodes	holocyclus	0	0	0	11	NSW							Member of public
PI 214	214HLF	Haemaphysalis	longicornis	0	0	0	3	NSW							Member of public
PI 214	214IHM	Ixodes	holocyclus	0	0	5	0	NSW							Member of public
PI 214	214HLN	Haemaphysalis	longicornis	0	7	0	0	NSW							Member of public
PI 215	215IHF	Ixodes	holocyclus	0	0	0	2	NSW							Member of public
PI 215	215HLF	Haemaphysalis	longicornis	0	0	0	1	NSW							Member of public
PI 215	215IHM	Ixodes	holocyclus	0	0	3	0	NSW							Member of public
PI 215	215HLN	Haemaphysalis	longicornis	0	4	0	0	NSW							Member of public

PI 221	221IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 221	221HLN	Haemaphysalis	longicornis	0	9	0	0	NSW							Member of public
PI 227	227IHF	Ixodes	holocyclus	0	0	0	1	QLD							Member of public
PI 235	235IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 236	236IHF	Ixodes	holocyclus	0	0	0	2	NSW							Member of public
PI 236	236IHM	Ixodes	holocyclus	0	0	2	0	NSW							Member of public
PI 236	236IHN	Ixodes	holocyclus	0	7	0	0	NSW							Member of public
PI 236	236HLN	Haemaphysalis	longicornis	0	1	0	0	NSW							Member of public
PI 237	237IHF	Ixodes	holocyclus	0	0	0	14	NSW							Member of public
PI 237	237HLF	Haemaphysalis	longicornis	0	0	0	1	NSW							Member of public
PI 237	237IHM	Ixodes	holocyclus	0	0	2	0	NSW							Member of public
PI 237	237IHN	Ixodes	holocyclus	0	20	0	0	NSW							Member of public
PI 237	237HLN	Haemaphysalis	longicornis	0	2	0	0	NSW							Member of public
PI 238	238IHF	Ixodes	holocyclus	0	0	0	5	NSW							Member of public
PI 238	238HLF	Haemaphysalis	longicornis	0	0	0	1	NSW							Member of public
PI 242	242IHF	Ixodes	holocyclus	0	0	0	11	NSW							Member of public
PI 242	242IHN	Ixodes	holocyclus	0	1	0	0	NSW							Member of public
PI 243	243IHF	Ixodes	holocyclus	0	0	0	6	NSW							Member of public
PI 244	244IHF	Ixodes	holocyclus	0	0	0	13	NSW							Member of public
PI 244	244HLF	Haemaphysalis	longicornis	0	0	0	1	NSW							Member of public
PI 244	244IHN	Ixodes	holocyclus	0	4	0	0	NSW							Member of public

PI 259	259RSF	Rhipicephalus	sanguineus	0	0	0	3	NT	831	Blackwell	2/60 Hudson Terrace	-12.480066	130.984006	Palmerston	PARAP Vet Hospital
PI 259	259RSM	Rhipicephalus	sanguineus	0	0	2	0	NT	831	Blackwell	2/60 Hudson Terrace	-12.480066	130.984006	Palmerston	PARAP Vet Hospital
PI 260	260RSF	Rhipicephalus	sanguineus	0	0	0	2	NT	832	Gunn	17 Camfield St	-12.484288	130.993234	Palmerston	PARAP Vet Hospital
PI 260	260RSM	Rhipicephalus	sanguineus	0	0	3	0	NT	832	Gunn	17 Camfield St	-12.484288	130.993234	Palmerston	PARAP Vet Hospital
PI 261	261RSF	Rhipicephalus	sanguineus	0	0	0	2	NT	830	Woodroffe	52 Gunter Circuit	-12.499222	130.980538	Palmerston	PARAP Vet Hospital
PI 261	261RSM	Rhipicephalus	sanguineus	0	0	3	0	NT	830	Woodroffe	52 Gunter Circuit	-12.499222	130.980538	Palmerston	PARAP Vet Hospital
PI 262	262RSF	Rhipicephalus	sanguineus	0	0	0	3	NT	830	Palmerston	Palmerston Veterinary Hospital, 7 Rolyat Street	-12.481763	130.986142	Palmerston	PARAP Vet Hospital
PI 262	262RSM	Rhipicephalus	sanguineus	0	0	5	0	NT	830	Palmerston	Palmerston Veterinary Hospital, 7 Rolyat Street	-12.481763	130.986142	Palmerston	PARAP Vet Hospital
PI 263	263RSF	Rhipicephalus	sanguineus	0	0	0	2	NT	832	Bakewell	10 Rail Close	-12.497515	130.989012	Palmerston	PARAP Vet Hospital
PI 263	263RSM	Rhipicephalus	sanguineus	0	0	3	0	NT	832	Bakewell	10 Rail Close	-12.497515	130.989012	Palmerston	PARAP Vet Hospital
PI 264	264RSF	Rhipicephalus	sanguineus	0	0	0	1	NT	810	Alawa	37 Bald Circuit	-12.381569	130.871371	Darwin	PARAP Vet Hospital
PI 264	264RSM	Rhipicephalus	sanguineus	0	0	1	0	NT	810	Alawa	37 Bald Circuit	-12.381569	130.871371	Darwin	PARAP Vet Hospital
PI 265	265RSF	Rhipicephalus	sanguineus	0	0	0	4	NT	820	Bayview	25 Latrobe St	-12.440944	130.856539	Darwin	PARAP Vet Hospital
PI 265	265RSM	Rhipicephalus	sanguineus	0	0	4	0	NT	820	Bayview	25 Latrobe St	-12.440944	130.856539	Darwin	PARAP Vet Hospital
PI 266	266RSF	Rhipicephalus	sanguineus	0	0	0	26	NT	820	Darwin	Darwin Sailing Club	-12.429577	130.836378	Darwin	PARAP Vet Hospital
PI 266	266RSM	Rhipicephalus	sanguineus	0	0	34	0	NT	820	Darwin	Darwin Sailing Club	-12.429577	130.836378	Darwin	PARAP Vet Hospital

PI 266	266RSN	Rhipicephalus	sanguineus	0	2	0	0	NT	820	Darwin	Darwin Sailing Club	-12.429577	130.836378	Darwin	PARAP Vet Hospital
PI 267	267RSF	Rhipicephalus	sanguineus	0	0	0	6	NT	820	Parap	85 May st	-12.432338	130.850156	Darwin	PARAP Vet Hospital
PI 267	267RSM	Rhipicephalus	sanguineus	0	0	7	0	NT	820	Parap	85 May st	-12.432338	130.850156	Darwin	PARAP Vet Hospital
PI 268	268RSN	Rhipicephalus	sanguineus	0	4	0	0	NT	820	Bayview	11 Castlemain close	-12.440034	130.85853	Darwin	PARAP Vet Hospital
PI 272	272IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 279	279IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 280	280IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 280	280IHN	Ixodes	holocyclus	0	2	0	0	NSW							Member of public
PI 282	282RSF	Rhipicephalus	sanguineus	0	0	0	1	WA	6149	Bull Creek	7 Sellars Way	-32.054589	115.866256	Perth	Member of public
PI 285	285RSF	Rhipicephalus	sanguineus	0	0	0	1	WA	6149	Bull Creek	7 Sellars Way	-32.054589	115.866256	Perth	Member of public
PI 286	286RSM	Rhipicephalus	sanguineus	0	0	1	0	WA	6149	Bull Creek	7 Sellars Way	-32.054589	115.866256	Perth	Member of public
PI 289	289IHN	Ixodes	holocyclus	0	2	0	0	TAS							Forbes St Vet Clinic
PI 290	290IHF	Ixodes	holocyclus	0	0	0	2	TAS							Forbes St Vet Clinic
PI 293	293IHF	Ixodes	holocyclus	0	0	0	1	TAS							Forbes St Vet Clinic
PI 308	308ATF	Amblyomma	triguttatum (triguttatum)	0	0	0	1	WA							Mt Helena Vet Clinic
PI 309	309ATN	Amblyomma	triguttatum (triguttatum)	0	1	0	0	WA							Mt Helena Vet Clinic
PI 310	310ATN	Amblyomma	triguttatum (triguttatum)	0	1	0	0	WA							Mt Helena Vet Clinic
PI 311	311ATN	Amblyomma	triguttatum (triguttatum)	0	3	0	0	WA							Mt Helena Vet Clinic
PI 312	312ATF	Amblyomma	triguttatum (triguttatum)	0	0	0	1	WA							Mt Helena Vet Clinic
PI 314	314IHF	Ixodes	holocyclus	0	0	0	1	TAS							Forbes St Vet Clinic
PI 316	316ITF	Ixodes	tasmani	0	0	0	2	TAS							Forbes St Vet Clinic

PI 316	316IHN	Ixodes	holocyclus	0	1	0	0	TAS								Forbes St Vet Clinic
PI 316	316ITN	Ixodes	tasmani	0	1	0	0	TAS								Forbes St Vet Clinic
PI 318	318ITF	Ixodes	tasmani	0	0	0	1	TAS								Forbes St Vet Clinic
PI 322	322RSF	Rhipicephalus	sanguineus	0	0	0	2	NT	850	N/A	21 Bernard St	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 322	322RSM	Rhipicephalus	sanguineus	0	0	4	0	NT	850	N/A	21 Bernard St	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 322	322RSN	Rhipicephalus	sanguineus	0	2	0	0	NT	850	N/A	21 Bernard St	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 323	323RSF	Rhipicephalus	sanguineus	0	0	0	3	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 323	323RSM	Rhipicephalus	sanguineus	0	0	4	0	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 324	324RSF	Rhipicephalus	sanguineus	0	0	0	2	NT	850	N/A	3 Neal St	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 324	324RSM	Rhipicephalus	sanguineus	0	0	3	0	NT	850	N/A	3 Neal St	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 325	325RSF	Rhipicephalus	sanguineus	0	0	0	1	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 325	325RSN	Rhipicephalus	sanguineus	0	3	0	0	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 326	326RSF	Rhipicephalus	sanguineus	0	0	0	4	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 326	326RSM	Rhipicephalus	sanguineus	0	0	2	0	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 327	327RSF	Rhipicephalus	sanguineus	0	0	0	6	NT	850	Katherine East	40 Acacia Dr	-14.470843	132.283503	Katherine	Katherine Vet Care
PI 327	327RSM	Rhipicephalus	sanguineus	0	0	5	0	NT	850	Katherine East	40 Acacia Dr	-14.470843	132.283503	Katherine	Katherine Vet Care
PI 327	327RSN	Rhipicephalus	sanguineus	0	2	0	0	NT	850	Katherine East	40 Acacia Dr	-14.470843	132.283503	Katherine	Katherine Vet Care
PI 328	328RSF	Rhipicephalus	sanguineus	0	0	0	7	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 328	328RSM	Rhipicephalus	sanguineus	0	0	13	0	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 329	329RSF	Rhipicephalus	sanguineus	0	0	0	13	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 329	329RSM	Rhipicephalus	sanguineus	0	0	10	0	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 330	330RSF	Rhipicephalus	sanguineus	0	0	0	17	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care

PI 330	330RSM	Rhipicephalus	sanguineus	0	0	17	0	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 330	330RSN	Rhipicephalus	sanguineus	0	3	0	0	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 331	331RSF	Rhipicephalus	sanguineus	0	0	0	2	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 331	331RSM	Rhipicephalus	sanguineus	0	0	7	0	NT	850	N/A	N/A	-14.464497	132.262021	Katherine	Katherine Vet Care
PI 349	349IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 350	350IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 351	351IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 352	352IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 353	353IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 354	354IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 355	355IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 356	356(ORIG)	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 360	360IHF	Ixodes	holocyclus	0	0	0	4	QLD							DermCare Vet
PI 360	360HLN	Haemaphysalis	longicornis	0	1	0	0	QLD							DermCare Vet
PI 361	361IHM	Ixodes	holocyclus	0	0	1	0	QLD							DermCare Vet
PI 365	365IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 369	369IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 370	370IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 371	371IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 372	372IHF	Ixodes	holocyclus	0	0	0	1	QLD							DermCare Vet
PI 373	373IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 374	374IHF	Ixodes	holocyclus	0	0	0	1	QLD							DermCare Vet

