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SAN DIEGO STATE UNIVERISTY

Interference and epidemiology of *Rickettsia*, endosymbionts and microbiomes within *Longitarsus* flea beetle and *Dermacentor occidentalis* tick hosts.

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Biology

by

Adam Nikos Gurfield

Committee in charge:

University of California, San Diego

Professor Douglas Bartlett
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San Diego State University

Professor Scott T. Kelley, Chair
Professor Richard Bizzoco
Professor David Lipson

2016

The Dissertation of Adam Nikos Gurfield is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

San Diego State University

2016

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Chapter 1 was prepared as part of a submission for a broader publication that investigates the microbial ecology in a plant, insect, soil ecosystem. The dissertation author was the primary investigator and author of this section of the paper. Its coauthors were Professor Susanne Dobler from Institute of Zoology, University of Hamburg, Hamburg, Germany and Professor Scott T. Kelley from the Department of Biology, San Diego State University, San Diego, CA, USA. I also wish to thank Karin Meyer, Carsten Winkler and Aleksandra Tomic for help with extractions and PCR.

Chapter 2 was prepared for submission for publication regarding endosymbiotic bacterial phenomena in *Dermacentor occidentalis* ticks. The dissertation author was the primary investigator and author of this material. Its coauthors were Drs. Saran Grewal and Lynnie Cua from the Department of Environmental Health, County of San Diego, San Diego, CA USA, and Pedro Torres and Professor Scott T. Kelley from the Department of Biology, San Diego State University, San Diego, CA, USA.

Chapter 3 was prepared for submission for publication as a description of the epidemiology of spotted fever group *Rickettsia* in San Diego County. The dissertation author was the primary investigator and author of this material. Its coauthors were Drs. Saran Grewal and Dr. Lynnie Cua from the Department of Environmental Health, County of San Diego, San Diego, CA USA and Professor Scott T. Kelley from the Department of Biology, San Diego State University, San Diego, CA, USA. Victoria Nguyen is thanked for help with DNA extractions.

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FIELDS OF STUDY

Major field: Microbiology

Studies in vector-borne zoonotic diseases and microbiomes

Professor Scott T. Kelley

ABSTRACT OF THE DISSERTATION

Interference and epidemiology of *Rickettsia*, endosymbionts and microbiomes within *Longitarsus* flea beetle and *Dermacentor occidentalis* tick hosts.

by

Adam Nikos Gurfield

Doctor of Philosophy in Biology

University of California, San Diego, 2016

San Diego State University, 2016

Professor Scott T. Kelley, Chair

Rickettsiae are small, gram negative, rod-shaped, obligate intracellular, endosymbiotic alphaproteobacteria that are responsible for several of the oldest zoonoses known to man. Over the past 10 years, genetic analysis of rickettsiae found in arthropod vectors, mammal reservoirs and clinical disease specimens has resulted in the recognition of new pathogenic rickettsial species and a re-evaluation of disease caused by species previously thought to be non-pathogenic, including, most recently

Rickettsia philipii strain 364D. Interference between different *Rickettsia* species co-infecting ticks has been described, although, the mechanisms are unknown. Interference is thought to lead to dramatic epidemiological consequences such as defining the geographic distribution of disease. In this dissertation, the association and possible interference between *Rickettsia* spp. and other bacteria were investigated using two different invertebrate models, namely, insect flea beetles of the *Longitarsus* genus and the arachnid tick *Dermacentor occidentalis*. *Longitarsus* flea beetles are herbivorous and complete their entire life cycle on and around an individual plant and its soil, in contrast to *Dermacentor* ticks that imbibe blood from three different vertebrate hosts to complete their life cycle. PCR amplification and Sanger sequencing of partial rickettsial ompA and IGR genes and PCR of 16S rRNA gene sequences followed by next generation sequencing, respectively, were used to identify rickettsias and other bacteria that constituted the microbiome of these invertebrate hosts. The Quantitative Insights Into Microbial Ecology (QIIME) open source bioinformatics pipeline was used for sequence data analysis and to explore the different intermicrobial and microbe-host relationships of *Rickettsia*. We found that while the species of *Longitarsus* was related to the make-up of their *Rickettsia* and *Wolbachia* endosymbionts, in contrast, in *Dermacentor* ticks, other *Francisella*-like endosymbionts and, to a lesser extent, non-endosymbiotic organisms appeared to "interfere" with rickettsial infection. An additional goal of this investigation was to describe the prevalence and distribution of *Rickettsias* in *Dermacentor occidentalis* in San Diego County. Both pathogenic and nonpathogenic *Rickettsias* were detected in

ticks collected from different locations over several years. These findings are described in the dissertation that follows.

CHAPTER 1

Endosymbionts and microbial diversity in three *Longitarsus* flea beetle species (Coleoptera, Chrysomelidae)

Abstract

Previous studies have demonstrated complex associations of flea beetle gut microbiomes with their insect hosts and environments rather than host plant chemistry. Using an ecologically controlled study, we compared the microbial community diversity (MCD) of three different *Longitarsus* beetle species (*L. luridis*, *L. melanocephalus* and *L. pratensis*) feeding on the same plant in three different locales in Germany in order to separate the influences of locale and host species on the beetle MCD. We determined that *Rickettsia* and/or *Wolbachia* bacteria dominate the MCD of the beetles and that multiple species of *Rickettsia* and *Wolbachia* co-infect the beetles. BLAST analysis of partial 16S rRNA gene sequences demonstrated 99-100% similarity to *Rickettsia* and *Wolbachia* of other Coleopteran and Hemipteran insects. Phylogenetic analysis showed that they were introduced into the beetle microbiomes at different phylogenetic points. In contrast, the rest of the non-*Rickettsia* and non-*Wolbachia* microbiome components were not correlated with the species of beetle or *Rickettsia* and *Wolbachia* populations. The results of this study indicate that endosymbiont microbial communities within *Longitarsus* beetles are shaped by host

species rather than geographic location or host plant and they are dominated by multiple, phylogenetically distinct lineages of *Rickettsia* and *Wolbachia* secondary endosymbionts.

Introduction

Herbivorous flea beetles (Coleoptera, Chrysomelidae) comprise over 600 species with a near global distribution (Gruev and Döberl 2005, Furth 2007). Many species within the *Longitarsus* genus accumulate noxious secondary compounds synthesized by their host plants, such as iridoid glycosides or pyrrolizine alkaloids, for protection from predators (Dobler et al. 2000, Willinger and Dobler 2001). This ability has shaped the evolution of the genus leading to many independent switches to plants offering these compounds (Dobler 2001). Recognizing the importance that microbiota play in the physiology of their host insects, such as termites (Warnecke et al. 2007), ants (Van Borm et al. 2002), and other beetles (Suh et al., 2005, Hu et al. 2014), Kelley and Dobler examined the microbiome of *Longitarsus* species using culture-independent deep sequencing molecular methods to assess if the microbial colony diversity correlated with beetle phylogeny or soil microbial diversity (Kelley and Dobler 2011). They determined that environmental factors were important in shaping beetle microbial diversity but found little evidence that host-plant chemistry played an important role in shaping the insect microbial diversity.

While Kelley and Dobler reported Enterobacteriaceae as the most common family of bacteria in the beetle microbiome, secondary endosymbiotic *Rickettsia* and *Wolbachia* genera bacteria were also detected in samples derived from whole beetle extractions. *Rickettsia* and *Wolbachia* spp. are well known secondary endosymbionts of insects that, depending on species, can be vertically or horizontally transmitted, are not necessary for host survival and reside in cells outside of the bacteriome (Tsuchida et al. 2002, Thao and Baumann 2004, Baumann 2005, Bing et al. 2014). As part of a comprehensive study of the microbiome of plants, soil, rhizosphere and associated beetles, we collected multiple *Longitarsus* spp. beetles feeding on the same host plant (*Plantago lanceolata*) in three separate locales in Germany in order to discern the effects of insect species and environment on the beetle microbiome. Using culture-independent parallel-tagged deep sequencing methods we elucidated the microbiomes present in each beetle species and locale. We found that different microbiomes in each *Longitarsus* species were dominated by one or two bacterial endosymbiont species whose abundances were inversely related. Moreover, in contrast to the non-endosymbiotic fractions, the endosymbionts had a greater association with host beetle species than with the geographic locale from which the beetles were derived.

Materials and Methods

Sample Collection. Three species of flea beetles, *Longitarsus luridis*, *L. melanocephalus* and *L. pratensis*, were collected from the same host plant species (*Plantago lanceolata*) in each of three different locations in Germany: Aumühle in Schleswig-Holstein (53°52' N, 10°32' E), Schönberg near Freiburg, Baden-Württemberg (47°95'N, 7°78'E) and Westerhever in Schleswig-Holstein (54°38'N, 8°68'E) in April, June and July 2011 and July and Aug 2013 (Table 1). *L. luridus* has a broad diet range that includes host plant species in several different plant families (Plantaginaceae, Ranunculaceae, Asteraceae) whereas *L. pratensis* and *L. melanocephalus* are specialists on *Plantago* species (Plantaginaceae). Detailed collection and DNA processing procedures are described in an earlier study (Kelley and Dobler 2011). Briefly, beetles were collected by sweep-nets or aspirators and identified by S. Dobler. All samples were returned live to the lab at the University of Hamburg before being frozen at -80 °C. Wings and elytra from beetles were removed and their carcasses washed in 10% hydrogen peroxide for 10 s to remove external bacteria and DNA contamination. According to species and location, two to three pools of beetles containing three to five beetles each were formed and then freeze-dried overnight.

DNA Extraction, PCR Amplification and Next Generation Sequencing. DNA was extracted from beetle pools using PowerSoil DNA Isolation Kits (MoBio Laboratories Inc., Carlsbad) per manufacturer's directions. A segment of the

conserved bacterial 16S rRNA gene was amplified using universal 779F/1115R universal primers that flank the 16S rRNA V5 region (Redford et al., 2010). These primers also contained a unique 12-nucleotide Golay ‘‘barcode’’ for each sample that allowed us to pool the PCR products from all the samples into one Illumina MiSeq sequencing run. PCR reactions were conducted using Taq98® Hot Start 2X Master Mix (Lucigen, Middleton, WI) with primer concentrations at 0.2 μM. PCR cycling conditions were: denaturation at 98 °C for 2 min followed by thirty-five cycles of 98 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; then final extension at 72 °C for 10 min before holding the PCR product at 4 °C. The PCR products were visualized under UV light on 1% agarose gels stained with ethidium bromide before being normalized and sequenced on an Illumina MiSeq instrument by The Scripps Research Institute DNA Array Core Facility using their standard protocols (TSRI, San Diego).