PI 375	375IHF	Ixodes	holocyclus	0	0	0	1	QLD							DermCare Vet
PI 376	376IHN	Ixodes	holocyclus	0	2	0	0	QLD							DermCare Vet
PI 376	376HLN	Haemaphysalis	longicornis	0	2	0	0	QLD							DermCare Vet
PI 378	378IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 379	379IHF	Ixodes	holocyclus	0	0	0	3	NSW							DermCare Vet
PI 380	380IHN	Ixodes	holocyclus	0	3	0	0	NSW							DermCare Vet
PI 380	380HLN	Haemaphysalis	longicornis	0	11	0	0	NSW							DermCare Vet
PI 381	381IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 382	382IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 383	383IHF	Ixodes	holocyclus	0	0	0	2	NSW							DermCare Vet
PI 384	384IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 384	384IHN	Ixodes	holocyclus	0	4	0	0	NSW							DermCare Vet
PI 384	384HLL	Haemaphysalis	longicornis	2	0	0	0	NSW							DermCare Vet
PI 384	384HLN	Haemaphysalis	longicornis	0	9	0	0	NSW							DermCare Vet
PI 385	385IHF	Ixodes	holocyclus	0	0	0	2	NSW							DermCare Vet
PI 395	395IHF	Ixodes	holocyclus	0	0	0	2	NSW							DermCare Vet
PI 396	396IHF	Ixodes	holocyclus	0	0	0	3	NSW							DermCare Vet
PI 398	398IHF	Ixodes	holocyclus	0	0	0	1	NSW							DermCare Vet
PI 399	399RSM	Rhipicephalus	sanguineus	0	0	1	0	WA	6726	CABLE BEACH	N/A	-17.950181	122.196423	Cable Beach	Member of public
PI 400	400RSM	Rhipicephalus	sanguineus	0	0	1	0	WA	6726	CABLE BEACH	N/A	-17.950181	122.196423	Cable Beach	Member of public
PI 401	401RSF	Rhipicephalus	sanguineus	0	0	0	1	WA	6726	CABLE BEACH	N/A	-17.950181	122.196423	Cable Beach	Member of public
PI 401	401RSN	Rhipicephalus	sanguineus	0	1	0	0	WA	6726	CABLE BEACH	N/A	-17.950181	122.196423	Cable Beach	Member of public

PI 402	402RSF	Rhipicephalus	sanguineus	0	0	0	1	WA	6726	CABLE BEACH	N/A	-17.950181	122.196423	Cable Beach	Member of public
PI 403	403RSF	Rhipicephalus	sanguineus	0	0	0	1	WA	6726	CABLE BEACH	N/A	-17.950181	122.196423	Cable Beach	Member of public
PI 403	403RSM	Rhipicephalus	sanguineus	0	0	2	0	WA	6726	CABLE BEACH	N/A	-17.950181	122.196423	Cable Beach	Member of public
PI 403	403RSN	Rhipicephalus	sanguineus	0	18	0	0	WA	6726	CABLE BEACH	N/A	-17.950181	122.196423	Cable Beach	Member of public
PI 575	575IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 577	577IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 579	579IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 580	580IHF	Ixodes	holocyclus	0	0	0	2	QLD							Member of public
PI 580	580IHM	Ixodes	holocyclus	0	0	1	0	QLD							Member of public
PI 599	599IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 600	600IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 601	601IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 602	602IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 603	603IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 604	604IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 606	606IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 607	607IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 608	608RSF	Rhipicephalus	sanguineus	0	0	0	6	WA	6714	Karratha	N/A	-20.736709	116.846295	Karratha	Member of public
PI 609	609IHF	Ixodes	holocyclus	0	0	0	1	QLD							Member of public
PI 612	612IHF	Ixodes	holocyclus	0	0	0	5	NSW							Member of public
PI 612	612HLF	Haemaphysalis	longicornis	0	0	0	3	NSW							Member of public
PI 612	612IHM	Ixodes	holocyclus	0	0	9	0	NSW							Member of public

PI 612	612HLN	Haemaphysalis	longicornis	0	4	0	0	NSW							Member of public
PI 613	613IHF	Ixodes	holocyclus	0	0	0	4	NSW							Member of public
PI 613	613HLF	Haemaphysalis	longicornis	0	0	0	1	NSW							Member of public
PI 613	613IHM	Ixodes	holocyclus	0	0	3	0	NSW							Member of public
PI 613	613HLN	Haemaphysalis	longicornis	0	5	0	0	NSW							Member of public
PI 614	614IHF	Ixodes	holocyclus	0	0	0	2	NSW							Member of public
PI 614	614HLF	Haemaphysalis	longicornis	0	0	0	1	NSW							Member of public
PI 614	614IHM	Ixodes	holocyclus	0	0	4	0	NSW							Member of public
PI 614	614HLN	Haemaphysalis	longicornis	0	4	0	0	NSW							Member of public
PI 615	615IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 615	615HLF	Haemaphysalis	longicornis	0	0	0	1	NSW							Member of public
PI 615	615HLN	Haemaphysalis	longicornis	0	3	0	0	NSW							Member of public
PI 616	616IHF	Ixodes	holocyclus	0	0	0	4	NSW							Member of public
PI 616	616IHM	Ixodes	holocyclus	0	0	1	0	NSW							Member of public
PI 616	616HLN	Haemaphysalis	longicornis	0	6	0	0	NSW							Member of public
PI 617	617IHF	Ixodes	holocyclus	0	0	0	20	NSW							Member of public
PI 617	617HLF	Haemaphysalis	longicornis	0	0	0	4	NSW							Member of public
PI 617	617IHM	Ixodes	holocyclus	0	0	6	0	NSW							Member of public
PI 617	617HLN	Haemaphysalis	longicornis	0	8	0	0	NSW							Member of public
PI 618	618IHF	Ixodes	holocyclus	0	0	0	15	NSW							Member of public
PI 618	618HLF	Haemaphysalis	longicornis	0	0	0	4	NSW							Member of public
PI 618	618IHM	Ixodes	holocyclus	0	0	7	0	NSW							Member of public

PI 618	618HLN	Haemaphysalis	longicornis	0	16	0	0	NSW							Member of public
PI 619	619IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 619	619HLN	Haemaphysalis	longicornis	0	2	0	0	NSW							Member of public
PI 620	620HLF	Haemaphysalis	longicornis	0	0	0	2	NSW							Member of public
PI 620	620IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 620	620HLN	Haemaphysalis	longicornis	0	2	0	0	NSW							Member of public
PI 621	621IHF	Ixodes	holocyclus	0	0	0	20	NSW							Member of public
PI 621	621HLF	Haemaphysalis	longicornis	0	0	0	6	NSW							Member of public
PI 621	621IHM	Ixodes	holocyclus	0	0	2	0	NSW							Member of public
PI 621	621HLN	Haemaphysalis	longicornis	0	8	0	0	NSW							Member of public
PI 622	622IHF	Ixodes	holocyclus	0	0	0	14	NSW							Member of public
PI 622	622HLF	Haemaphysalis	longicornis	0	0	0	6	NSW							Member of public
PI 622	622IHM	Ixodes	holocyclus	0	0	5	0	NSW							Member of public
PI 622	622HLN	Haemaphysalis	longicornis	0	9	0	0	NSW							Member of public
PI 623	623IHF	Ixodes	holocyclus	0	0	0	9	NSW							Member of public
PI 623	623HLF	Haemaphysalis	longicornis	0	0	0	4	NSW							Member of public
PI 623	623IHM	Ixodes	holocyclus	0	0	5	0	NSW							Member of public
PI 623	623HLN	Haemaphysalis	longicornis	0	9	0	0	NSW							Member of public
PI 624	624IHF	Ixodes	holocyclus	0	0	0	3	NSW							Member of public
PI 624	624HLF	Haemaphysalis	longicornis	0	0	0	3	NSW							Member of public
PI 624	624IHM	Ixodes	holocyclus	0	0	1	0	NSW							Member of public
PI 624	624HLN	Haemaphysalis	longicornis	0	7	0	0	NSW							Member of public

PI 625	625HLN	Haemaphysalis	longicornis	0	2	0	0	NSW							Member of public
PI 626	626HLF	Haemaphysalis	longicornis	0	0	0	1	NSW							Member of public
PI 626	626IHM	Ixodes	holocyclus	0	0	1	0	NSW							Member of public
PI 626	626HLN	Haemaphysalis	longicornis	0	1	0	0	NSW							Member of public
PI 627	627IHF	Ixodes	holocyclus	0	0	0	2	NSW							Member of public
PI 627	627HBF	Haemaphysalis	bancrofti	0	0	0	1	NSW							Member of public
PI 627	627HLF	Haemaphysalis	longicornis	0	0	0	1	NSW							Member of public
PI 627	627IHM	Ixodes	holocyclus	0	0	6	0	NSW							Member of public
PI 628	628IHM	Ixodes	holocyclus	0	0	3	0	NSW							Member of public
PI 628	628HLN	Haemaphysalis	longicornis	0	1	0	0	NSW							Member of public
PI 629	629IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 631	631IHF	Ixodes	holocyclus	0	0	0	2	NSW							Member of public
PI 632	632IHF	Ixodes	holocyclus	0	0	0	1	NSW							Member of public
PI 637	637RSF	Rhipicephalus	sanguineus	0	0	0	2	NT	830	Farrar	N/A	-12.480219	130.997607	Palmerston	Palmerston Vet Hospital
PI 640	640RSF	Rhipicephalus	sanguineus	0	0	0	4	NT	810	Alawa	11 Young Cres	-12.379233	130.870895	Darwin	Parap Vet Hospital
PI 640	640RSM	Rhipicephalus	sanguineus	0	0	2	0	NT	810	Alawa	11 Young Cres	-12.379233	130.870895	Darwin	Parap Vet Hospital
PI 641	641RSF	Rhipicephalus	sanguineus	0	0	0	13	NT	810	Coconut Grove	20 Orchard Rd	-12.396794	130.847718	Darwin	Parap Vet Hospital
PI 641	641RSM	Rhipicephalus	sanguineus	0	0	17	0	NT	810	Coconut Grove	20 Orchard Rd	-12.396794	130.847718	Darwin	Parap Vet Hospital
PI 641	641RSN	Rhipicephalus	sanguineus	0	5	0	0	NT	810	Coconut Grove	20 Orchard Rd	-12.396794	130.847718	Darwin	Parap Vet Hospital
PI 644	644RSF	Rhipicephalus	sanguineus	0	0	0	46	NT	820	Parap	1/6 Drysdale St	-12.433179	130.844498	Darwin	Parap Vet Hospital
PI 644	644RSM	Rhipicephalus	sanguineus	0	0	63	0	NT	820	Parap	1/6 Drysdale St	-12.433179	130.844498	Darwin	Parap Vet Hospital