Computational and statistical analyses. The sequence data was analyzed using the QIIME (Quantitative Insights Into Microbial Ecology) version 1.8.0 software program (Caporaso et al. 2010a). The sequence and mapping data files are available on Figshare: http://dx.doi.org/10.6084/m9.figshare.1553418, http://dx.doi.org/10.6084/m9.figshare.1554842, respectively. Raw sequence data was demultiplexed into samples by barcode and filtered by mean quality score below 25, homopolymers greater than 6, uncorrected barcodes, barcodes not found in the mapping file, chimeric sequences and mismatched primers. Each sequence was

grouped into operational taxonomic units (OTUs) at the 97% sequence similarity level using UCLUST (Edgar 2010) and a consensus taxonomic classification was assigned to each representative OTU using the UCLUST classifier with a Greengenes 13_8 reference database (DeSantis et al. 2006) in which at least 90% of the sequences within the OTU matched the consensus taxonomic classification 16S rRNA gene. Sequences were aligned using PyNAST (Caporaso 2010b) against the Greengenes 13_8 reference core set and a phylogenetic tree of the OTUs inferred using FastTree (Price, Dehal and Arkin 2009). In order to remove spurious OTU's and samples with low numbers of sequences, OTU's that occurred only once in the data and samples with less than 150 OTUs were removed. *Rickettsia* and *Wolbachia* taxonomic sequence identifications were crosschecked against the NCBI nucleotide database using BLAST.

After rarefying the OTU dataset to an even sampling depth of 8000, weighted and unweighted UniFrac distance measures between all pairs of microbial communities were calculated in order to cluster microbial communities based on their UniFrac distances and visualized by principal coordinate analyses (PCoA) (Lozupone and Knight 2005). *Rickettsia* and *Wolbachia* OTU sequences were aligned with other *Rickettsia* and *Wolbachia* 16S rRNA sequences found in the RDP Database (Cole et al. 2013) and aligned using MUSCLE in MEGA 6.01 software (Edgar 2004, Tamura et al. 2013). Maximum-likelihood phylogenetic trees were constructed using MEGA

6.01. Lastly, the *Rickettsia* and *Wolbachia* populations were compared to the non-*Rickettsia* and non-*Wolbachia* bacterial populations using a Procrustes analysis (Gower 1966) in QIIME to determine dependencies of the populations.

Results

All extracted beetle samples produced visible PCR products. After quality filtering the total number of sequences for all samples was 682,132 with sample depths ranging from 8,891 to 79,218 sequences per sample (Supplemental table 1.1). Three hundred seventy-three taxa, including one unassigned taxon, were identified (Supplemental table 1.2). Interestingly, *Rickettsia* and *Wolbachia* genera were the most abundant genera in the beetles, representing 43.2%% and 41.4%% of all taxa, respectively. In individual pooled samples, however, *Rickettsia* or *Wolbachia* constituted up to 85% (PR11WH) and 95% (ME13SB) of the total microbiome, respectively (Supplemental table 1.2). The vast majority of the two genera were comprised of just five OTUs: denovo561498 and denovo338079 comprised 87.4% of the total *Rickettsia* OTUs; and denovo68494, denovo54077, and denovo196440 comprised 66.7% of all *Wolbachia* OTUs (Supplementary table 1.2). All other *Rickettsia* and *Wolbachia* OTUs were 100 to 10,000 times less abundant than the five dominant OTUs (Figures 1.1.A & 1.1.B). Other genera and family present in quantities of at least 1% of the microbiome were *Gluconacetobacter* (2.2%),

Streptococcus (2.0%), *Rickettsiaceae* (non-*Rickettsia* or *Wolbachia*) (1.7%), and *Lactococcus* (1.1%) (Supplemental table 1.2). All other taxa comprised less than 1% of the total microbiome.

Rickettsia and *Wolbachia* were negatively correlated in the beetles (Pearson product-moment correlation coefficient after log transformation R=-0.534, p=0.033). The proportion of *Rickettsia* and *Wolbachia* in *L. melanocephalus* and *L. pratensis*, in particular, were highly negatively correlated (R=-0.809, p<0.001; Figure 1.2).

GenBank BLAST searches of the predominant *Rickettsia* and *Wolbachia* OTU sequences revealed 99-100% similar identities to 16S rRNA gene sequences of *Rickettsia* and *Wolbachia* endosymbionts of other insects including *Curculio* sp. and *Sitona obsoletus* (*Rickettsia* OTU 561498), *Propylea japonica*, *Coccotrypes dactyliperda*, and *Onychiurus sinensis* (*Rickettsia* OTU 338079), *Hyposoter horticola*, *Drosophila simulans* and *Kleidocerys resedae* (*Wolbachia* OTU 68494), *Cosmoscarta heros* and *Curculio okumai* (*Wolbachia* OTU 54077), and *Diprion pini*, *Drosophila simulans* and *Curculio hachijoensis* (Wolbachia OTU 96440) (Table 1.2). The partial 16S rRNA gene sequences of *Rickettsia* OTUs denovo561498 and denovo338079 and *Wolbachia* OTUs denovo68494, denovo54077, and denovo196440 were aligned to a spectrum of pathogenic and nonpathogenic *Rickettsia* and *Wolbachia* species from the

RDP database using MUSCLE as implemented by MEGA 6 software. Phylogenetic trees were constructed using a maximum likelihood Kimura 2-parameter model with gamma distribution (K2+G) (Figures 1.3 and 1.4, respectively).

We found significant dissimilarity between the microbial community diversity of the three different beetle species as measured by weighted and unweighted UniFrac distances and visualized by PCoA plots (Figures 1.5.A & 1.5.B, respectively). The UniFrac distance measures the combined phylogenetic distances of microbes within a community and enables comparisons of the overall diversity between different bacterial communities. Analysis of similarity (ANOSIM) of weighted and unweighted UniFrac distances between the beetle species was R=0.42, p=0.005; and R=0.65, p=0.001, respectively. This difference was due primarily to the *Rickettsia* and *Wolbachia* OTUs rather than the rest of the microbiome; weighted and unweighted ANOSIM R results were 0.667 and 0.452, p=0.001, respectively, when only *Rickettsia* and *Wolbachia* OTUs were analyzed. When the two highest abundance *Rickettsia* OTUs and three highest abundance *Wolbachia* OTUs were analyzed together without the lower abundance *Rickettsia* and *Wolbachia* OTUs, weighted ANOSIM results remained significant R=0.454, p=0.004, but unweighted ANOSIM results were not statistically significant. Therefore, the *Rickettsia* and *Wolbachia* OTUs are associated with insect species while the remaining bacterial taxa are not associated with either insect species or location. In contrast, weighted and unweighted ANOSIM

comparisons of non-*Rickettsia* and non-*Wolbachia* communities between beetle species were not statistically significant. Furthermore, comparisons of the UniFrac distances of total microbial communities, *Rickettsia* and *Wolbachia* microbial communities, and non-*Rickettsia* non-*Wolbachia* microbial communities by location or collection date were not significantly different. Procrustes analysis of both weighted and unweighted *Rickettsia* plus *Wolbachia* communities in the beetles compared to beetle microbiomes without *Rickettsia* and *Wolbachia* revealed that the microbial community diversity was more dependent on the beetle species in which they occurred than each other ($p=0.672$, $M^2=0.918$, $p=0.205$, $M^2=0.807$, weighted and unweighted Procrustes comparisons, respectively, Figure 1.6).

Discussion

The goals of this study were to elucidate the microbiomes of three different *Longitarsus* beetle species in three different regions of Germany and to determine the respective roles that geographic locale and beetle host species play in shaping the beetles' microbial community diversity. The beetles were collected from the same species of plant host at each location to negate the effect of plant host variation on beetle microbiomes. The predominant bacteria detected belonged to the Rickettsiaceae family with the most common genera being *Rickettsia* and *Wolbachia* that combined

comprised 45-99% of the bacterial microbiome (Figures 1.1 and 1.2). This is in contrast to the findings of our prior study of 11 different *Longitarsus* species that fed on five different plant species (Kelley and Dobler 2011). In this prior study, Enterobacteriaceae was found to be the most common family in the beetle species overall with *Rickettsia* and *Wolbachia* contributing lesser quantities to the microbiomes. The difference in genera prevalence in the earlier study may have been a factor of different beetle species assayed or, perhaps more likely, the bias introduced by using a different extraction method and a different set of "universal" bacterial PCR primers (Frank et al. 2008, Klindworth et al. 2012, Winsley et al. 2012, Guo and Zang 2013). In the Kelley and Dobler study, the DNeasy Tissue Kit (Qiagen, Hilden, Germany) and universal primers 27F/338R were used to purify and amplify 16S rRNA, respectively. This combination of methods resulted in over 50% of the DNA sequences amplified being chloroplast DNA. In order to avoid amplifying chloroplast DNA, we chose 779F/1115R universal primers that flank the 16S rRNA V5 region (Redford et al., 2010). The three different beetle species harbored different proportions of *Rickettsia* and *Wolbachia*. The pools of *L. luridis* contained an average of 30% *Rickettsia* and 50% *Wolbachia*, whereas *L. melanocephalus* had an average of 1% *Rickettsia* and 86% *Wolbachia* and *L. pratensis* had an average of 81% *Rickettsia* and 4% *Wolbachia* (Figure 1.2). *Rickettsia* and *Wolbachia* were strongly inversely proportional in *L. melanocephalus* and *L. pratensis* (Pearson product moment correlation coefficient after log transformation, $R=-0.809$, $p<0.01$). The results

indicate that although the same assortment of *Rickettsia* and *Wolbachia* species are present in all locales, their proportions are not population specific but, rather, are related to beetle species, much more so than geographic location or presence of other *Rickettsia* or *Wolbachia* bacteria. Determining whether competitive inhibition occurs between *Rickettsia* or *Wolbachia* within these *Longitarsus* bettles requires further investigation. Weighted and unweighted PCoA plots of the UniFrac phylogenetic distances of bacterial taxa within the beetles demonstrated association with beetle species both quantitatively and qualitatively (Figures 1.5.A & 1.5.B). Interestingly, the microbiome of *L. pratensis* (a *Plantago* specialist feeder) clusters most closely with *L. luridis* (a generalist feeder) rather than *L. melanocephalus* (also a *Plantago* specialist feeder). Although *L. pratensis* and *L. melanocephalus* both specialize on feeding on *Plantago lanceolata*, they represent independent evolutionary shifts to the same host plant. This further supports the conclusion that microbial community diversity in *Longitarsus* beetles is more shaped by beetle species than the host plant.

Rickettsia and *Wolbachia* are obligate intracellular bacteria that can be found in many arthropods and can sometimes be found co-infecting the same insect host (Weinert et al. 2009; Skaljac et al. 2010). Co-infection with multiple species of the same genus has been documented in other insects, such as *Wolbachia* in hemipterans and *Rickettsia* in ticks (Burgdorfer 1988, Macaluso et al. 2002, Kikuchi and Fukatsu 2003, Bing et al. 2014). In the case of *Wolbachia*, *Wolbachia* was found both within

and outside of bacteriocytes suggesting that the *Wolbachia* species were transmitted both vertically and horizontally. In the *Altica* leaf beetle genus, horizontal transmission was found to be the predominant mode of *Wolbachia* transmission (Jäckel et al. 2013). However, in ticks co-infected with two different *Rickettsia* species, infection with one *Rickettsia* interfered with a second *Rickettsia* from infecting the ovaries and prevented vertical transmission. In contrast to *Altica* flea beetles, which are notorious for their female biased sex ratio and heavily infected with *Wolbachia* (Jäckel et al. 2013), sex ratios in *Longitarsus* are not clearly skewed towards females (S. Dobler, personal observation). Given the effects of some *Rickettsia* and *Wolbachia* species on their insect hosts' fitness, reproduction and speciation, and the fact that they dominate the microbiome of *Longitarsus* beetles, their possible effects on their host beetles is intriguing and remains to be further explored (Sakurai et al. 2005, Werren, Baldo and Clark 2008, Zilber-Rosenberg and Rosenberg 2008).