PI 646	646RSF	Rhipicephalus	sanguineus	0	0	0	14	NT	810	Rapid Creek	N/A	-12.387231	130.864402	Darwin	Parap Vet Hospital
PI 646	646RSM	Rhipicephalus	sanguineus	0	0	17	0	NT	810	Rapid Creek	N/A	-12.387231	130.864402	Darwin	Parap Vet Hospital
PI 646	646RSN	Rhipicephalus	sanguineus	0	1	0	0	NT	810	Rapid Creek	N/A	-12.387231	130.864402	Darwin	Parap Vet Hospital
PI 647	647RSF	Rhipicephalus	sanguineus	0	0	0	1	NT	830	Driver	24 Driver Ave	-12.484239	130.977186	Palmerston	Parap Vet Hospital
PI 647	647RSM	Rhipicephalus	sanguineus	0	0	2	0	NT	830	Driver	24 Driver Ave	-12.484239	130.977186	Palmerston	Parap Vet Hospital
PI 649	649RSF	Rhipicephalus	sanguineus	0	0	0	6	NT	830	Gray	13 Mianorelli Crt	-12.487317	130.984983	Palmerston	Palmerston Vet Hospital
PI 649	649RSM	Rhipicephalus	sanguineus	0	0	4	0	NT	830	Gray	13 Mianorelli Crt	-12.487317	130.984983	Palmerston	Palmerston Vet Hospital
PI 650	650RSF	Rhipicephalus	sanguineus	0	0	0	2	NT	830	Gray	N/A	-12.384847	130.872981	Darwin	Palmerston Vet Hospital
PI 650	650RSM	Rhipicephalus	sanguineus	0	0	3	0	NT	830	Gray	N/A	-12.384847	130.872981	Darwin	Palmerston Vet Hospital
PI 651	651RSM	Rhipicephalus	sanguineus	0	0	3	0	NT	830	Driver	24 Driver Ave	-12.480219	130.997607	Palmerston	Palmerston Vet Hospital
PI 651	651RSN	Rhipicephalus	sanguineus	0	2	0	0	NT	830	Driver	24 Driver Ave	-12.480219	130.997607	Palmerston	Palmerston Vet Hospital
PI 652	652RSF	Rhipicephalus	sanguineus	0	0	0	3	NT	830	Woodroffe	N/A	-12.504958	130.981903	Palmerston	Palmerston Vet Hospital
PI 652	652RSM	Rhipicephalus	sanguineus	0	0	3	0	NT	830	Woodroffe	N/A	-12.504958	130.981903	Palmerston	Palmerston Vet Hospital
PI 653	653RSF	Rhipicephalus	sanguineus	0	0	0	1	NT	832	Rosebery	57 Kenbi Place	-12.508809	130.994663	Palmerston	Palmerston Vet Hospital
PI 653	653RSM	Rhipicephalus	sanguineus	0	0	1	0	NT	832	Rosebery	57 Kenbi Place	-12.508809	130.994663	Palmerston	Palmerston Vet Hospital
PI 654	654RSF	Rhipicephalus	sanguineus	0	0	0	4	NT	830	Driver	N/A	-12.487233	130.972637	Palmerston	Palmerston Vet Hospital

PI 654	654RSM	Rhipicephalus	sanguineus	0	0	1	0	NT	830	Driver	N/A	-12.487233	130.972637	Palmerston	Palmerston Vet Hospital
PI 655	655RSF	Rhipicephalus	sanguineus	0	0	0	5	NT	830	Woodroffe	N/A	-12.487233	130.972637	Palmerston	Palmerston Vet Hospital
PI 656	656RSL	Rhipicephalus	sanguineus	3	0	0	0	NT	832	Rosebery	1/59 Kenbi Place	-12.509025	130.994619	Palmerston	Palmerston Vet Hospital
PI 656	656RSM	Rhipicephalus	sanguineus	0	0	2	0	NT	832	Rosebery	1/59 Kenbi Place	-12.509025	130.994619	Palmerston	Palmerston Vet Hospital
PI 659	659RSF	Rhipicephalus	sanguineus	0	0	0	15	NT	872	Mutitjulu (East of Uluru)	N/A	-25.351457	131.063954	Mutitjulu	Sydney Animal Hospital
PI 659	659RSM	Rhipicephalus	sanguineus	0	0	16	0	NT	872	Mutitjulu (East of Uluru)	N/A	-25.351457	131.063954	Mutitjulu	Sydney Animal Hospital
PI 659	659RSN	Rhipicephalus	sanguineus	0	20	0	0	NT	872	Mutitjulu (East of Uluru)	N/A	-25.351457	131.063954	Mutitjulu	Sydney Animal Hospital
PI 660	660RSF1	Rhipicephalus	sanguineus	0	0	0	17	NT	872	Mutitjulu (East of Uluru)	N/A	-25.351457	131.063954	Mutitjulu	Sydney Animal Hospital
PI 660	660RSF2	Rhipicephalus	sanguineus	0	0	0	5	NT	872	Mutitjulu (East of Uluru)	N/A	-25.351457	131.063954	Mutitjulu	Sydney Animal Hospital
PI 660	660RSM	Rhipicephalus	sanguineus	0	0	17	0	NT	872	Mutitjulu (East of Uluru)	N/A	-25.351457	131.063954	Mutitjulu	Sydney Animal Hospital
PI 660	660RSN	Rhipicephalus	sanguineus	0	11	0	0	NT	872	Mutitjulu (East of Uluru)	N/A	-25.351457	131.063954	Mutitjulu	Sydney Animal Hospital
PI 661	661RSF1	Rhipicephalus	sanguineus	0	0	0	13	NT	872	Mutitjulu (East of Uluru)	N/A	-25.351457	131.063954	Mutitjulu	Sydney Animal Hospital
PI 661	661RSF2	Rhipicephalus	sanguineus	0	0	0	2	NT	872	Mutitjulu (East of Uluru)	N/A	-25.351457	131.063954	Mutitjulu	Sydney Animal Hospital
PI 661	661RSM	Rhipicephalus	sanguineus	0	0	38	0	NT	872	Mutitjulu (East of Uluru)	N/A	-25.351457	131.063954	Mutitjulu	Sydney Animal Hospital

PI 661	661RSN	Rhipicephalus	sanguineus	0	26	0	0	NT	872	Mutitjulu (East of Uluru)	N/A	-25.351457	131.063954	Mutitjulu	Sydney Animal Hospital
PI 663	663IHF	Ixodes	holocyclus	0	0	0	1	QLD							Member of public
PI 674	674IHF	Ixodes	holocyclus	0	0	0	1	WA							Member of public
PI 688	688IHF	Ixodes	holocyclus	0	0	0	1	QLD							Member of public
PI 695	695RSM	Rhipicephalus	sanguineus	0	0	1	0	WA	6110	Southern River	109 Territt Pl	-32.119577	115.957193	Perth	Animal Protection Society
PI 696	696RSF	Rhipicephalus	sanguineus	0	0	0	2	WA	6110	Southern River	109 Territt Pl	-32.119577	115.957193	Perth	Animal Protection Society
PI 697	697RSF	Rhipicephalus	sanguineus	0	0	0	4	WA	6164	Attwell	29 Minyon Circuit	-32.130633	115.86281	Perth	Animal Protection Society
PI 697	697RSM	Rhipicephalus	sanguineus	0	0	1	0	WA	6164	Attwell	29 Minyon Circuit	-32.130633	115.86281	Perth	Animal Protection Society
PI 698	698RSF	Rhipicephalus	sanguineus	0	0	0	1	WA	6164	Attwell	29 Minyon Circuit	-32.130633	115.86281	Perth	Animal Protection Society
PI 699	699RSF	Rhipicephalus	sanguineus	0	0	0	2	WA	6110	Southern River	109 Territt Pl	-32.119577	115.957193	Perth	Animal Protection Society
PI 699	699RSM	Rhipicephalus	sanguineus	0	0	1	0	WA	6110	Southern River	109 Territt Pl	-32.119577	115.957193	Perth	Animal Protection Society
PI 718	718IHN	Ixodes	holocyclus	0	1	0	0	QLD							Member of public
PI 719	719IHF	Ixodes	holocyclus	0	0	0	3	QLD							Member of public
PI 720	720IHF	Ixodes	holocyclus	0	0	0	1	QLD							Member of public
PI 721	721IHN	Ixodes	holocyclus	0	1	0	0	QLD							Member of public
PI 723	723IHN	Ixodes	holocyclus	0	1	0	0	QLD							Member of public
PI 724	724IHN	Ixodes	holocyclus	0	2	0	0	QLD							Member of public
PI 726	726IHF	Ixodes	holocyclus	0	0	0	3	QLD							Member of public

PI 726	726IHN	Ixodes	holocyclus	0	6	0	0	QLD							Member of public
PI 727	727IHN	Ixodes	holocyclus	0	1	0	0	QLD							Member of public
PI 728	728IHF	Ixodes	holocyclus	0	0	0	2	QLD							Member of public
PI 728	728IHN	Ixodes	holocyclus	0	1	0	0	QLD							Member of public
PI 729	729IHF	Ixodes	holocyclus	0	0	0	1	QLD							Member of public
PI 730	730IHN	Ixodes	holocyclus	0	10	0	0	QLD							Member of public
PI 731	731IHF	Ixodes	holocyclus	0	0	0	1	QLD							Member of public
PI 732	732IHF	Ixodes	holocyclus	0	0	0	3	QLD							Member of public
PI 732	732IHN	Ixodes	holocyclus	0	1	0	0	QLD							Member of public
PI 733	733IHF	Ixodes	holocyclus	0	0	0	1	QLD							Member of public
PI 736	736ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 737	737ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 738	738ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 740	740ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 741	741ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 744	744ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 745	745ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 746	746ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 747	747ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 748	748ITF	Ixodes	tasmani	0	0	0	2	TAS							Forbes St Vet Clinic
PI 749	749IHF	Ixodes	holocyclus	0	0	0	1	QLD							Member of public

PI 750	750RSF	Rhipicephalus	sanguineus	0	0	0	2	WA	6701	Carnarvon	12 Bassett Way	-24.871625	113.675619	Carnarvon	Coral Coast Vet Hospital
PI 751	751RSM	Rhipicephalus	sanguineus	0	0	2	0	WA	6701	Carnarvon	12 Bassett Way	-24.871625	113.675619	Carnarvon	Coral Coast Vet Hospital
PI 752	752RSF	Rhipicephalus	sanguineus	0	0	0	1	WA	6701	Carnarvon	12 Bassett Way	-24.871625	113.675619	Carnarvon	Coral Coast Vet Hospital
PI 753	753RSF	Rhipicephalus	sanguineus	0	0	0	6	WA	6701	Carnarvon	12 Bassett Way	-24.871625	113.675619	Carnarvon	Coral Coast Vet Hospital
PI 753	753RSM	Rhipicephalus	sanguineus	0	0	4	0	WA	6701	Carnarvon	12 Bassett Way	-24.871625	113.675619	Carnarvon	Coral Coast Vet Hospital
PI 754	754RSF	Rhipicephalus	sanguineus	0	0	0	19	WA	6701	Carnarvon	12 Bassett Way	-24.871625	113.675619	Carnarvon	Coral Coast Vet Hospital
PI 754	754RSM	Rhipicephalus	sanguineus	0	0	8	0	WA	6701	Carnarvon	12 Bassett Way	-24.871625	113.675619	Carnarvon	Coral Coast Vet Hospital
PI 770	770RSF	Rhipicephalus	sanguineus	0	0	0	2	WA	6025	Craigie	N/A	-31.7848	115.7678	Perth	Murdoch Uni Vet Hospital
PI 770	770RSM	Rhipicephalus	sanguineus	0	0	37	0	WA	6025	Craigie	N/A	-31.7848	115.7678	Perth	Murdoch Uni Vet Hospital
PI 776	776HLF	Haemaphysalis	longicornis	0	0	0	1	NSW							Member of public
PI 777	777RSF	Rhipicephalus	sanguineus	0	0	0	24	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 777	777RSM	Rhipicephalus	sanguineus	0	0	21	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 777	777RSN	Rhipicephalus	sanguineus	0	1	0	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 778	778RSF	Rhipicephalus	sanguineus	0	0	0	19	NT	830	Palmerston (15 mile)	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC

										Indigenous Village					
PI 778	778RSM	Rhipicephalus	sanguineus	0	0	17	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 778	778RSN	Rhipicephalus	sanguineus	0	1	0	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 779	779RSF	Rhipicephalus	sanguineus	0	0	0	29	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 779	779RSM	Rhipicephalus	sanguineus	0	0	6	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 779	779RSN	Rhipicephalus	sanguineus	0	2	0	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 780	780RSF	Rhipicephalus	sanguineus	0	0	0	33	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 780	780RSM	Rhipicephalus	sanguineus	0	0	17	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 781	781RSF1	Rhipicephalus	sanguineus	0	0	0	13	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 781	781RSF2	Rhipicephalus	sanguineus	0	0	0	4	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 781	781RSN	Rhipicephalus	sanguineus	0	1	0	0	NT	0830	Palmerston (15 mile) Indegenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 781	781RSL	Rhipicephalus	sanguineus	1	0	0	0	NT	830	Palmerston (15 mile)	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC

										Indigenous Village					
PI 781	781RSM	Rhipicephalus	sanguineus	0	0	8	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 782	782RSF	Rhipicephalus	sanguineus	0	0	0	33	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 782	782RSM	Rhipicephalus	sanguineus	0	0	69	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 782	782RSN	Rhipicephalus	sanguineus	0	1	0	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 783	783RSF	Rhipicephalus	sanguineus	0	0	0	56	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 783	783RSM	Rhipicephalus	sanguineus	0	0	53	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 784	784RSF	Rhipicephalus	sanguineus	0	0	0	23	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 784	784RSM	Rhipicephalus	sanguineus	0	0	8	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 784	784RSN	Rhipicephalus	sanguineus	0	1	0	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 785	785RSF	Rhipicephalus	sanguineus	0	0	0	17	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 785	785RSL	Rhipicephalus	sanguineus	13	0	0	0	NT	830	Palmerston (15 mile)	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC

										Indigenous Village					
PI 785	785RSM	Rhipicephalus	sanguineus	0	0	15	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 785	785RSN	Rhipicephalus	sanguineus	0	46	0	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 786	786RSF	Rhipicephalus	sanguineus	0	0	0	35	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 786	786RSL	Rhipicephalus	sanguineus	1	0	0	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 786	786RSM	Rhipicephalus	sanguineus	0	0	37	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 786	786RSN	Rhipicephalus	sanguineus	0	1	0	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 787	787RSF	Rhipicephalus	sanguineus	0	0	0	12	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 787	787RSL	Rhipicephalus	sanguineus	1	0	0	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 787	787RSM	Rhipicephalus	sanguineus	0	0	3	0	NT	830	Palmerston (15 mile) Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 788	788RSF	Rhipicephalus	sanguineus	0	0	0	29	NT	0872	Mutitjulu Indigenous Community	N/A	-25.3523	131.0667	Mutitjulu	AMRRIC
PI 788	788RSM	Rhipicephalus	sanguineus	0	0	26	0	NT	0872	Mutitjulu Indigenous Community	N/A	-25.3523	131.0667	Mutitjulu	AMRRIC

PI 788	788RSN	Rhipicephalus	sanguineus	0	1	0	0	NT	0872	Mutitjulu Indigenous Community	N/A	-25.3523	131.0667	Mutitjulu	AMRRIC
PI 789	789RSF	Rhipicephalus	sanguineus	0	0	0	19	NT	0873	Mutitjulu Indigenous Community	N/A	-25.3523	131.0667	Mutitjulu	AMRRIC
PI 789	789RSM	Rhipicephalus	sanguineus	0	0	28	0	NT	0873	Mutitjulu Indigenous Community	N/A	-25.3523	131.0667	Mutitjulu	AMRRIC
PI 790	790RSF	Rhipicephalus	sanguineus	0	0	0	35	NT	0874	Mutitjulu Indigenous Community	N/A	-25.3523	131.0667	Mutitjulu	AMRRIC
PI 790	790RSM	Rhipicephalus	sanguineus	0	0	15	0	NT	0874	Mutitjulu Indigenous Community	N/A	-25.3523	131.0667	Mutitjulu	AMRRIC
PI 791	791RSF	Rhipicephalus	sanguineus	0	0	0	28	NT	0875	Mutitjulu Indigenous Community	N/A	-25.3523	131.0667	Mutitjulu	AMRRIC
PI 791	791RSM	Rhipicephalus	sanguineus	0	0	23	0	NT	0875	Mutitjulu Indigenous Community	N/A	-25.3523	131.0667	Mutitjulu	AMRRIC
PI 792	792RSF	Rhipicephalus	sanguineus	0	0	0	24	NT	0876	Mutitjulu Indigenous Community	N/A	-25.3523	131.0667	Mutitjulu	AMRRIC
PI 792	792RSM	Rhipicephalus	sanguineus	0	0	19	0	NT	0876	Mutitjulu Indigenous Community	N/A	-25.3523	131.0667	Mutitjulu	AMRRIC
PI 792	792RSN	Rhipicephalus	sanguineus	0	2	0	0	NT	0876	Mutitjulu Indigenous Community	N/A	-25.3523	131.0667	Mutitjulu	AMRRIC
PI 793	793RSF	Rhipicephalus	sanguineus	0	0	0	2	NT	0877	Yuendumu	293km NW of Alice Springs	-22.253296	131.795945	Yuendumu	AMRRIC
PI 793	793RSL	Rhipicephalus	sanguineus	1	0	0	0	NT	0877	Yuendumu	293km NW of Alice Springs	-22.253296	131.795945	Yuendumu	AMRRIC
PI 793	793RSM	Rhipicephalus	sanguineus	0	0	30	0	NT	0877	Yuendumu	293km NW of Alice Springs	-22.253296	131.795945	Yuendumu	AMRRIC
PI 793	793RSN	Rhipicephalus	sanguineus	0	22	0	0	NT	0877	Yuendumu	293km NW of Alice Springs	-22.253296	131.795945	Yuendumu	AMRRIC

PI 794	794RSF	Rhipicephalus	sanguineus	0	0	0	11	NT	0878	Yuendumu	293km NW of Alice Springs	-22.253296	131.795945	Yuendumu	AMRRIC
PI 794	794RSM	Rhipicephalus	sanguineus	0	0	17	0	NT	0878	Yuendumu	293km NW of Alice Springs	-22.253296	131.795945	Yuendumu	AMRRIC
PI 795	795RSF	Rhipicephalus	sanguineus	0	0	0	12	NT	0879	Nyirripi	160km WSW of Yuendumu	-22.6475681	130.5494494	Nyirripi	AMRRIC
PI 795	795RSM	Rhipicephalus	sanguineus	0	0	43	0	NT	0879	Nyirripi	160km WSW of Yuendumu	-22.6475681	130.5494494	Nyirripi	AMRRIC
PI 795	795RSN	Rhipicephalus	sanguineus	0	12	0	0	NT	0879	Nyirripi	160km WSW of Yuendumu	-22.6475681	130.5494494	Nyirripi	AMRRIC
PI 796	796RSF	Rhipicephalus	sanguineus	0	0	0	2	NT	0880	Yuelamu (Mt Allan)	290km NW of Alice Springs	-22.257958	132.204607	Yuelamu	AMRRIC
PI 796	796RSM	Rhipicephalus	sanguineus	0	0	4	0	NT	0880	Yuelamu (Mt Allan)	290km NW of Alice Springs	-22.257958	132.204607	Yuelamu	AMRRIC
PI 798	798RSF	Rhipicephalus	sanguineus	0	0	0	7	NT	0860	Ali Curung	via Tennart Creek	-19.648306	134.186642	Tennant Creek	AMRRIC
PI 798	798RSM	Rhipicephalus	sanguineus	0	0	7	0	NT	0860	Ali Curung	via Tennart Creek	-19.648306	134.186642	Tennant Creek	AMRRIC
PI 823	823IHF	Ixodes	holocyclus	0	0	0	1	NSW							Lake Rd Vet Clinic
PI 828	828HLF	Haemaphysalis	longicornis	0	0	0	1	NSW							Member of public
PI 828	828HLN	Haemaphysalis	longicornis	0	1	0	0	NSW							Member of public
PI 829	829HLF	Haemaphysalis	longicornis	0	0	0	2	NSW							Member of public
PI 830	830HLF	Haemaphysalis	longicornis	0	0	0	4	NSW							Member of public
PI 831	831HLF	Haemaphysalis	longicornis	0	0	0	2	NSW							Member of public
PI 832	832HLF	Haemaphysalis	longicornis	0	0	0	1	NSW							Member of public
PI 833	833HLN	Haemaphysalis	longicornis	0	1	0	0	NSW							Member of public
PI 834	834(ORIG)	Amblyomma	triguttatum (triguttatum)	0	0	0	1	WA							Member of public

PI 836	836RSF	Rhipicephalus	sanguineus	0	0	0	21	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding
PI 836	836RSM	Rhipicephalus	sanguineus	0	0	46	0	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding
PI 836	836RSN	Rhipicephalus	sanguineus	0	3	0	0	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding
PI 837	837RSF	Rhipicephalus	sanguineus	0	0	0	1	SA	5723	Coober Pedy	N/A	-29.037845	134.723814	Coober Pedy	Redgum Vet and Boarding
PI 837	837RSM	Rhipicephalus	sanguineus	0	0	1	0	SA	5723	Coober Pedy	N/A	-29.037845	134.723814	Coober Pedy	Redgum Vet and Boarding
PI 838	838RSF	Rhipicephalus	sanguineus	0	0	0	5	SA	5723	Coober Pedy	N/A	-29.037845	134.723814	Coober Pedy	Redgum Vet and Boarding
PI 838	838RSM	Rhipicephalus	sanguineus	0	0	18	0	SA	5723	Coober Pedy	N/A	-29.037845	134.723814	Coober Pedy	Redgum Vet and Boarding
PI 838	838RSN	Rhipicephalus	sanguineus	0	1	0	0	SA	5723	Coober Pedy	N/A	-29.037845	134.723814	Coober Pedy	Redgum Vet and Boarding
PI 839	839RSF	Rhipicephalus	sanguineus	0	0	0	1	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding
PI 840	840RSF	Rhipicephalus	sanguineus	0	0	0	1	SA	5723	Coober Pedy	N/A	-29.037845	134.723814	Coober Pedy	Redgum Vet and Boarding
PI 840	840RSM	Rhipicephalus	sanguineus	0	0	1	0	SA	5723	Coober Pedy	N/A	-29.037845	134.723814	Coober Pedy	Redgum Vet and Boarding
PI 841	841RSF	Rhipicephalus	sanguineus	0	0	0	19	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding
PI 841	841RSM	Rhipicephalus	sanguineus	0	0	47	0	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding
PI 841	841RSN	Rhipicephalus	sanguineus	0	33	0	0	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding

PI 842	842RSM	Rhipicephalus	sanguineus	0	0	2	0	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding
PI 843	843RSF	Rhipicephalus	sanguineus	0	0	0	40	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding
PI 843	843RSM	Rhipicephalus	sanguineus	0	0	70	0	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding
PI 843	843RSN	Rhipicephalus	sanguineus	0	2	0	0	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding
PI 844	844RSF	Rhipicephalus	sanguineus	0	0	0	7	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding
PI 844	844RSL	Rhipicephalus	sanguineus	1	0	0	0	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding
PI 844	844RSM	Rhipicephalus	sanguineus	0	0	14	0	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding
PI 844	844RSN	Rhipicephalus	sanguineus	0	1	0	0	SA	5734	Oodnadatta	N/A	-27.546529	135.447026	Oodnadatta	Redgum Vet and Boarding
PI 850	850RSF	Rhipicephalus	sanguineus	0	0	0	3	NT		Kalaluk	Coconut Grove	-12.398	130.852	Darwin	AMRRIC
PI 850	850RSL	Rhipicephalus	sanguineus	10	0	0	0	NT		Kalaluk	Coconut Grove	-12.398	130.852	Darwin	AMRRIC
PI 850	850RSM	Rhipicephalus	sanguineus	0	0	4	0	NT		Kalaluk	Coconut Grove	-12.398	130.852	Darwin	AMRRIC
PI 850	850RSN	Rhipicephalus	sanguineus	0	1	0	0	NT		Kalaluk	Coconut Grove	-12.398	130.852	Darwin	AMRRIC
PI 851	851RSF	Rhipicephalus	sanguineus	0	0	0	9	NT	872	Lake Nash = Alpurrurulam	N/A	-20.981094	137.861604	Lake Nash	AMRRIC
PI 851	851RSM	Rhipicephalus	sanguineus	0	0	14	0	NT	872	Lake Nash = Alpurrurulam	N/A	-20.981094	137.861604	Lake Nash	AMRRIC
PI 852	852RSF	Rhipicephalus	sanguineus	0	0	0	3	NT	872	Lake Nash = Alpurrurulam	N/A	-20.981094	137.861604	Lake Nash	AMRRIC
PI 852	852RSM	Rhipicephalus	sanguineus	0	0	3	0	NT	872	Lake Nash = Alpurrurulam	N/A	-20.981094	137.861604	Lake Nash	AMRRIC
PI 853	853RSF	Rhipicephalus	sanguineus	0	0	0	10	NT	872	Lake Nash = Alpurrurulam	N/A	-20.981094	137.861604	Lake Nash	AMRRIC
PI 853	853RSM	Rhipicephalus	sanguineus	0	0	9	0	NT	872	Lake Nash = Alpurrurulam	N/A	-20.981094	137.861604	Lake Nash	AMRRIC