The *Rickettsia* and *Wolbachia* endosymbionts of *Longitarsus* are polyphyletic, (Figures 1.3 & 1.4). Rickettsia OTU 561498 shares a most recent common ancestor with *Rickettsia* from other Coleoptera insects. In contrast, *Rickettsia* OTU 338079 shares a common ancestor with *Rickettsia canadensis*, a *Rickettsia* found in rabbit ticks and occasionally infecting humans (Eremeeva et al. 2005). Similarly, *Wolbachia* OTUs in the beetles demonstrate multiple ancestries whose descendants have been

found in Coleopteran and Hemipteran insects. Interestingly, one study of *Rickettsia* in whiteflies demonstrated *Rickettsia* transmission between whiteflies and a plant intermediate host with the *Rickettsia* transiting through the phloem of infected plants (Caspi-Fluger 2011). This raises a putative novel mechanism for horizontal transmission of *Rickettsia* and perhaps, other endosymbiotic bacteria, between herbivorous arthropods through the plants they feed upon.

In terms of the rest of the non-*Rickettsia* non-*Wolbachia* microbiome, the most abundant genera were *Gluconacetobacter*, *Streptococcus* and *Lactococcus*. These genera have been found in the guts of other insects, including Coleoptera, and are thought to play a role in digestion (Martin and Mundt 1972; Crotti et al. 2010; Tagliavia et al. 2013). In contrast to *Rickettsia* and *Wolbachia*, beetle species does not appear to be the significantly associated with the diversity of these other bacterial genera. However, due to the difficulty in collecting beetle samples, and their relatively small size, higher numbers of beetles were not collected or tested individually that would have allowed finer resolution of specific geographic location influence on individual beetle species' microbiomes.

Procrustes comparison of *Rickettsia* and *Wolbachia* components of the beetle microbiomes to beetle microbiomes without *Rickettsia* and *Wolbachia* demonstrated

that *Rickettsia* and *Wolbachia* populations are more associated with beetle species rather than to each other (Figure 1.6). This is congruent with previous studies that have demonstrated the dominant role of host species in dictating *Wolbachia* infection densities (Poinsot et al. 1998, Kondo, Shimada and Fukatsu 2005). Our previous study (Kelley and Dobler 2011) also gave some indication that host plant species might influence the gut community in oligophagous *Longitarsus* species, yet this effect was not the subject of the present study design.

Taken as a whole, the results of this study indicate that dominant endosymbiotic microbial communities within *Longitarsus* beetles are driven by host species rather than geographic location or inter-bacterial interactions. Furthermore, they are dominated by multiple, co-infecting *Rickettsia* and/or *Wolbachia* secondary endosymbionts that are derived from different phylogenetic ancestors and can comprise over 95% of a pooled beetle microbiome.

Acknowledgement

Chapter 1 was prepared as part of a submission for a broader publication that investigates the microbial ecology in a plant, insect, soil ecosystem. The dissertation

author was the primary investigator and author of this section of the paper. Its coauthors were Professor Susanne Dobler from Institute of Zoology, University of Hamburg, Hamburg, Germany and Professor Scott T. Kelley from the Department of Biology, San Diego State University, San Diego, CA, USA. I also thank Karin Meyer, Carsten Winkler and Aleksandra Tomic for help with extractions and PCR.

Table 1.1. Predominant *Rickettsia* and *Wolbachia* OTUs in *Longitarsus* flea beetles and percent similarity with *Rickettsia* and *Wolbachia* 16S rRNA sequences found in other insects.

OTU	Genus	GenBank Acc#	Endosymbiont host	Match (%)	GenBank Acc#
			Curculio* sp.	99	AB756411
561498	*Rickettsia*	KU253792	***Sitona obsoletus***	99	KJ494867
			Curculio aino	99	AB604674
			Propylea japonica	100	FN550103
338079	*Rickettsia*	KU253793	***Coccotrypes dactyliperda***	99	AY961085
			Onychiurus sinensis	99	AY712949
			Hyposoter horticola	100	KJ150624
68494	*Wolbachia*	KU253794	*Drosophila simulas*	100	CP003883
			Kleidocerys resedae	100	JQ726770
			Cosmoscarta heros	99	AB772264
54077	*Wolbachia*	KU253795	***Curculio morimotoi***	99	AB746400
			Curculio okumai	99	AB746402
			Diprion pini	99	HE814622
196440	*Wolbachia*	KU253796	*Drosophila simulans*	99	CP003884
			Curculio hachijoensis	99	AB746399

*Hosts in **BOLD** type are Coleoptera

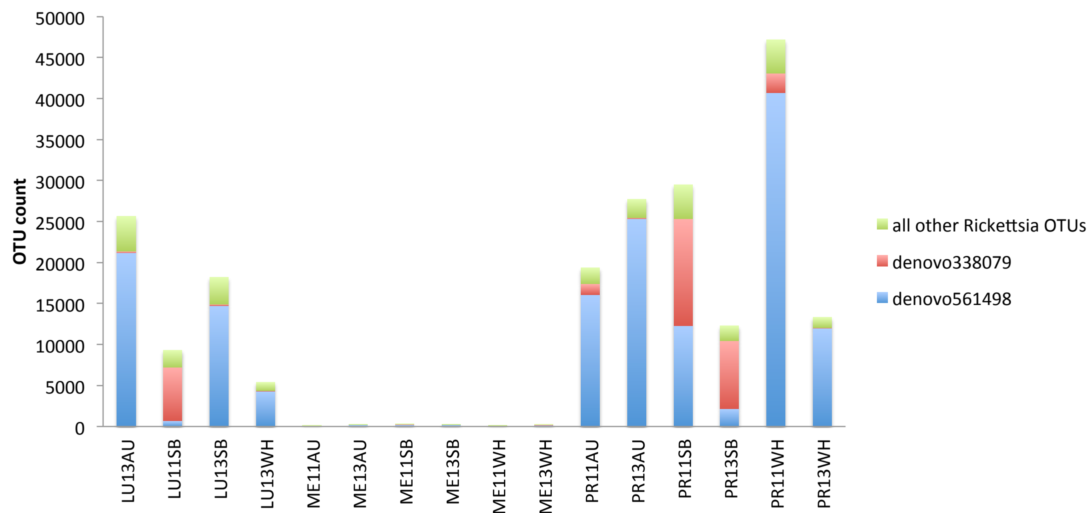

Figure 1.1.A. *Rickettsia* OTU abundance in flea beetle species. Eighty seven percent of all *Rickettsia* OTUs in *Longitarsus* beetles consist of denovoOTUs 561498 (blue) and 338079 (red). *L. luridis* and *L. pratensis* pools are dominated by OTU 561498, except for samples from Schönberg (LU11SB, PR11SB, and PR13SB). Sample names are abbreviated by two alphanumeric codes for species of beetle, followed by the year collected and the location. LU=*L. luridis*; ME=*L. melanocephalus*; PR=*L. pratensis*; 11=2013; 13=2013; AU= Aumühle; SB= Schönberg; WH=Westerhever.

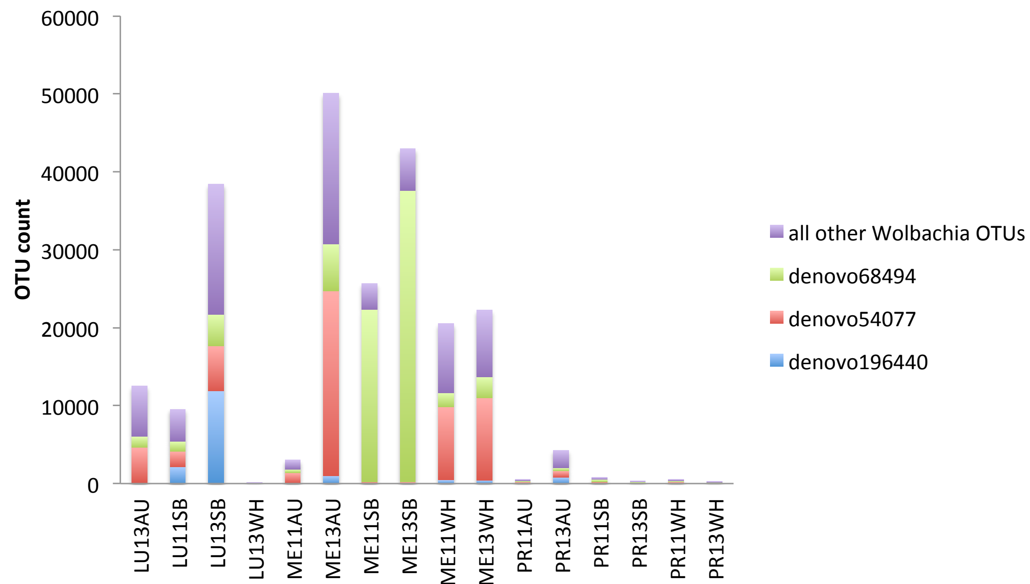

Figure 1.1.B. *Wolbachia* OTU abundance in flea beetle species. Sixty seven percent of all *Wolbachia* OTUs in the beetle pools consist of three OTUs: denovo68494 (green), denovo54077 (red), and denovo196440 (blue). Pools ME11SB and ME13SB are dominated by denovo68494. The Y-axis is the abundance of each OTU in each sample. Sample names are abbreviated by two alphanumeric codes for species of beetle, followed by the year collected and the location. LU=*L. luridis*; ME=*L. melanocephalus*; PR=*L. pratensis*; 11=2013; 13=2013; AU= Aumühle; SB= Schönberg; WH=Westerhever.

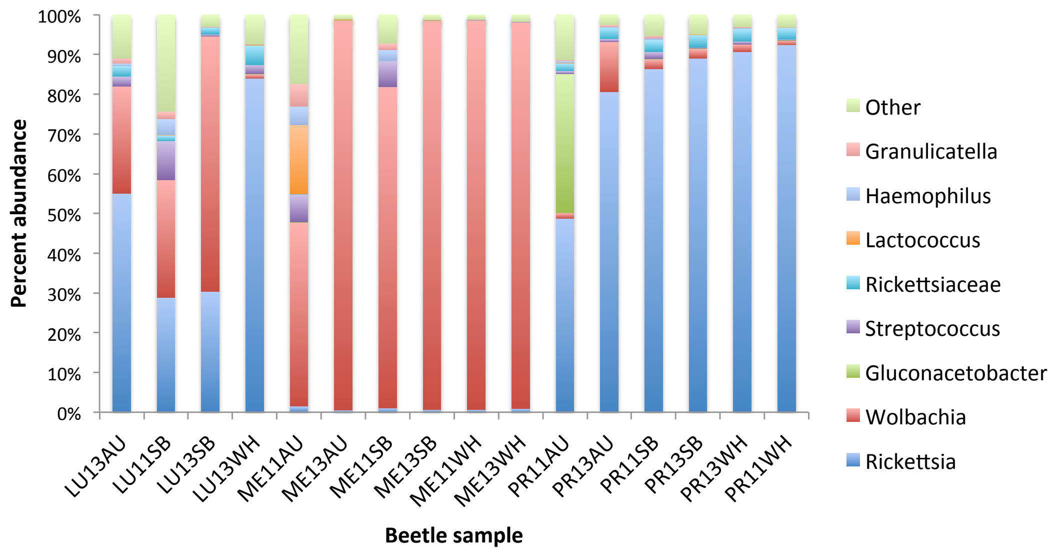

Figure 1.2. Relative abundances of *Rickettsia*, *Wolbachia* and six other most abundant overall bacterial taxa in *Longitarsus* beetle species as determined by 16S rRNA gene sequences. Sample names are abbreviated by two alphanumeric codes for species of beetle, followed by the year collected and the location. LU=*L. luridis*; ME=*L. melanocephalus*; PR=*L. pratensis*; 11=2013; 13=2013; AU= Aumühle; SB= Schönberg; WH=Westerhever.

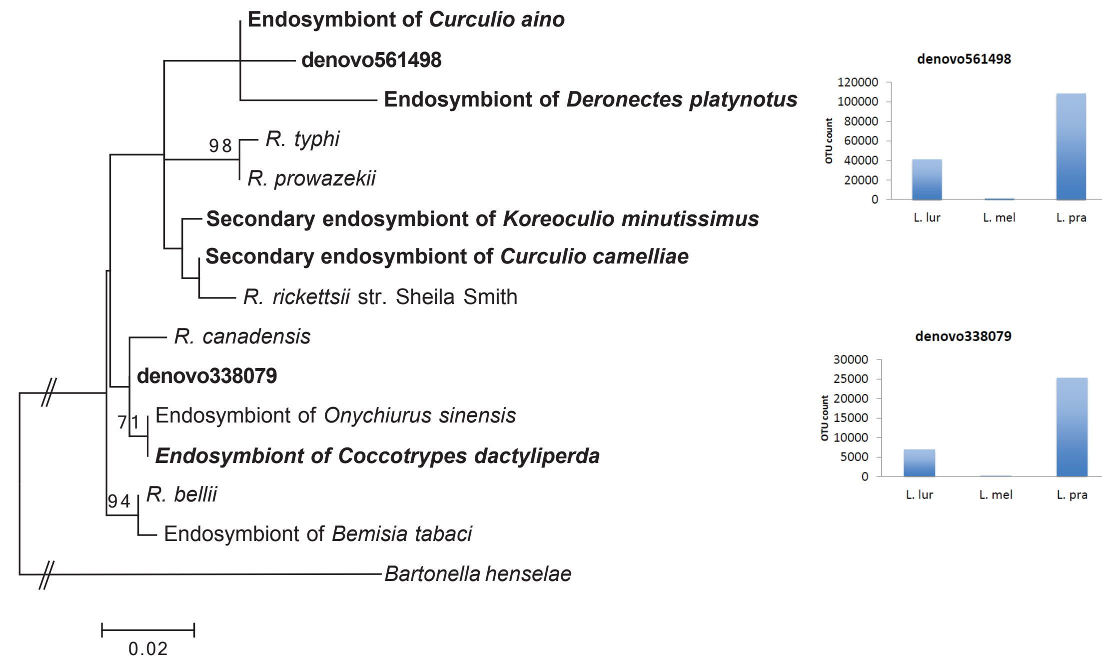

Figure 1.3. Phylogenetic tree of most abundant *Rickettsia* OTUs and their relationship with other *Rickettsia* endosymbionts and species. Names in bold face indicate species that were found in Coleoptera insects. Counts of the OTUs in different *Longitarsus* species are given at the right. The tree was constructed using a maximum likelihood Kimura 2-parameter model with gamma distribution. Numbers besides branches are percent bootstrap values above 70 after 1000 replications. L. lur=*L. luridis*; L. mel=*L. melanocephalus*; L. pra=*L. pratensis*.

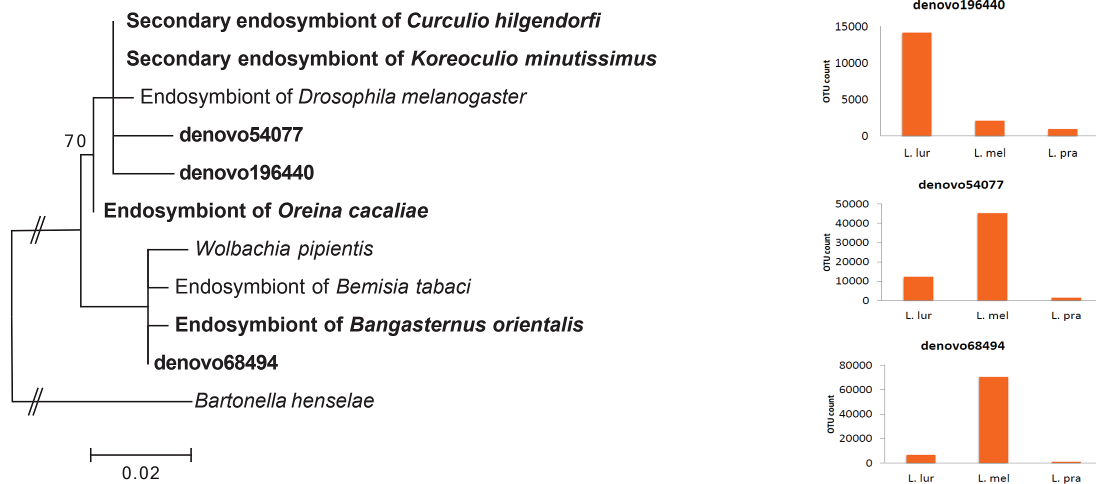

Figure 1.4. Phylogenetic tree of most abundant *Wolbachia* OTUs and their relationship with other *Wolbachia* endosymbionts and species. Names in bold face indicate species that are found in Coleoptera insects. Counts of the OTUs in different *Longitarsus* species are given at the right. The tree was constructed using a maximum likelihood Kimura 2-parameter model with gamma distribution. Numbers besides branches are percent bootstrap values above 70 after 1000 replications. L. lur=*L. luridis*; L. mel=*L. melanocephalus*; L. pra=*L. pratensis*.

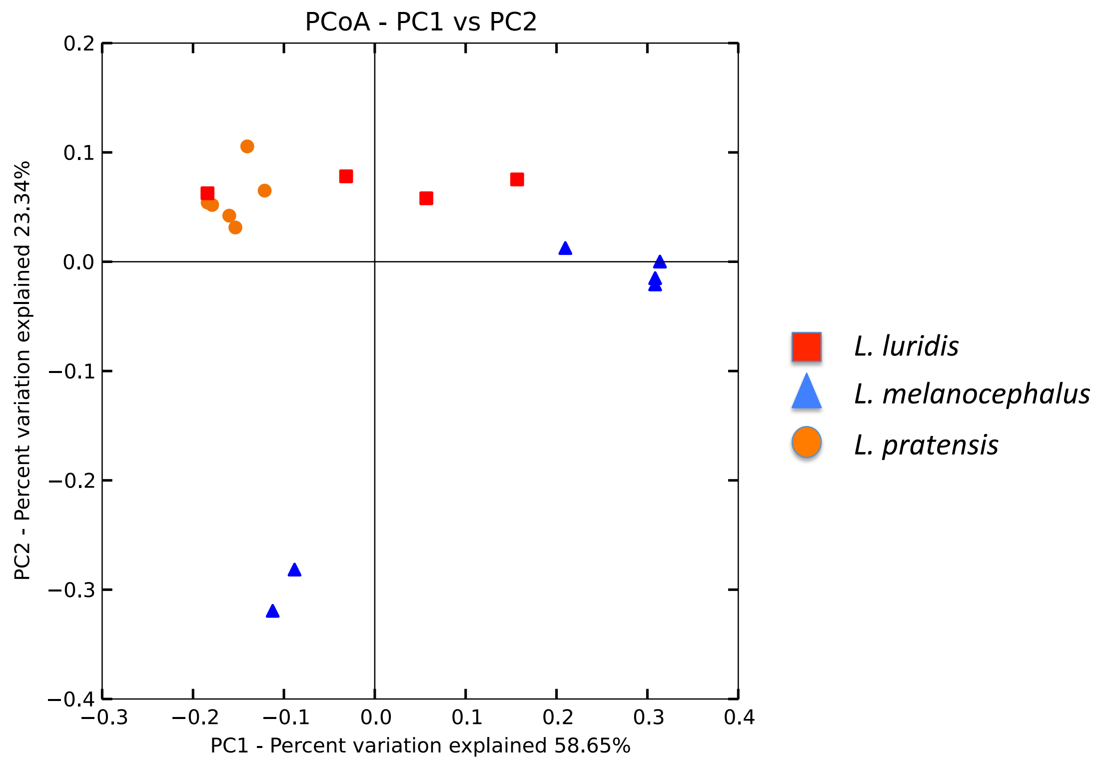

Figure 1.5.A. PCoA plot of weighted UniFrac distances of beetle microbiomes. Clustering of microbiome UniFrac measures on the plot by beetle species indicates that the beta diversity of the beetle microbiomes is associated with the beetle species in which they occur and that each beetle species's microbiome is different than the other species's (ANOSIM R=0.42, p=0.005). The two blue triangles in the PC1/PC2 negative quadrant are *L. melanocephalus* samples from Schönberg.