PI 853	853RSN	Rhipicephalus	sanguineus	0	2	0	0	NT	872	Lake Nash = Alpurrurulam	N/A	-20.981094	137.861604	Lake Nash	AMRRIC
PI 854	854RSM	Rhipicephalus	sanguineus	0	0	1	0	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 854	854RSN	Rhipicephalus	sanguineus	0	32	0	0	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 855	855RSF	Rhipicephalus	sanguineus	0	0	0	7	NT	828	Knuckey Lagoon	N/A	-12.426825	130.934141	Knuckey Lagoon	AMRRIC
PI 856	856RSN	Rhipicephalus	sanguineus	0	15	0	0	NT		Minmarama	N/A	-12.41118055	130.8491806	Darwin	AMRRIC
PI 858	858ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 859	859ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 860	860ITN	Ixodes	tasmani	0	1	0	0	TAS							Forbes St Vet Clinic
PI 863	863ITL	Ixodes	tasmani	5	0	0	0	TAS							Forbes St Vet Clinic
PI 864	864ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 865	865ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 866	866ITF	Ixodes	tasmani	0	0	0	1	TAS							Forbes St Vet Clinic
PI 867	867ITL	Ixodes	tasmani	11	0	0	0	TAS							Forbes St Vet Clinic
PI 870	870RSF	Rhipicephalus	sanguineus	0	0	0	13	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 870	870RSM	Rhipicephalus	sanguineus	0	0	13	0	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 870	870RSN	Rhipicephalus	sanguineus	0	4	0	0	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 871	871RSF	Rhipicephalus	sanguineus	0	0	0	9	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 871	871RSM	Rhipicephalus	sanguineus	0	0	18	0	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 871	871RSN	Rhipicephalus	sanguineus	0	3	0	0	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 872	872RSF	Rhipicephalus	sanguineus	0	0	0	18	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 872	872RSM	Rhipicephalus	sanguineus	0	0	13	0	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 872	872RSN	Rhipicephalus	sanguineus	0	7	0	0	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 873	873RSF	Rhipicephalus	sanguineus	0	0	0	10	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 873	873RSM	Rhipicephalus	sanguineus	0	0	12	0	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC

PI 873	873RSN	Rhipicephalus	sanguineus	0	2	0	0	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 874	874RSF	Rhipicephalus	sanguineus	0	0	0	8	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 874	874RSM	Rhipicephalus	sanguineus	0	0	17	0	NT	820	Bagot	N/A	-12.415	130.856	Darwin	AMRRIC
PI 875	875RSF	Rhipicephalus	sanguineus	0	0	0	29	NT	0820	Palmerston Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 875	875RSM	Rhipicephalus	sanguineus	0	0	33	0	NT	0820	Palmerston Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 875	875RSN	Rhipicephalus	sanguineus	0	1	0	0	NT	0820	Palmerston Indigenous Village	N/A	-12.48809722	131.0116806	Palmerston	AMRRIC
PI 879	879RSF	Rhipicephalus	sanguineus	0	0	0	1	WA	6122	Byford	N/A	-32.221725	116.0072	Perth	Foothills Animal Hospital
PI 880	880RSF	Rhipicephalus	sanguineus	0	0	0	1	WA	6112	Armadale	Foothills Animal Hospital, 23 Thomas St	-32.154628	116.016502	Perth	Foothills Animal Hospital
PI 881	881RSF	Rhipicephalus	sanguineus	0	0	0	1	WA	6112	Armadale	Foothills Animal Hospital, 23 Thomas St	-32.154628	116.016502	Perth	Foothills Animal Hospital
PI 882	882RSF	Rhipicephalus	sanguineus	0	0	0	1	WA	6112	Armadale	Foothills Animal Hospital, 23 Thomas St	-32.154628	116.016502	Perth	Foothills Animal Hospital
PI 883	883(ORIG)	Amblyomma	triguttatum (triguttatum)	0	0	0	1	WA							Foothills Animal Hospital
SUM				56	528	1291	1401								
TOTAL															

Table A.2: Number of overall submissions from each state and territory.

State/Territory	Number of Submissions
New South Wales (NSW)	105
Northern Territory (NT)	71
Queensland (QLD)	23
South Australia (SA)	9
Tasmania (TAS)	34
Victoria (VIC)	1
Western Australia (WA)	43
Total	**286**

Appendix B.

Table B.1: Mapping file with MID-tag combinations

#SampleID	BarcodeSequence	RBarcodeSequence	Genus	Species	Pooling	Sex	HostGenus	HostSpecies	PostCodeAus	GeoLocation	OriginalTubeID	F_Tag	R_Tag
264RSM	TATGCGAC	ACTGTG	Rhipicephalus	sanguineus	1xMale	Male	Canis	lupis	0810	Darwin	PI 264	F441	R37
640RSM	TATGCGAC	AACAAC	Rhipicephalus	sanguineus	2xMale	Male	Canis	lupis	0810	Darwin	PI 640	F441	R38
646RSM	TATGCGAC	ACTTGA	Rhipicephalus	sanguineus	2xMale	Male	Canis	lupis	0810	Darwin	PI 646	F441	R39
650RSM	TATGCGAC	GGTGTT	Rhipicephalus	sanguineus	2xMale	Male	Canis	lupis	0830	Darwin	PI 650	F441	R40
641RSM	TATGCGAC	AGAAGA	Rhipicephalus	sanguineus	2xMale	Male	Canis	lupis	0810	Darwin	PI 641	F441	R41
265RSM	TATGCGAC	TTGAAG	Rhipicephalus	sanguineus	1xMale	Male	Canis	lupis	0820	Darwin	PI 265	F441	R42
644RSM	TATGCGAC	AACTTG	Rhipicephalus	sanguineus	5xMale	Male	Canis	lupis	0820	Darwin	PI 644	F441	R43
267RSM	TATGCGAC	AAGACA	Rhipicephalus	sanguineus	3xMale	Male	Canis	lupis	0820	Darwin	PI 267	F441	R44
266RSM	TGATCGAC	ACTGTG	Rhipicephalus	sanguineus	2xMale	Male	Canis	lupis	0820	Darwin	PI 266	F442	R37
264RSF	TGATCGAC	AACAAC	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	0810	Darwin	PI 264	F442	R38
640RSF	TGATCGAC	ACTTGA	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	0810	Darwin	PI 640	F442	R39
646RSF	TGATCGAC	GGTGTT	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	0810	Darwin	PI 646	F442	R40
650RSF	TGATCGAC	AGAAGA	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	0830	Darwin	PI 650	F442	R41
641RSF	TGATCGAC	TTGAAG	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	0810	Darwin	PI 641	F442	R42
265RSF	TGATCGAC	AACTTG	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	0820	Darwin	PI 265	F442	R43
644RSF	TGATCGAC	AAGACA	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	0820	Darwin	PI 644	F442	R44
267RSF	AGCTCGAC	ACTGTG	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	0820	Darwin	PI 267	F443	R37
266RSF	AGCTCGAC	AACAAC	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	0820	Darwin	PI 266	F443	R38
697RSM	AGCTCGAC	ACTTGA	Rhipicephalus	sanguineus	1xMale	Male	Canis	lupis	6164	Perth	PI 697	F443	R39
695RSM	AGCTCGAC	GGTGTT	Rhipicephalus	sanguineus	1xMale	Male	Canis	lupis	6110	Perth	PI 695	F443	R40

699RSM	AGCTCGAC	AGAAGA	Rhipicephalus	sanguineus	1xMale	Male	Canis	lupis	6110	Perth	PI 699	F443	R41
136RSM	AGCTCGAC	TTGAAG	Rhipicephalus	sanguineus	5xMale	Male	Canis	lupis	6150	Perth	PI 136	F443	R42
286RSM	AGCTCGAC	AACTTG	Rhipicephalus	sanguineus	1xMale	Male	Canis	lupis	6149	Perth	PI 286	F443	R43
770RSM	AGCTCGAC	AAGACA	Rhipicephalus	sanguineus	6xMale	Male	Canis	lupis	6025	Perth	PI 770	F443	R44
879RSF	ATCATGAC	ACTGTG	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	6122	Perth	PI 879	F444	R37
880RSF	ATCATGAC	AACAAC	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	6112	Perth	PI 880	F444	R38
881RSF	ATCATGAC	ACTTGA	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	6112	Perth	PI 881	F444	R39
882RSF	ATCATGAC	GGTGTT	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	6112	Perth	PI 882	F444	R40
697RSF	ATCATGAC	AGAAGA	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	6164	Perth	PI 697	F444	R41
698RSF	ATCATGAC	TTGAAG	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	6164	Perth	PI 698	F444	R42
696RSF	ATCATGAC	AACTTG	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	6110	Perth	PI 696	F444	R43
699RSF	ATCATGAC	AAGACA	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	6110	Perth	PI 699	F444	R44
76RSF	TATATGAC	ACTGTG	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	6162	Perth	PI **076**	F445	R37
198RSF	TATATGAC	AACAAC	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	6150	Perth	PI 198	F445	R38
282RSF	TATATGAC	ACTTGA	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	6149	Perth	PI 282	F445	R39
285RSF	TATATGAC	GGTGTT	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	6149	Perth	PI 285	F445	R40
770RSF	TATATGAC	AGAAGA	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	6025	Perth	PI 770	F445	R41
Darwin Extraction Control	TATATGAC	TTGAAG	ExControl	ExControl	ExControl	ExControl	ExControl	ExControl	ExControl	ExControl	ExControl	F445	R42
Perth Extraction Control	TATATGAC	AACTTG	ExControl	ExControl	ExControl	ExControl	ExControl	ExControl	ExControl	ExControl	ExControl	F445	R43
Palmerston Extraction Control	TATATGAC	AAGACA	ExControl	ExControl	ExControl	ExControl	ExControl	ExControl	ExControl	ExControl	ExControl	F445	R44
Clean Lab NTC	ACGCTGAC	ACTGTG	NTControl	NTControl	NTControl	NTControl	NTControl	NTControl	NTControl	NTControl	NTControl	F446	R37
Cryptick Lab NTC	ACGCTGAC	AACAAC	NTControl	NTControl	NTControl	NTControl	NTControl	NTControl	NTControl	NTControl	NTControl	F446	R38

649RSM	TCTCTGAC	ACTGTG	Rhipicephalus	sanguineus	2xMale	Male	Canis	lupis	0830	Palmerston	PI 649	F447	R37
654RSM	TCTCTGAC	AACAAC	Rhipicephalus	sanguineus	1xMale	Male	Canis	lupis	0830	Palmerston	PI 654	F447	R38
260RSM	TCTCTGAC	ACTTGA	Rhipicephalus	sanguineus	1xMale	Male	Canis	lupis	0832	Palmerston	PI 260	F447	R39
262RSM	TCTCTGAC	GGTGTT	Rhipicephalus	sanguineus	3xMale	Male	Canis	lupis	0830	Palmerston	PI 262	F447	R40
651RSM	TCTCTGAC	AGAAGA	Rhipicephalus	sanguineus	2xMale	Male	Canis	lupis	0830	Palmerston	PI 651	F447	R41
259RSM	TCTCTGAC	TTGAAG	Rhipicephalus	sanguineus	1xMale	Male	Canis	lupis	0831	Palmerston	PI 259	F447	R42
656RSM	TCTCTGAC	AACTTG	Rhipicephalus	sanguineus	1xMale	Male	Canis	lupis	0832	Palmerston	PI 656	F447	R43
653RSM	TCTCTGAC	AAGACA	Rhipicephalus	sanguineus	1xMale	Male	Canis	lupis	0833	Palmerston	PI 653	F447	R44
652RSM	TCAGTGAC	ACTGTG	Rhipicephalus	sanguineus	2xMale	Male	Canis	lupis	0830	Palmerston	PI 652	F448	R37
261RSM	TCAGTGAC	AACAAC	Rhipicephalus	sanguineus	2xMale	Male	Canis	lupis	0830	Palmerston	PI 261	F448	R38
263RSM	TCAGTGAC	ACTTGA	Rhipicephalus	sanguineus	2xMale	Male	Canis	lupis	0832	Palmerston	PI 263	F448	R39
649RSF	TCAGTGAC	GGTGTT	Rhipicephalus	sanguineus	3xFemale	Female	Canis	lupis	0830	Palmerston	PI 649	F448	R40
654RSF	TCAGTGAC	AGAAGA	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	0830	Palmerston	PI 654	F448	R41
655RSF	TCAGTGAC	TTGAAG	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	0830	Palmerston	PI 655	F448	R42
260RSF	TCAGTGAC	AACTTG	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	0832	Palmerston	PI 260	F448	R43
647RSF	TCAGTGAC	AAGACA	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	0830	Palmerston	PI 647	F448	R44
262RSF	ATAGTGAC	ACTGTG	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	0830	Palmerston	PI 262	F449	R37
637RSF	ATAGTGAC	AACAAC	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	0830	Palmerston	PI 637	F449	R38
259RSF	ATAGTGAC	ACTTGA	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	0831	Palmerston	PI 259	F449	R39
653RSF	ATAGTGAC	GGTGTT	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	0832	Palmerston	PI 653	F449	R40
652RSF	ATAGTGAC	AGAAGA	Rhipicephalus	sanguineus	2xFemale	Female	Canis	lupis	0830	Palmerston	PI 652	F449	R41
261RSF	ATAGTGAC	TTGAAG	Rhipicephalus	sanguineus	1xFemale	Female	Canis	lupis	0830	Palmerston	PI 261	F449	R42

Table B.2: Relative quantification C_T values and pooling volume for Darwin and Perth samples with Bact16S27F/338R primers

Sample	C_T rep1	C_T rep2	C_T Mean	Ref C_T	Ratio	Volume (µL)	Pool #
264RSM	27.19	27.14	27.165	21.61	1.26	12.57	1
640RSM	23.09	22.78	22.935	21.61	1.06	10.61	1
646RSM	24.91	24.19	24.55	21.61	1.14	11.36	1
650RSM	22.61	22.14	22.375	21.61	1.04	9.35	1
641RSM	21.4	21.4	21.4	21.61	0.99	8.90	1
265RSM	28.41	28.19	28.3	21.61	1.31	13.10	1
644RSM	18.76		18.76	21.61	0.87	7.68	1
267RSM	21.36	27.51	24.435	21.61	1.13	11.31	1
266RSM	27.96	27.57	27.765	21.61	1.28	12.85	2
264RSF		29.22	29.22	21.61	1.35	13.52	2
640RSF	28.06	27.06	27.56	21.61	1.28	12.75	2
646RSF	27.48	27.32	27.4	21.61	1.27	12.68	2
650RSF	20.98	22.6	21.79	21.61	1.01	9.08	2
641RSF	21.35	21.87	21.61	21.61	1.00	10.00	2
265RSF	27.26	27.76	27.51	21.61	1.27	12.73	2
644RSF	17.56	16.78	17.17	21.61	0.79	6.95	2
267RSF	29.09	29.74	29.415	21.61	1.36	13.61	3
266RSF	24.34	23.91	24.125	21.61	1.12	11.16	3
697RSM	23.68	24.244	23.962	21.61	1.11	11.09	3
695RSM	26.03	26.73	26.38	21.61	1.22	12.21	3
699RSM	27.82	28.64	28.23	21.61	1.31	13.06	3
136RSM	26.44	26.122	26.2812	21.61	1.22	12.16	3
286RSM	29.09	28.87	28.98	21.61	1.34	13.41	3
770RSM	26.07	25.93	26	21.61	1.20	12.03	3
Amp plateaued - volume adjusted manually							
Reference Sample							

Table B.3: Relative quantification C_T values and pooling volume for Perth samples with Bact16S27F/338R primers

Sample	C_T rep1	C_T rep2	C_T Mean	Ref C_T	Ratio	Volume (µL)	Pool #
879RSF	24.3965	25.5432	24.96985	22.43	1.11	11.13	1
880RSF	25.2661	26.0458	25.65595	22.43	1.14	11.44	1
881RSF	28.3408	27.9919	28.16635	22.43	1.26	12.56	1
882RSF		25.8615	25.8615	22.43	1.15	11.53	1
697RSF	23.8173	23.6041	23.7107	22.43	1.06	10.57	1
698RSF	27.545	26.049	26.797	22.43	1.19	11.95	1
696RSF	23.6489	23.286	23.46745	22.43	1.05	10.46	1
699RSF	26.5253	25.3863	25.9558	22.43	1.16	11.57	2
76RSF	27.1928	27.6422	27.4175	22.43	1.22	12.22	2
198RSF	28.1554	28.2919	28.22365	22.43	1.26	12.58	2
282RSF	22.8037	22.0656	22.43465	22.43	1.00	10.00	2
285RSF	23.1949	23.1192	23.15705	22.43	1.03	10.32	2
770RSF	27.5545	27.4551	27.5048	22.43	1.23	12.26	2
Darwin Ext Ctrl	31.0663		31.0663	22.43	1.39	13.85	3
Perth Ext Ctrl	30.3197	30.9662	30.64295	22.43	1.37	13.66	3
Palmerston Ext Ctrl	31.1187	30.1839	30.6513	22.43	1.37	13.67	3
NTC	30.9391		30.9391	22.43	1.38	13.79	3
NTC	31.9095		31.9095	22.43	1.42	14.23	3
Amp plateaued - volume adjusted manually							
Reference Sample							

Table B.4: Relative quantification CT values and pooling volume for Palmerston samples with Bact16S27F/338R primers.

Sample	C_T rep1	C_T rep2	C_T Mean	Ref C_T	Ratio	Volume (µL)	Pool #
649RSM	29.9158	28.4868	29.2013	26.8849	1.09	10.86	1
654RSM	31.8514	29.1968	30.5241	26.8849	1.14	11.35	1
260RSM	28.9719	28.5115	28.7417	26.8849	1.07	10.69	1
262RSM	26.2669	26.7292	26.49805	26.8849	0.99	9.86	1
651RSM	28.0021	27.0614	27.53175	26.8849	1.02	10.24	1
259RSM	30.7988	28.8829	29.84085	26.8849	1.11	11.10	1
656RSM	29.9647	29.7731	29.8689	26.8849	1.11	11.11	1
653RSM	30.3955	29.2445	29.82	26.8849	1.11	11.09	1
652RSM	27.9534	27.2133	27.58335	26.8849	1.03	10.26	2
261RSM	29.3841	29.8346	29.60935	26.8849	1.10	11.01	2
263RSM	28.342	29.8603	29.10115	26.8849	1.08	10.82	2
649RSF	29.3343	28.431	28.88265	26.8849	1.07	10.74	2
654RSF	29.8684	30.0162	29.9423	26.8849	1.11	11.14	2
655RSF	27.9441	27.1623	27.5532	26.8849	1.02	10.25	2
260RSF	26.5578	27.212	26.8849	26.8849	1.00	10.00	2
647RSF	26.4008	27.6803	27.04055	26.8849	1.01	10.06	2
262RSF	28.39	28.4454	28.4177	26.8849	1.06	10.57	3
637RSF	29.4722	30.521	29.9966	26.8849	1.12	11.16	3
259RSF	28.4152	28.1685	28.29185	26.8849	1.05	10.52	3
653RSF	30.3977	30.3683	30.383	26.8849	1.13	11.30	3
652RSF	30.4664	30.1867	30.32655	26.8849	1.13	11.28	3
261RSF	30.9892	30.0791	30.53415	26.8849	1.14	11.36	3
Reference Sample							

Table B.5: Absolute Quantification C_T values and calculations for final volume.

	C_T Rep1	C_T Rep 2	C_T Rep3	C_T Mean		C_T Rep1	C_T Rep2	C_T Rep3	C_T Mean	Copies/µL	Copies	Volume to add (uL):
					0.01	8.85	8.1	7.99	8.313333	193,099,641	386,199,282	3.4
1000000000	6.95	6.78	6.95	6.893333	0.001	11.68	11.75	11.86	11.76333	14,541,395	29,082,791	44.7
100000000	10.3		10	10.15	0.0005	12.93	12.71	12.49	12.71	7,151,730	14,303,460	90.9
10000000	13.21	13.23	13.36	13.26667	0.00025	14.01	14.43	14.07	14.17	2,393,846	4,787,693	271.5
1000000	16.6	16.26	16.48	16.44667	0.000125	14.63	14.73	14.44	14.6	1,734,229	3,468,457	374.8
100000	19.52	19.13	19.09	19.24667	6.25E-05	15.6	16.29	15.44	15.77667	717,849	1,435,698	905.5
10000	22.08	22.98	21.86	22.30667	3.13E-05	16.59	16.51	16.6	16.56667	397,047	794,094	1637.1
1000					1.56E-05	17.69	17.42	17.39	17.5	197,237	394,474	3295.5
Target Copy #	650,000,000		**Dilution used**	1in100		**Volume Added**	3.4ul Library + 21.6ul water					

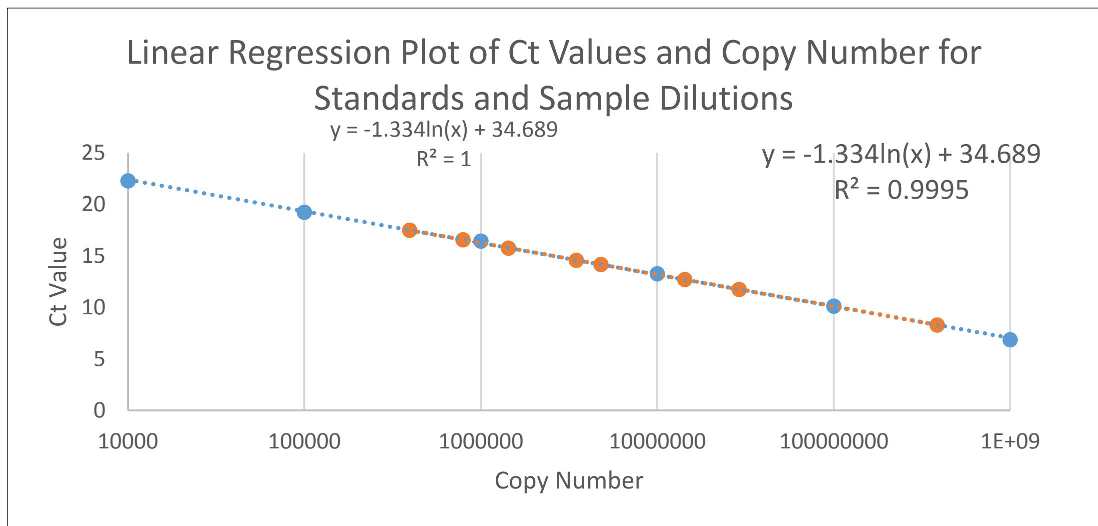

Figure B: Absolute quantification standard curve.

Appendix C.