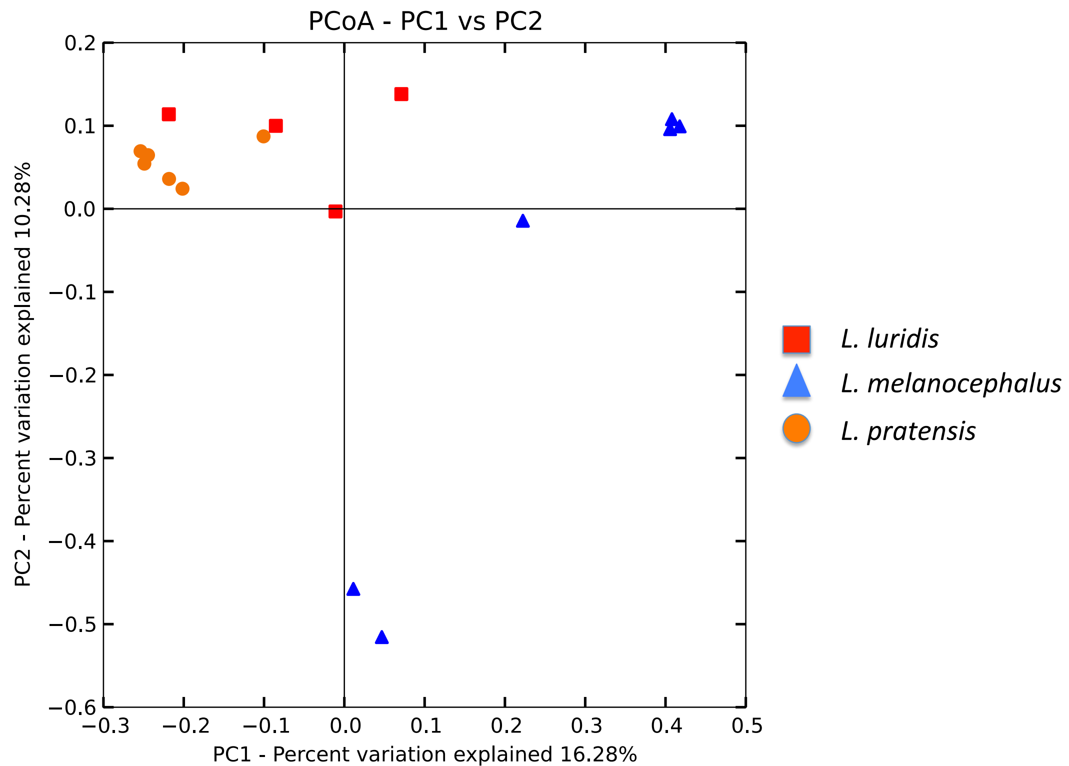

Figure 1.5.B. PCoA plot of unweighted UniFrac beetle microbiome distances. Clustering of microbiome UniFrac measures on the plot by beetle species indicates that the beta diversity of the beetle microbiomes is correlated with the beetle species in which they occur (ANOSIM R=0.65, p=0.001). The two blue triangles in the PC1 positive/PC2 negative quadrant are *L. melanocephalus* samples from Schönberg and Aumühle.

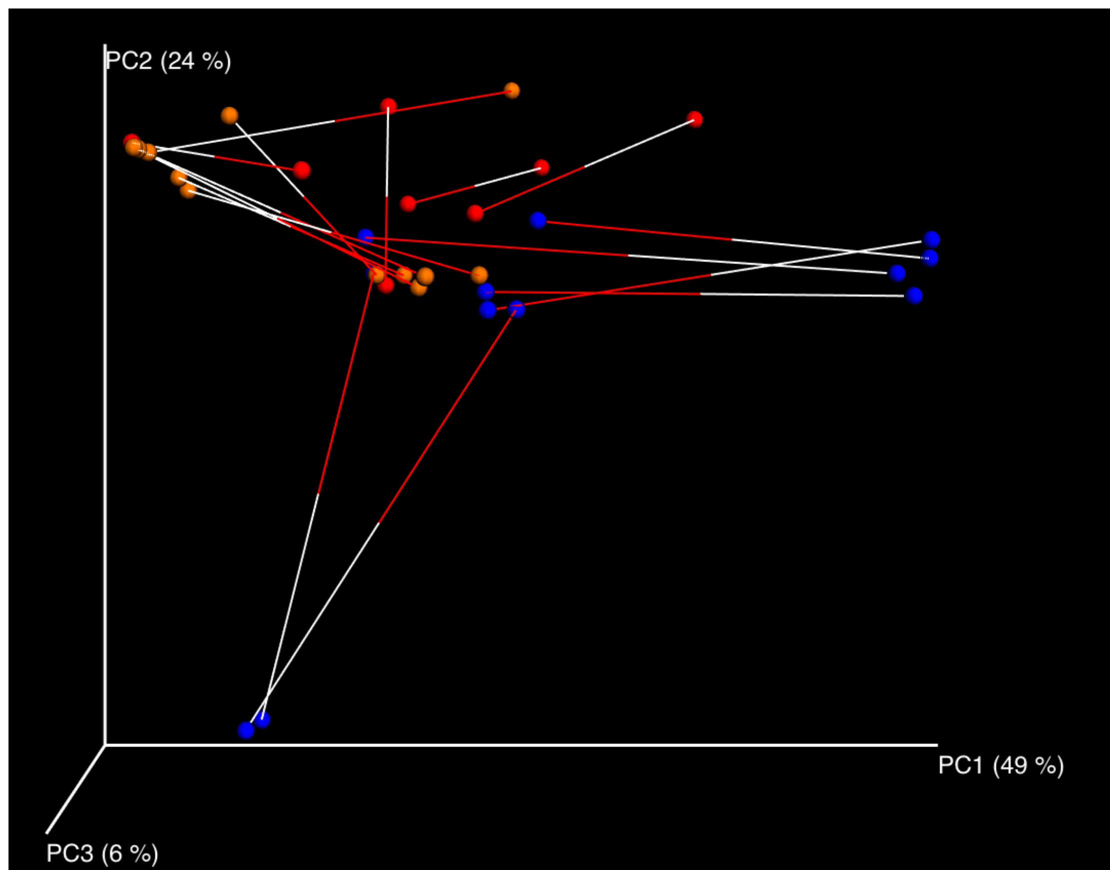

Figure 1.6. Procrustes comparison of weighted beetle *Rickettsia* and *Wolbachia* PCoA distributions (white end of bars) versus PCoA distribution of beetle microbiomes without *Rickettsia* and *Wolbachia* (red end of bars). There is a large shift in the distribution of the UniFrac distances on the three dimensional PCoA axes which indicates that the *Rickettsia* and *Wolbachia* microbiomes are not similarly associated with beetle species as the non-*Rickettsia* and non-*Wolbachia* microbiomes. PCoA = Principle Coordinate Analysis; red = *L. luridis*; blue = *L. melanocephalus*; orange = *L. pratensis*.

Supplemental table 1.1. Sample descriptions and number of 16S rRNA sequences generated per sample.

Sample name	Species	Year	Location	Sequences
LU13AU	*L. luridis*	2013	Aumühle	62196
LU11SB		2011	Schönberg	46899
LU13SB		2013	Schönberg	79218
LU13WH		2013	Westerhever	8891
ME11AU	*L. melanocephalus*	2011	Aumühle	10886
ME13AU		2013	Aumühle	64485
ME11SB		2011	Schönberg	45232
ME13SB		2013	Schönberg	53886
ME11WH		2011	Westerhever	29341
ME13WH		2013	Westerhever	29159
PR11AU	*L. pratensis*	2011	Aumühle	51727
PR13AU		2013	Aumühle	43430
PR11SB		2011	Schönberg	50105
PR13SB		2013	Schönberg	19901
PR11WH		2011	Westerhever	65704
PR13WH		2013	Westerhever	21072

Supplemental table 1.2. Percent abundance of taxa within samples.

Taxon	Total % in all samples	% of all taxa	PR11SB	LU13SB	PR13AU	ME11SB	PR11WH	ME11WH	ME13WH	ME13AU	ME11AU	LU13AU	LU11SB	PR11AU	PR13SB	ME13SB	PR13WH	LU13WH
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;g__Rickettsia	6.91341597	0.432088498	0.864291574	0.303974096	0.806567963	0.009786337	0.923818292	0.006301618	0.00894962	0.005053473	0.014777575	0.550427571	0.28826262	0.48757122	0.890451678	0.006515994	0.907108294	0.839558045
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;g__Wolbachia	6.616612778	0.413538299	0.023789992	0.641346619	0.12478548	0.808552818	0.01122451	0.979710698	0.971754126	0.981490187	0.463132236	0.268853938	0.2957572	0.014067463	0.023852679	0.978902761	0.018810059	0.010582011
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Gluconacetobacter	0.358195548	0.022387222	0.000849643	0.000317127	0.000319963	0.000346141	0.000724794	0.000190958	0.000436567	0.000430916	0.000457038	0.000386747	0.000836172	0.349972268	0.001740013	6.83E-05	0.000340762	0.000778089
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus	0.332605031	0.020787814	0.017988984	0.005691586	0.007562756	0.065042953	0.002056847	0.000859312	0.000829477	0.000568026	0.069622182	0.02516007	0.09764633	0.006806837	0.002175016	0.000592363	0.006815239	0.023187053
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;g__	0.278179269	0.017386204	0.029503106	0.017241667	0.028069461	0.000188804	0.028737096	0.000190958	0.000305597	0.000391742	0.000914077	0.026685574	0.013719418	0.019664196	0.031392735	0.000318965	0.031213794	0.049642079
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus	0.180757939	0.011297371	0.00058596	0.000233672	8.73E-05	0.000566412	0.000626849	0.000143219	0	0.00013711	0.174283973	0.000171888	0.001052958	0.000605052	0.000580004	6.83E-05	0.000681524	0.00093707
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Haemophilus	0.136514004	0.008532125	0.004218915	0.000250363	0.002065214	0.027691243	0.000215479	0.000238698	8.73E-05	0.000254632	0.046008531	0.005564866	0.041127284	0.005319417	0.000362503	0.000227832	0.002726096	0.000155618
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae;g__Granulicatella	0.122494479	0.007655905	0.006006094	0.000166909	0.005177579	0.015639259	0.000430958	0.000143219	0.000174627	0.000176284	0.056368068	0.013127927	0.018457727	0.003025261	0.000725005	0.000227832	0.002180876	0.000466853
Unassigned;Other;Other;Other;Other;Other	0.094791729	0.005924483	0.006855737	0.003755445	0.00305419	0.014349098	0.004368352	0.001623144	0.001353357	0.001566968	0.010359537	0.003652615	0.01551564	0.011622044	0.003552527	0.003713661	0.005247734	0.004201681
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;Other	0.094408558	0.005900535	0.000791047	0.000634253	0.00011635	0.000377608	0.000313424	9.55E-05	0.003317908	0.000254632	0.001066423	0.000128916	0.08073707	0.00597489	0.000217502	9.11E-05	0.000136305	0.000155618
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Staphylococcus	0.05954594	0.003721621	0.000175788	6.68E-05	0.000378138	0.003776079	0.000293835	9.55E-05	0	0	0.00274223	0.003867475	0.047321152	0.000252105	0.000145001	9.11E-05	0.000340762	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae;g__Propionibacterium	0.049587097	0.003099194	0.001025431	0.001385342	0.001454376	0.000975487	0.000959862	0.000572874	4.37E-05	9.79E-05	0.032906764	0.000193374	0.008268814	0.000352947	0.000217502	9.11E-05	0.000885981	0.000155618
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__	0.045324793	0.0028328	0.005683816	0.001435415	0.001977952	0.002643255	0.001919724	0.001336707	0.001615297	0.0012144	0.005789153	0.004748399	0.003561474	0.003302577	0.004640035	0.001116377	0.002317181	0.002023031
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__	0.035437529	0.002214846	0.002226649	0.004890424	8.73E-05	0.000409075	0.006758213	0.000143219	0.001484327	0.000156697	0.002285192	0.000236346	0.004614432	0.009403519	0.002537519	6.83E-05	0.000136305	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	0.035085502	0.002192844	0.001962967	0.00327141	0.000901713	0.000220271	5.88E-05	0.000143219	8.73E-05	7.83E-05	0.024223035	0.002621288	0.000526479	0.000226895	0.000290002	4.56E-05	0.00027261	0.000155618
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;Other	0.034675457	0.002167216	8.79E-05	1.67E-05	8.73E-05	9.44E-05	9.79E-05	0	0	7.83E-05	0	2.15E-05	6.19E-05	0.033555186	0.000217502	4.56E-05	0	0.000311236
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae];g__Chryseobacterium	0.030513171	0.001907073	0.000498066	0.000150218	0.001338026	0.00094402	0.000215479	0.000238698	8.73E-05	0.000156697	0.005179768	0.00021486	0.000371632	0.000403368	0.000652505	0.000113916	0.000340762	0.019607843
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas	0.023519619	0.001469976	0.001962967	0.000600871	0.000901713	0.000755216	0.000881506	0.000716093	0.000436567	0.000509265	0.003808653	0.002836148	0.002725302	0.002016841	0.001957515	0.000364531	0.002112724	0.00093707
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Rothia	0.023275732	0.001454733	0.001728583	8.35E-05	0.000203613	0.002202712	5.88E-05	0	0	5.88E-05	0.006246191	0.000644579	0.010684422	0.000378158	0.000217502	0	0.000613371	0.000155618
k__Bacteria;p__Firmicutes;c__Bacilli;o__Gemellales;Other;Other	0.019713167	0.001232073	0.001084027	8.35E-05	5.82E-05	0.002832059	0	4.77E-05	4.37E-05	0.000509265	0.008379037	0.005178119	0.000185816	0.000655473	7.25E-05	0	0.00027261	0.000311236
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium	0.018921311	0.001182582	0.001347709	0.001318579	0.00058175	0.000597879	0.000744383	0.000525135	0.00078582	0.000568026	0.001828154	0.002492372	0.001176835	0.001184894	0.00398753	0.000410098	0.000749676	0.000622471
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__	0.015576532	0.000973533	0.000908239	0.000367199	0.000378138	0.000534944	0.000430958	0.000238698	0.00013097	0.000176284	0.000914077	0.002105625	0.000526479	0.000554631	0.000580004	0.000182266	0.000545219	0.0070002801
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus	0.015576311	0.000973519	0.000527364	0.000267054	0.000319963	0.000566412	0.000313424	0.000286437	0.00026194	0.000117523	0.001218769	0.009733144	0.000433571	0.000378158	0.000652505	9.11E-05	0.000408914	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;Other;Other	0.013543979	0.000846499	0.001025431	3.34E-05	8.73E-05	0.002422984	5.88E-05	0	0	1.96E-05	0.005332115	0.000386747	0.003127903	0.000453789	0.000145001	2.28E-05	0.00027261	0.000155618
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__	0.011373145	0.000710822	0.001054729	0.00038389	0.000959888	0.00223418	0.000372191	0.000238698	0.000349253	0.000215458	0.000609385	0.000472691	0.000650356	0.000857157	0.000870007	0.000159482	0.000545219	0.00140056

Supplemental table 1.2. Continued.

k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Erwinia	0.011117459	0.000694841	0.000615258	0.003955736	8.73E-05	9.44E-05	7.84E-05	0	0	9.79E-05	0.000609385	6.45E-05	0.002322701	0.000907578	0.002102516	4.56E-05	0.000136305	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__	0.010897336	0.000681084	0.000380874	0.000116836	2.91E-05	0.000157337	0.000235068	4.77E-05	0.000174627	0.00013711	0.000457038	0.002578316	0.002013007	0.000529421	0.001160009	0.000478447	6.82E-05	0.000233426 7
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;Other	0.010403473	0.000650217	0.00029298	5.01E-05	0.000145438	0.000188804	5.88E-05	4.77E-05	0.000174627	1.96E-05	0.000304692	0.000171888	0.003995045	0.000252105	0	9.11E-05	0.000408914	0.000420168 1
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Shewanellaceae;g__Shewanella	0.009973508	0.000623344	2.93E-05	3.34E-05	2.91E-05	3.15E-05	7.84E-05	0	4.37E-05	1.96E-05	0	0	3.10E-05	0.009605203	7.25E-05	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Microbacterium	0.009344523	0.000584033	0.000205086	5.01E-05	2.91E-05	0.000157337	0.000156712	4.77E-05	0.00078582	5.88E-05	0.001371115	0.002298999	0.000371632	0.000327737	0.000725005	0.000113916	0	0.000264550 3
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Corynebacteriaceae;g__Corynebacterium	0.009313611	0.000582101	0.000380874	0.00020029	0.000290875	0.000314673	0.000783561	0	0	1.96E-05	0.001066423	0.000601607	0.003685352	0.000201684	0.000652505	0.001048027	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__	0.008716084	0.000544755	0.000322278	6.68E-05	5.82E-05	0.000125869	9.79E-05	0.000143219	0	0.000117523	0	0.006230931	0.000309693	0.000151263	0.000580004	4.56E-05	0	0.000046685 3
k__Bacteria;p__TM7;c__TM7-3;o__;f__;g__	0.007935219	0.000495951	0.000908239	0.000216981	0.000174525	0.000597879	0.000430958	0.000381916	0.00026194	0.000332981	0.000609385	0.000472691	0.000743264	0.000479	0.000942507	0.000205049	0.001022286	0.000015561 8
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Aerococcaceae;g__Facklamia	0.007515989	0.000469749	0.00029298	0	0	3.15E-05	0	0	0	0	0	0.001826307	3.10E-05	5.04E-05	0.005147539	0	0.000136305	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Photobacterium	0.007513842	0.000469615	0.000380874	1.67E-05	5.82E-05	9.44E-05	3.92E-05	0	0	1.96E-05	0.000152346	8.59E-05	0.001207804	0.004890839	0	2.28E-05	0.000545219	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Rhodoplanes	0.007465511	0.000466594	0.000556662	0.000250363	0.0002327	0.001132824	0.000293835	9.55E-05	0.000305597	0.000176284	0.000152346	0.000794981	0.001362651	0.000403368	0.000942507	0.000113916	0.000340762	0.000031123 6
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;Other	0.007024842	0.000439053	0.000351576	6.68E-05	0.0001635	0.000409075	0.000470137	9.55E-05	0.000218283	0.000117523	0.001371115	0.000644579	0.000650356	0.000252105	0.0013 0501	2.28E-05	0	0.000093370 7
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus	0.00678344	0.000423965	5.86E-05	3.34E-05	2.91E-05	0.001353095	1.96E-05	0	0	0	0.000914077	0.004146792	0	5.04E-05	0	2.28E-05	0	0.000015561 8
k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__;g__	0.006598673	0.000412417	0.00058596	0.000150218	0.000174525	0.000251739	0.000215479	0.000190958	0.00013097	0.000235045	0.000457038	0.00111727	0.001300712	0.000378158	0.000580004	9.11E-05	0.00027261	0.000046685 3
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Flavobacterium	0.006272874	0.000392055	2.93E-05	0	0	0	3.92E-05	0	0	1.96E-05	0.006093845	4.30E-05	0	2.52E-05	0	2.28E-05	0	0
k__Bacteria;p__TM7;c__TM7-1;o__;f__;g__	0.006208587	0.000388037	0.000234384	3.34E-05	8.73E-05	0.005003304	7.84E-05	0	0	7.83E-05	0.000152346	0.00010743	0.000216785	0.000126053	0	2.28E-05	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;Other;Other;Other	0.005471042	0.00034194	5.86E-05	3.34E-05	0	0.000188804	0	0	0.000742164	0	0.000609385	2.15E-05	0.000774234	0.00297484	0	0	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Janthinobacterium	0.005261637	0.000328852	0.00014649	1.67E-05	2.91E-05	0.002391516	3.92E-05	0	0	0	0	0.002191569	0.000123877	5.04E-05	0	6.83E-05	0.000204457	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae;g__	0.004873206	0.000304575	0.000322278	0.000116836	0.000174525	0.000188804	0.00019589	0.000190958	0.000218283	0.00013711	0.000304692	0.000236346	0.001610406	0.000352947	0.000217502	2.28E-05	0.00027261	0.000031123 6
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__BD7-3;f__;g__	0.004833709	0.000302107	2.93E-05	0	5.82E-05	0.004562762	1.96E-05	4.77E-05	0	0	0	0	0	2.52E-05	0	2.28E-05	6.82E-05	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Micrococcus	0.004674391	0.000292149	0	0.000116836	0.000727188	0.000409075	0.000293835	4.77E-05	0	0	0.001371115	0	0.001455559	2.52E-05	0	0.000227832	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae;Other	0.004495701	0.000280981	0.000703152	0	0.000145438	0.002171245	1.96E-05	0	0	1.96E-05	0.000609385	8.59E-05	0.000247755	0.000201684	0	0	0.000136305	0.000015561 8
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Flavisolibacter	0.0042527	0.000265794	0.000234384	6.68E-05	0	9.44E-05	3.92E-05	4.77E-05	0.00013097	0.000117523	0.002894576	0.000365261	9.29E-05	0.000100842	0	0	6.82E-05	0
k__Bacteria;p__TM7;c__;o__;f__;g__	0.004248243	0.000265515	0.000556662	0.00020029	8.73E-05	0.000409075	0.000156712	9.55E-05	4.37E-05	0.000235045	0.000304692	0.00021486	0.000681325	0.000327737	0.000435003	0.000227832	0.00027261	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Candidatus Regiella	0.004014497	0.000250906	0	0	0	0	0	0	0	0	0	0	0.003964076	5.04E-05	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Hymenobacter	0.004008401	0.000250525	0.000234384	0.000116836	5.82E-05	0.000629346	9.79E-05	0.000190958	0	9.79E-05	0.000152346	0.001074298	0.000278724	0.000176474	0.000362503	2.28E-05	0.000204457	0.000031123 6
k__Bacteria;p__Chloroflexi;c__Chloroflexi;o__[Roseiflexales];f__[Kouleothrixaceae];g__	0.003893647	0.000243353	5.86E-05	1.67E-05	0	9.44E-05	1.96E-05	0	0.00013097	3.92E-05	0	0	9.29E-05	0.000100842	7.25E-05	0	0	0.000326797 4
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Frigoribacterium	0.003892212	0.000243263	5.86E-05	1.67E-05	0	3.15E-05	0	0	4.37E-05	0	0.000152346	0	0	2.52E-05	7.25E-05	0	6.82E-05	0.000342359 2
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae;g__Mycobacterium	0.003785738	0.000236609	0.000263682	3.34E-05	8.73E-05	0.000220271	0.000137123	4.77E-05	8.73E-05	3.92E-05	0.000914077	0.001095784	0.00046454	0.000126053	0	4.56E-05	6.82E-05	0.000015561 8
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;g__Actinomyces	0.003713022	0.000232064	0.000175788	1.67E-05	0	0.000283206	0	0	0	0	0.001523461	0	0.001641375	0	7.25E-05	0	0	0