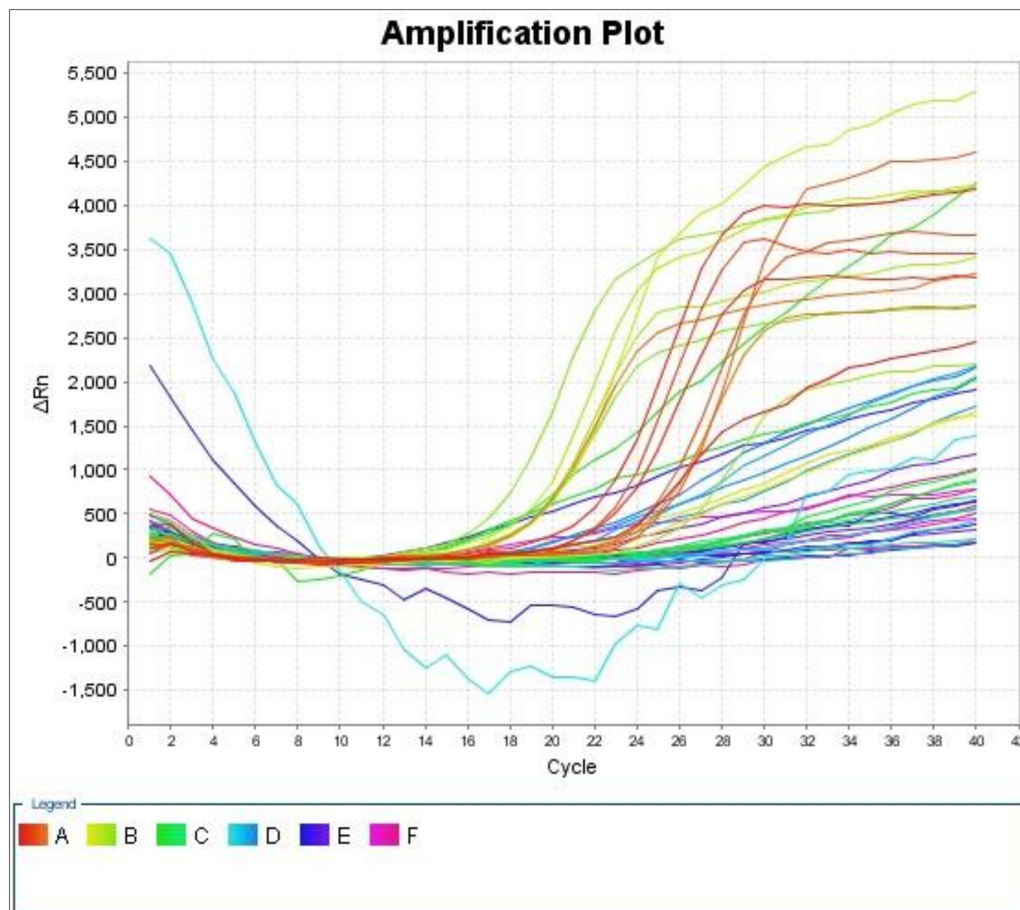

Figure C.1: Amplification plot for Darwin undiluted DNA samples with Bact16S27F/338R primers.

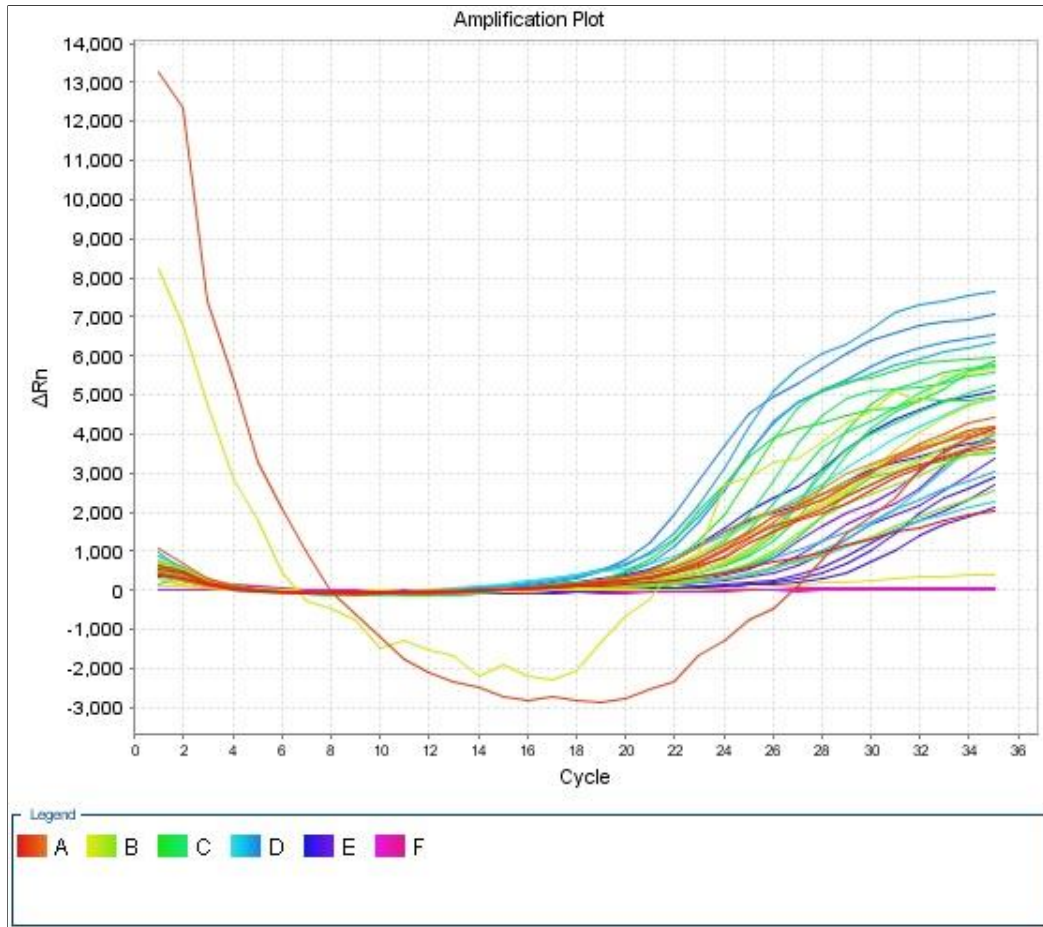

Figure C.2: Amplification plot for Perth undiluted DNA samples with Bact16S27F/338R primers.

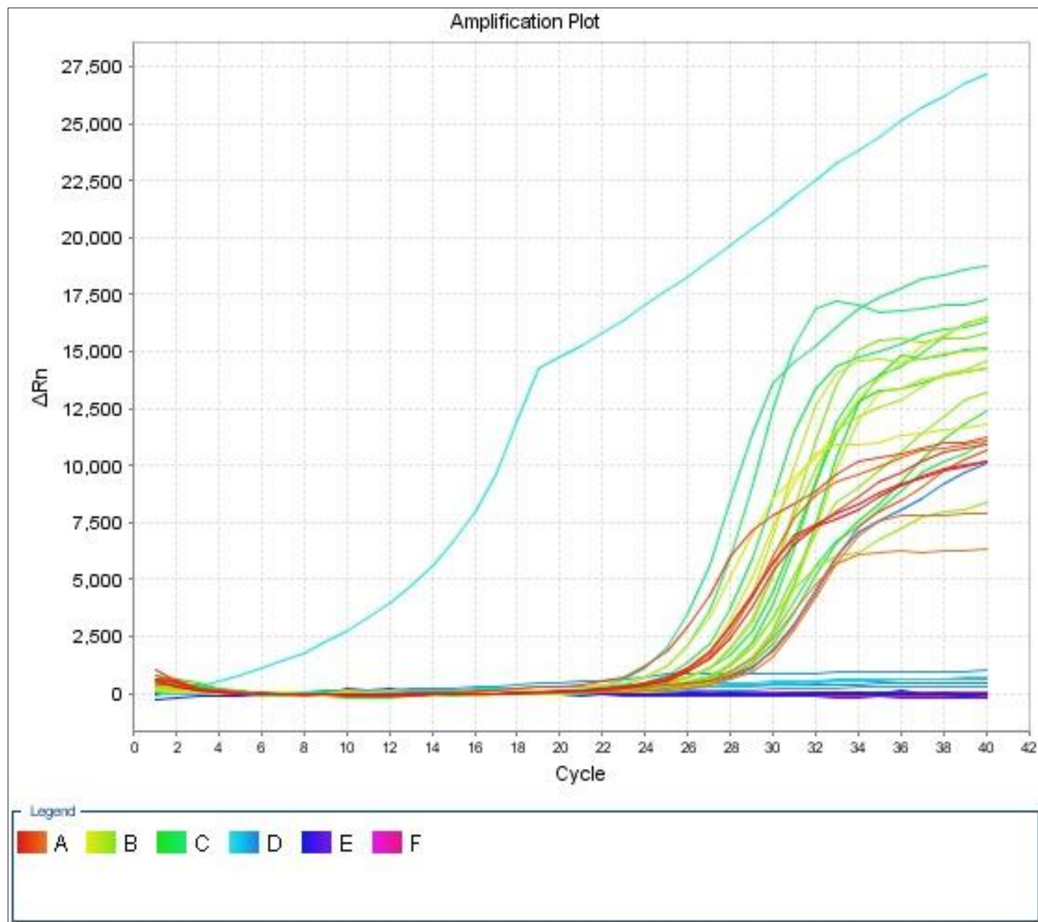

Figure C.3: Amplification plot for Palmerston undiluted DNA samples with Bact16S27F/338R primers.

Appendix D.

Table D.1: Percent of *Coxiella* spp. reads for each sample.

Sample ID	Geographical Location	Sex	Number of Ticks	(Genus) Coxiella %
644RSM	Darwin	Male	5	0.00%
641RSM	Darwin	Male	2	0.30%
640RSM	Darwin	Male	2	0.40%
646RSM	Darwin	Male	2	1.20%
650RSM	Darwin	Male	2	1.80%
266RSM	Darwin	Male	2	18.30%
267RSM	Darwin	Male	3	31.40%
265RSM	Darwin	Male	1	44.60%
264RSM	Darwin	Male	1	48.00%
644RSF	Darwin	Female	2	0.00%
646RSF	Darwin	Female	2	0.10%
650RSF	Darwin	Female	2	0.10%
641RSF	Darwin	Female	2	0.90%
640RSF	Darwin	Female	1	1.80%
264RSF	Darwin	Female	1	3.20%
267RSF	Darwin	Female	2	10.70%
265RSF	Darwin	Female	1	74.60%
266RSF	Darwin	Female	2	91.40%
262RSM	Palmerston	Male	3	0.00%
651RSM	Palmerston	Male	2	0.00%
652RSM	Palmerston	Male	2	1.30%
259RSM	Palmerston	Male	1	1.60%
653RSM	Palmerston	Male	1	1.60%
649RSM	Palmerston	Male	2	3.40%
261RSM	Palmerston	Male	2	3.70%
654RSM	Palmerston	Male	1	7.50%
656RSM	Palmerston	Male	1	22.20%
263RSM	Palmerston	Male	2	34.40%
260RSM	Palmerston	Male	1	64.90%
261RSF	Palmerston	Female	1	0.00%
637RSF	Palmerston	Female	1	0.00%
652RSF	Palmerston	Female	2	0.20%
654RSF	Palmerston	Female	2	0.50%
262RSF	Palmerston	Female	2	0.70%
649RSF	Palmerston	Female	3	9.70%
653RSF	Palmerston	Female	1	12.30%
655RSF	Palmerston	Female	2	30.20%
260RSF	Palmerston	Female	1	49.20%
259RSF	Palmerston	Female	2	51.70%
647RSF	Palmerston	Female	1	86.40%
699RSM	Perth	Male	1	11.80%
770RSM	Perth	Male	6	19.40%
286RSM	Perth	Male	1	36.40%
697RSM	Perth	Male	1	62.90%
136RSM	Perth	Male	5	74.70%

695RSM	Perth	Male	1	81.40%
198RSF	Perth	Female	1	7.90%
699RSF	Perth	Female	2	11.40%
696RSF	Perth	Female	2	13.20%
770RSF	Perth	Female	2	38.10%
698RSF	Perth	Female	1	45.50%
697RSF	Perth	Female	2	53.90%
881RSF	Perth	Female	1	54.00%
76RSF	Perth	Female	1	73.00%
879RSF	Perth	Female	1	84.90%
880RSF	Perth	Female	1	92.80%
282RSF	Perth	Female	1	97.40%
882RSF	Perth	Female	1	98.90%
285RSF	Perth	Female	1	99.20%
Darwin Extraction Control	N/A	N/A	N/A	0.00%
Palmerston Extraction Control	N/A	N/A	N/A	0.10%
Perth Extraction Control	N/A	N/A	N/A	0.20%
Cryptick Lab NTC	N/A	N/A	N/A	0.00%
Clean Lab NTC	N/A	N/A	N/A	0.00%

Table D.2: Legend for taxonomic assignment in QIIME.