Supplemental table 1.2. Continued.

k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Rathayibacter	0.003573685	0.000223355	8.79E-05	0	2.91E-05	6.29E-05	0	0	0	1.96E-05	0	2.15E-05	6.19E-05	0	0	2.28E-05	0	0.0003267974
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	0.003541593	0.00022135	8.79E-05	0.000283745	0	9.44E-05	1.96E-05	0	0	0	0.001523461	0	0.000247755	5.04E-05	0.000145001	0	0	0.0001089325
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;Other	0.003521875	0.000220117	0.000410172	0.000166909	0.0002327	0.000125869	0.00019589	0.000143219	0.00013097	0.00013711	0.000152346	0.00010743	0.000371632	0.000277316	0.000797506	6.83E-05	0.000204457	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Curtobacterium	0.003483036	0.00021769	5.86E-05	1.67E-05	2.91E-05	6.29E-05	1.96E-05	0	0.000174627	0	0.000152346	0.002148597	0.000650356	2.52E-05	0.000145001	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Burkholderia	0.003368308	0.000210519	8.79E-05	1.67E-05	2.91E-05	6.29E-05	3.92E-05	0	4.37E-05	0.000156697	0	0.002492372	9.29E-05	5.04E-05	7.25E-05	6.83E-05	0	0.0000155618
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;Other	0.003336851	0.000208553	5.86E-05	3.34E-05	0	3.15E-05	0	0	0	1.96E-05	0.000152346	0.000386747	3.10E-05	0.000126053	7.25E-05	2.28E-05	6.82E-05	0.0002334267
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Pedobacter	0.003329418	0.000208089	0.00043947	0.000116836	0.0002327	0.000188804	0.000137123	9.55E-05	8.73E-05	0.000215458	0.000152346	0.000150402	0.000216785	0.000201684	0.000507504	9.11E-05	0.000340762	0.0000155618
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__Peptoniphilus	0.003281445	0.00020509	0	0	0	0	0.000450548	0	4.37E-05	0	0	0	0.002787241	0	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__	0.003224174	0.000201511	0.000263682	0.000100145	0.000290875	0.000188804	9.79E-05	0.000190958	0.00013097	0.00013711	0.000152346	0.000171888	0.000247755	0.000327737	0.000362503	0.000113916	0.000136305	0.0000311236
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__;g__	0.003127382	0.000195461	0.000351576	0.000116836	0.000378138	0.000220271	0.000176301	0.000190958	0	5.88E-05	0.000457038	0.000171888	0.000185816	0.000201684	7.25E-05	0.000136699	0.000408914	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Oceanospirillaceae;g__	0.003110739	0.000194421	2.93E-05	0	0	0	0	0	0	0	0	0	3.10E-05	0.003050471	0	0	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Gemellales;f__;g__	0.003064479	0.00019153	0.00014649	1.67E-05	2.91E-05	6.29E-05	0	4.77E-05	0	0	0.002589884	4.30E-05	3.10E-05	2.52E-05	7.25E-05	0	0	0
k__Bacteria;p__TM7;c__TM7-3;o__I025;f__;g__	0.003054582	0.000190911	0	0	0	0	0	0	0	1.96E-05	0	0	0.003034995	0	0	0	0	0
k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__Ellin515;g__	0.002917662	0.000182354	5.86E-05	0.000100145	2.91E-05	0.000157337	9.79E-05	0	0	1.96E-05	0	0.001181728	0.000247755	0.000126053	7.25E-05	0	0.000204457	0.0000622471
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__	0.002890001	0.000180625	0	5.01E-05	0	6.29E-05	0	0	0	1.96E-05	0.0019805	0.000365261	0.000154847	2.52E-05	7.25E-05	2.28E-05	0.000136305	0
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae;g__	0.002828202	0.000176763	0.000322278	3.34E-05	2.91E-05	6.29E-05	0	9.55E-05	4.37E-05	5.88E-05	0.000761731	0.000580121	0.000185816	0.000100842	0.000145001	6.83E-05	0.000340762	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Kaistobacter	0.002819248	0.000176203	8.79E-05	1.67E-05	5.82E-05	9.44E-05	3.92E-05	0	4.37E-05	3.92E-05	0.000152346	0.001912251	0.000154847	7.56E-05	0.000145001	0	0	0
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__MIZ46;f__;g__	0.002754883	0.00017218	0	0	5.82E-05	0	0	0	0	0	0	0	0.002601425	0	7.25E-05	2.28E-05	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Sporosarcina	0.002645901	0.000165369	2.93E-05	0	0	0	1.96E-05	0	0	1.96E-05	0	0.002384943	3.10E-05	2.52E-05	0	0	0.000136305	0
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__;g__	0.002523748	0.000157734	0.000380874	6.68E-05	0.000174525	0.000534944	5.88E-05	4.77E-05	8.73E-05	0.00013711	0.000152346	0.000300804	0.000154847	0.000100842	0.000145001	4.56E-05	0.000136305	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;Other;Other	0.002461982	0.000153874	0.000205086	3.34E-05	0.000407225	0.000125869	1.96E-05	0	0	0	0.000457038	0.000472691	0.000371632	0.000201684	0.000145001	2.28E-05	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__;g__	0.00242402	0.000151501	5.86E-05	3.34E-05	0	0.000157337	7.84E-05	0.000143219	4.37E-05	5.88E-05	0.000152346	0.000623093	9.29E-05	0.000176474	0.000290002	6.83E-05	0.000136305	0.0000311236
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__SC-I-84;f__;g__	0.002331593	0.000145725	0.000234384	5.01E-05	2.91E-05	3.15E-05	9.79E-05	0.000190958	4.37E-05	5.88E-05	0.000152346	0.000644579	0.000185816	0.000176474	7.25E-05	2.28E-05	0.000340762	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__	0.002320435	0.000145027	0.000117192	8.35E-05	5.82E-05	0.000440542	0.000137123	4.77E-05	4.37E-05	5.88E-05	0.000761731	8.59E-05	0.000216785	0.000151263	7.25E-05	4.56E-05	0	0
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Solirubrobacteraceae;g__	0.002291282	0.000143205	0	0	0	0	1.96E-05	0	0	0	0.001828154	2.15E-05	0.000371632	5.04E-05	0	0	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Alicyclobacillaceae;g__Alicyclobacillus	0.002247794	0.000140487	0	0	0	0	0	0	0	1.96E-05	0.000152346	0.002019681	3.10E-05	2.52E-05	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae;g__	0.00220489	0.000137806	0	0	0	0	1.96E-05	0	0	0	0.002132846	2.15E-05	3.10E-05	0	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Saprospiraceae;g__	0.002190197	0.000136887	8.79E-05	8.35E-05	5.82E-05	6.29E-05	9.79E-05	0.000143219	0.00013097	3.92E-05	0.000304692	0.00010743	0.000216785	0.000151263	0.000145001	4.56E-05	0.000204457	0.0000311236
k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__Solibacteraceae;g__Candidatus Solibacter	0.002184982	0.000136561	2.93E-05	5.01E-05	0.00011635	3.15E-05	7.84E-05	0	4.37E-05	3.92E-05	0.000152346	0.000558635	0.000247755	0.000226895	7.25E-05	2.28E-05	0.000204457	0.0000311236
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__	0.002150196	0.000134387	0.000234384	3.34E-05	0	0.000157337	0.000137123	0	4.37E-05	9.79E-05	0	0.000794981	0.000123877	7.56E-05	7.25E-05	0	6.82E-05	0.0000311236
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__	0.002141074	0.000133817	0	1.67E-05	0.000203613	0.001510431	0	4.77E-05	4.37E-05	0	0.000152346	4.30E-05	0	0.000100842	0	2.28E-05	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Caulobacter	0.002140436	0.000133777	8.79E-05	1.67E-05	2.91E-05	3.15E-05	3.92E-05	0	0	0	0	0.001718878	0.000123877	2.52E-05	0	0	6.82E-05	0

Supplemental table 1.2. Continued.