Legend	Taxonomy
	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Friedmanniella
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas
	Unassigned
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Variovorax
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__
	k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Pelomonas
	k__Bacteria;p__OD1;c__SM2F11;o__;f__;g__
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;Other
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Ralstonia
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Burkholderia
	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae;g__Propionibacterium
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Stenotrophomonas
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;Other;Other
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__;g__
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Photobacterium
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;Other
	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus
	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Corynebacteriaceae;g__Corynebacterium
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__
	k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae];g__Chryseobacterium
	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Staphylococcus
	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus
	k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Flavobacterium
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__
	k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__;g__
	k__Bacteria;p__OD1;c__ZB2;o__;f__;g__
	k__Bacteria;p__Firmicutes;c__Bacilli;o__Turicibacterales;f__Turicibacteraceae;g__Turicibacter
	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Herbaspirillum
	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Veillonella
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Erythrobacteraceae;g__
	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;Other
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Rhizobium
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Haemophilus
	k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Streptophyta;f__;g__
	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Sporosarcina
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__

k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae;g__Rickettsiella
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;Other
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__
k__Bacteria;p__Cyanobacteria;c__4C0d-2;o__SM1D11;f__;g__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae;g__Coxiella
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Micrococcus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;Other
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Hymenobacter
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Jeotgalicoccus
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;Other;Other;Other
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Geodermatophilaceae;g__Geodermatophilus
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingobium
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Curtobacterium
k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__Gemmatimonadales;f__;g__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae;g__Rhodococcus
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Leucobacter
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Geodermatophilaceae;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptomycetaceae;g__Streptomyces
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;Other
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Aerococcaceae;g__
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Lautropia
k__Bacteria;p__Acidobacteria;c__Acidobacteria-5;o__;f__;g__
k__Bacteria;p__Acidobacteria;c__Acidobacteriia;o__Acidobacteriales;f__Acidobacteriaceae;g__
k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__Solibacteraceae;g__
k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__Solibacteraceae;g__Candidatus Solibacter
k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__PK29;f__;g__
k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__RB41;f__Ellin6075;g__
k__Bacteria;p__Acidobacteria;c__iii1-8;o__DS-18;f__;g__
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__;g__
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__C111;g__
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__EB1017;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;Other;Other
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Actinomyces
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Brevibacteriaceae;g__Brevibacterium
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Cellulomonadaceae;g__Cellulomonas
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Cellulomonadaceae;g__Demequina
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Dermacoccaceae;g__Dermacoccus
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Frankiaceae;g__

k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Geodermatophilaceae;g__Modestobacter
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Gordoniaceae;g__Gordonia
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae;Other
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae;g__Knoellia
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Kineosporiaceae;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Kineosporiaceae;g__Kineococcus
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;Other
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Agrococcus
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Candidatus Aquiluna
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Candidatus Rhodoluna
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Cryocola
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Microbacterium
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;Other
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Arthrobacter
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Kocuria
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Rothia
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;Other
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae;g__Mycobacterium
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;Other
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Aeromicrobium
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Kribbella
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Nocardioides
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae;Other
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae;g__Actinomycetospora
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae;g__Pseudonocardia
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Sporichthyaceae;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Thermomonosporaceae;g__Actinomadura
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__
k__Bacteria;p__Actinobacteria;c__MB-A2-108;o__0319-7L14;f__;g__
k__Bacteria;p__Actinobacteria;c__Nitriliruptoria;o__Euzebyales;f__Euzebyaceae;g__Euzebya
k__Bacteria;p__Actinobacteria;c__Rubrobacteria;o__Rubrobacterales;f__Rubrobacteraceae;g__Rubrobacter
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae;g__
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__;g__
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Conexibacteraceae;g__
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Patulibacteraceae;g__Patulibacter
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Solirubrobacteraceae;g__
k__Bacteria;p__Armatimonadetes;c__Armatimonadia;o__Armatimonadales;f__Armatimonadaceae;g__
k__Bacteria;p__Armatimonadetes;c__Chthonomonadetes;o__SJA-22;f__;g__
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides

k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Marinilabiaceae;g__
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Porphyromonas
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Tannerella
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cyclobacteriaceae;g__
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Adhaeribacter
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Flectobacillus
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Pontibacter
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Rhodocytophaga
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Spirosoma
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Flammeovirgaceae;g__
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Cryomorphaceae;g__
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Capnocytophaga
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Gillisia
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Myroides
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae];g__
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae];g__Cloacibacterium
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__;g__
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Pedobacter
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Sphingobacterium
k__Bacteria;p__Bacteroidetes;c__[Rhodothermi];o__[Rhodothermales];f__Rhodothermaceae;g__
k__Bacteria;p__Bacteroidetes;c__[Rhodothermi];o__[Rhodothermales];f__Rhodothermaceae;g__Rubricoccus
k__Bacteria;p__Bacteroidetes;c__[Rhodothermi];o__[Rhodothermales];f__[Balneolaceae];g__Balneola
k__Bacteria;p__Bacteroidetes;c__[Rhodothermi];o__[Rhodothermales];f__[Balneolaceae];g__KSA1
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__;g__
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Chitinophaga
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Flavisolibacter
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Sediminibacterium
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Segetibacter
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Saprospiraceae;g__
k__Bacteria;p__Chlorobi;c__OPB56;o__;f__;g__
k__Bacteria;p__Chloroflexi;c__Chloroflexi;o__Herpetosiphonales;f__;g__
k__Bacteria;p__Chloroflexi;c__Chloroflexi;o__[Roseiflexales];Other;Other
k__Bacteria;p__Chloroflexi;c__Chloroflexi;o__[Roseiflexales];f__[Kouleothrixaceae];g__
k__Bacteria;p__Chloroflexi;c__Ellin6529;o__;f__;g__
k__Bacteria;p__Chloroflexi;c__TK17;o__mle1-48;f__;g__
k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__JG30-KF-CM45;f__;g__
k__Bacteria;p__Cyanobacteria;c__4C0d-2;o__YS2;f__;g__
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__;f__;g__
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Stramenopiles;f__;g__
k__Bacteria;p__Cyanobacteria;c__ML635J-21;o__;f__;g__
k__Bacteria;p__Cyanobacteria;c__Nostocophycideae;o__Nostocales;f__Nostocaceae;g__Cylindrospermopsis
k__Bacteria;p__Cyanobacteria;c__Nostocophycideae;o__Stigonematales;f__Rivulariaceae;g__Calothrix
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;o__Chroococcales;f__Xenococcaceae;g__
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;Other;Other

k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Alicyclobacillaceae;g__Alicyclobacillus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Geobacillus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Virgibacillus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Listeriaceae;g__Brochothrix
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Ammoniphilus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Brevibacillus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Paenibacillus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;Other
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Planococcus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Planomicrobium
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Ureibacillus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Macrococcus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__[Exiguobacteraceae];g__Exiguobacterium
k__Bacteria;p__Firmicutes;c__Bacilli;o__Gemellales;f__Gemellaceae;g__Gemella
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Aerococcaceae;g__Aerococcus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae;g__Carnobacterium
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;g__Enterococcus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;g__Vagococcus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae;g__Leuconostoc
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__;g__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;Other
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Eubacteriaceae;g__Acetobacterium
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Gracilibacteraceae;Other
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;Other
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Ruminococcus]
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae;g__Desulfosporosinus
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae;g__Peptococcus
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__Filifactor
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__Peptostreptococcus
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Oscillospira
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__G07
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Megamonas
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Phascolarctobacterium
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Acidaminobacteraceae];g__Fusibacter
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];Other
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__Anaerococcus
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__Finegoldia
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__Helcococcus

k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__Parvimonas
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__Peptoniphilus
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__WAL_1855D
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Catenibacterium
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__[Eubacterium]
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae;g__Fusobacterium
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Leptotrichiaceae;g__Leptotrichia
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Leptotrichiaceae;g__Sneathia
k__Bacteria;p__GN02;c__BD1-5;o__;f__;g__
k__Bacteria;p__GN02;c__GKS2-174;o__;f__;g__
k__Bacteria;p__GN04;c__;o__;f__;g__
k__Bacteria;p__Gemmatimonadetes;c__;o__;f__;g__
k__Bacteria;p__Gemmatimonadetes;c__Gemm-1;o__;f__;g__
k__Bacteria;p__Gemmatimonadetes;c__Gemm-3;o__;f__;g__
k__Bacteria;p__Gemmatimonadetes;c__Gemm-5;o__;f__;g__
k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__;f__;g__
k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__KD8-87;f__;g__
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira
k__Bacteria;p__OD1;c__;o__;f__;g__
k__Bacteria;p__OP11;c__OP11-4;o__;f__;g__
k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__WD2101;f__;g__
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Isosphaeraceae;g__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__;f__;g__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__BD7-3;f__;g__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Mycoplana
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__RF32;f__;g__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__;g__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Aurantimonadaceae;Other
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Aurantimonadaceae;g__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bartonellaceae;g__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Balneimonas
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Devosia
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Rhodoplanes
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Agrobacterium
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Kaistia
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhodobiaceae;g__Afifella
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;Other
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;Other
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Amaricoccus
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Paracoccus
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Rubellimicrobium
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Roseomonas

k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingopyxis
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__;f__;g__
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;Other
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Comamonas
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Delftia
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Lampropedia
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Paucibacter
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__MND1;f__;g__
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae;Other
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae;g__
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae;g__Conchiformibius
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae;g__Kingella
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Nitrosomonadales;f__Nitrosomonadaceae;Other
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__Hydrogenophilus
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobulbaceae;g__Desulfobulbus
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfohalobiaceae;g__
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__;g__
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Nannocystaceae;g__Nannocystis
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Polyangiaceae;g__
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae;g__
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__[Entotheonellales];f__[Entotheonellaceae];g__
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae;g__Campylobacter
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cardiobacteriales;f__Cardiobacteriaceae;g__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Proteus
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__;g__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__Legionella
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Pasteurella
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Moraxella
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Psychrobacter
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae;g__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Luteimonas
k__Bacteria;p__SR1;c__;o__;f__;g__
k__Bacteria;p__Synergistetes;c__Synergistia;o__Synergistales;f__Dethiosulfovibrionaceae;g__TG5
k__Bacteria;p__TM6;c__SJA-4;o__;f__;g__
k__Bacteria;p__TM7;c__SC3;o__;f__;g__
k__Bacteria;p__TM7;c__TM7-1;o__;f__;g__
k__Bacteria;p__TM7;c__TM7-3;o__;f__;g__
k__Bacteria;p__TM7;c__TM7-3;o__CW040;f__;g__
k__Bacteria;p__TM7;c__TM7-3;o__CW040;f__F16;g__
k__Bacteria;p__TM7;c__TM7-3;o__EW055;f__;g__
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Mycoplasmatales;f__Mycoplasmataceae;g__Mycoplasma
k__Bacteria;p__WS3;c__PRR-12;o__Sediment-1;Other;Other

k__Bacteria;p__[Thermi];c__Deinococci;o__Deinococcales;f__Deinococcaceae;g__Deinococcus

k__Bacteria;p__[Thermi];c__Deinococci;o__Deinococcales;f__Trueperaceae;g__Truepera