k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Renibacterium	0.002136565	0.000133535	2.93E-05	0.000367199	0	0	3.92E-05	0	0	0	0	0	3.10E-05	2.52E-05	0.000217502	0.000182266	0	0.001244942
k__Bacteria;p__Firmicutes;c__Bacilli;o__Gemellales;f__Gemellaceae;g__	0.002084603	0.000130288	8.79E-05	1.67E-05	0	3.15E-05	1.96E-05	0	0	0	0.000152346	6.45E-05	0.00142459	0.000151263	0	0	0.000136305	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__;g__	0.002079512	0.00012997	5.86E-05	1.67E-05	5.82E-05	0	1.96E-05	0	0	5.88E-05	0	0.00154699	0.000154847	2.52E-05	7.25E-05	0	6.82E-05	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Aerococcaceae;g__Aerococcus	0.002047961	0.000127998	8.79E-05	1.67E-05	0	0.000597879	1.96E-05	0	0	0	0.000761731	8.59E-05	0.000402601	7.56E-05	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Brevibacteriaceae;g__Brevibacterium	0.002045177	0.000127824	0	0	0.000261788	0.000188804	3.92E-05	0	0	0	0	0	0.000154847	0	0	0	0	0.001405056
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__;f__;g__	0.002015832	0.000125989	0.00029298	0	2.91E-05	0.000125869	0.000117534	9.55E-05	0.00013097	9.79E-05	0	0.000171888	0.000123877	0.000352947	0	6.83E-05	0.000408914	0
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__Finegoldia	0.002003512	0.000125219	0.000498066	0	0.001308939	3.15E-05	1.96E-05	4.77E-05	0	0	0	0	0	2.52E-05	7.25E-05	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Ellin6067;f__;g__	0.001997464	0.000124842	0.000351576	5.01E-05	2.91E-05	3.15E-05	7.84E-05	9.55E-05	8.73E-05	0	0.000152346	0.00010743	0.000247755	0.000302526	0.000217502	2.28E-05	6.82E-05	0.000155618
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;Other	0.001922706	0.000120169	5.86E-05	0	0	0.000534944	0	0	0	0	0.000914077	8.59E-05	0.000278724	5.04E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Rubellimicrobium	0.001911578	0.000119474	0	0	0	0	0	0	0	0	0.001828154	2.15E-05	6.19E-05	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Nocardioides	0.00190447	0.000119029	0.000205086	6.68E-05	2.91E-05	0	1.96E-05	0	0	7.83E-05	0.000914077	0	3.10E-05	0.000100842	0.000145001	2.28E-05	0.000136305	0.000155618
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__	0.00189279	0.000118299	2.93E-05	6.68E-05	8.73E-05	0.000566412	7.84E-05	4.77E-05	8.73E-05	3.92E-05	0	0.00021486	0.000185816	0.000126053	0	9.11E-05	0.00027261	0
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__Parvimonas	0.001889173	0.000118073	0	0	0	0	3.92E-05	0.000143219	0	0	0.001675807	0	3.10E-05	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Novosphingobium	0.001853936	0.000115871	0.000117192	0	5.82E-05	0	3.92E-05	4.77E-05	0	1.96E-05	0.000609385	0	0.000743264	0.000151263	0	0	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;Other	0.001816045	0.000113503	0.00029298	0.000100145	0.000174525	0	3.92E-05	0	0	3.92E-05	0	6.45E-05	0.000216785	0.000302526	0.000362503	0	6.82E-05	0.000155618
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Aurantimonadaceae;g__	0.001812388	0.000113274	2.93E-05	3.34E-05	8.73E-05	6.29E-05	9.79E-05	4.77E-05	8.73E-05	3.92E-05	0	0.000730523	0.000154847	0.000201684	0.000217502	2.28E-05	0	0
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae;g__Fusobacterium	0.001779196	0.000111 2	0.000117192	0	0	0	0	0	0	1.96E-05	0	0.001611448	3.10E-05	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Dialister	0.001732401	0.000108275	0	0	2.91E-05	0	0	0	0	0	0	0	0.001703314	0	0	0	0	0
k__Bacteria;p__Chloroflexi;c__TK10;o__AKYG885;f__Dolo_23;g__	0.001706263	0.000106641	5.86E-05	1.67E-05	0	0	1.96E-05	0	4.37E-05	0	0.001523461	2.15E-05	0	0	0	2.28E-05	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;g__Macrococcus	0.001689737	0.000105609	0	0	0	0	1.96E-05	4.77E-05	0	0	0	4.30E-05	0.001579436	0	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;Other	0.001634695	0.000102168	0.000175788	0	0.00011635	0.000125869	9.79E-05	0.000190958	4.37E-05	1.96E-05	0.000304692	0.00010743	0	7.56E-05	0.000217502	9.11E-05	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Shewanellaceae;Other	0.00161756	0.000101098	2.93E-05	0	0	0	0	0	0	0	0	0	0	0.001588262	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Kocuria	0.001595271	9.97044E-05	0	0	2.91E-05	3.15E-05	0	4.77E-05	0	0	0	0.00133213	0.000154847	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Salinivibrio	0.001595029	9.96893E-05	0.000117192	0	0	0.000125869	0	0	0	0	0.000761731	0	0.000216785	0.000100842	0	0	0.00027261	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Mycoplana	0.001577991	9.86244E-05	2.93E-05	0	2.91E-05	3.15E-05	3.92E-05	4.77E-05	0	0	0	0.000279318	0.000433571	2.52E-05	0.000507504	0	0	0.000155618
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Williamsiaceae;g__Williamsia	0.001563589	9.77243E-05	8.79E-05	5.01E-05	2.91E-05	0	0.000646438	0	4.37E-05	1.96E-05	0	2.15E-05	0	0.000252105	7.25E-05	0	0.000340762	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Rhizobium	0.001477001	9.23125E-05	0.000117192	6.68E-05	2.91E-05	9.44E-05	5.88E-05	4.77E-05	4.37E-05	5.88E-05	0.000304692	0.00010743	0.000185816	0.000176474	7.25E-05	4.56E-05	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__A21b;f__EB1003;g__	0.001396216	8.72635E-05	0.000175788	5.01E-05	8.73E-05	6.29E-05	3.92E-05	4.77E-05	4.37E-05	3.92E-05	0.000152346	8.59E-05	0.000154847	0.000126053	0.000217502	4.56E-05	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Ellin329;f__;g__	0.001395551	8.72219E-05	0.00014649	8.35E-05	5.82E-05	0.000125869	7.84E-05	0	0	1.96E-05	0	0.000279318	0.000154847	0.000126053	0.000145001	2.28E-05	0	0.000155618
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;Other;Other	0.001366322	8.53951E-05	0	1.67E-05	0.000145438	0.000849618	0	0	4.37E-05	0	0	0	6.19E-05	2.52E-05	0	0	6.82E-05	0.000155618
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__Parachlamydiaceae;Other	0.001299566	8.12229E-05	0	1.67E-05	5.82E-05	0	0	0	0	0	0	0.0012247	0	0	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae];g__Wautersiella	0.001297967	8.11229E-05	0	0	0	0	0.001253697	0	0	0	0	2.15E-05	0	0	0	2.28E-05	0	0
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Spirosoma	0.001273408	7.9588E-05	0.000205086	6.68E-05	0	3.15E-05	5.88E-05	9.55E-05	8.73E-05	1.96E-05	0	0.000193374	9.29E-05	5.04E-05	0.000145001	2.28E-05	0.000204457	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Methylibium	0.001272444	7.95278E-05	0.000117192	3.34E-05	5.82E-05	6.29E-05	0.000215479	9.55E-05	0	7.83E-05	0.000152346	4.30E-05	9.29E-05	5.04E-05	0	6.83E-05	0.000204457	0

Supplemental table 1.2. Continued.

k__Bacteria;p__Fibrobacteres;c__Fibrobacteria;o__258ds10;f__;g__	0.001269235	7.93272E-05	0	1.67E-05	2.91E-05	0	5.88E-05	0	0	0	0.000152346	0.000902411	6.19E-05	2.52E-05	0	2.28E-05	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Agrobacterium	0.001269178	7.93236E-05	0.00014649	1.67E-05	5.82E-05	9.44E-05	0.000117534	0	0	0	0	0.000386747	6.19E-05	0.000151263	0.000145001	2.28E-05	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__MND1;f__;g__	0.001238256	7.7391E-05	8.79E-05	5.01E-05	2.91E-05	9.44E-05	7.84E-05	0	0.00013097	3.92E-05	0	4.30E-05	0.000185816	5.04E-05	0.000290002	2.28E-05	0.000136305	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae;g__	0.00123137	7.69606E-05	5.86E-05	3.34E-05	2.91E-05	0	1.96E-05	9.55E-05	4.37E-05	0	0	0.000773495	6.19E-05	2.52E-05	0	2.28E-05	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Methylophilales;f__Methylophilaceae;g__	0.001221841	7.63651E-05	0.000205086	1.67E-05	0	6.29E-05	5.88E-05	4.77E-05	0.00013097	0	0.000152346	8.59E-05	0.000154847	2.52E-05	0.000145001	0	0.000136305	0
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__;g__	0.001182021	7.38763E-05	0.000117192	3.34E-05	2.91E-05	3.15E-05	3.92E-05	0	4.37E-05	0	0.000152346	0.000236346	3.10E-05	0.000176474	0	0	0.000136305	0.0000155618
k__Bacteria;p__Firmicutes;c__Bacilli;Other;Other;Other	0.001171613	7.32258E-05	5.86E-05	5.01E-05	0	0.000346141	0	4.77E-05	0	0	0.000304692	0.000171888	3.10E-05	2.52E-05	0	0	0.000136305	0
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Patulibacteraceae;g__	0.00113881	7.11756E-05	0	0	0	3.15E-05	0	0	0	0	0	0.000236346	9.29E-05	0	0	0	0	0.0000778089
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bacteriovoracaceae;g__	0.001135598	7.09749E-05	0.000175788	3.34E-05	8.73E-05	6.29E-05	1.96E-05	4.77E-05	0	1.96E-05	0	8.59E-05	6.19E-05	0.000100842	0.000145001	9.11E-05	0.000204457	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;Other	0.001130237	7.06398E-05	2.93E-05	0	0	0.000125869	1.96E-05	0	0	0	0.000761731	0	9.29E-05	0.000100842	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Alcaligenes	0.001112028	6.95018E-05	0	0.000484035	0	0	0	0	0	0	0	0.000472691	6.19E-05	2.52E-05	0	0	6.82E-05	0
k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__;g__	0.001108172	6.92607E-05	8.79E-05	0	0	3.15E-05	5.88E-05	4.77E-05	0	3.92E-05	0.000152346	6.45E-05	9.29E-05	0.000100842	7.25E-05	0	0.000204457	0.0000155618
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Veillonella	0.001098319	6.8645E-05	0.000234384	0	0.000378138	0.000283206	0	0	0	0	0	0	6.19E-05	0	7.25E-05	0	6.82E-05	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Frankiaceae;g__	0.001080363	6.75227E-05	0	0	0	0	0	4.77E-05	0	0	0	0.001009841	0	0	0	2.28E-05	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;Other	0.00104685	6.54281E-05	0	1.67E-05	0	6.29E-05	0.000215479	0	4.37E-05	0	0	0	3.10E-05	0	0.000290002	0.000318965	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingobium	0.001030359	6.43974E-05	5.86E-05	1.67E-05	0	0	0	0	0	0	0.000152346	2.15E-05	9.29E-05	2.52E-05	0.000507504	0	0	0.0000155618
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__	0.001028072	6.42545E-05	2.93E-05	0	0	3.15E-05	0	0	0	0	0	0.000794981	3.10E-05	5.04E-05	0	2.28E-05	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Paracoccus	0.001005113	6.28196E-05	0	0	0.000145438	0	9.79E-05	0	0	0	0.000761731	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__	0.00098018	6.12613E-05	0.000117192	1.67E-05	0	0.000440542	1.96E-05	0	4.37E-05	1.96E-05	0	4.30E-05	3.10E-05	2.52E-05	0	0	6.82E-05	0.0000155618
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Amaricoccus	0.000977335	6.10834E-05	2.93E-05	1.67E-05	0	0	0	0	0	0	0	0.000880925	0	5.04E-05	0	0	0	0
k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__[Pedosphaeraceae];g__Pedosphaera	0.000954203	5.96377E-05	2.93E-05	6.68E-05	0	6.29E-05	5.88E-05	0	4.37E-05	5.88E-05	0.000304692	4.30E-05	0	5.04E-05	0.000145001	2.28E-05	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Stenotrophomonas	0.000935531	5.84707E-05	0	0	2.91E-05	0	0	0	0	0	0	0.000515663	0.000123877	0.000126053	7.25E-05	6.83E-05	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__	0.000934405	5.84003E-05	0.000234384	0.000116836	8.73E-05	0.000125869	7.84E-05	0	4.37E-05	3.92E-05	0	8.59E-05	0	5.04E-05	7.25E-05	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Phenylobacterium	0.000933902	5.83689E-05	5.86E-05	1.67E-05	0	6.29E-05	0	0	0	3.92E-05	0.000152346	0.00032229	3.10E-05	0	7.25E-05	2.28E-05	0	0.0000155618
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae;g__	0.000921428	5.75893E-05	0.000175788	1.67E-05	0	0.000125869	1.96E-05	4.77E-05	8.73E-05	0	0	0	3.10E-05	7.56E-05	7.25E-05	4.56E-05	6.82E-05	0.0000155618
k__Bacteria;p__Acidobacteria;c__Acidobacteriia;o__Acidobacteriales;f__Koribacteraceae;g__	0.000921384	5.75865E-05	8.79E-05	0	2.91E-05	6.29E-05	0.000117534	0	8.73E-05	1.96E-05	0	0	0.000154847	0.000126053	0.000145001	9.11E-05	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__	0.000917808	5.7363E-05	5.86E-05	1.67E-05	8.73E-05	0	7.84E-05	4.77E-05	8.73E-05	1.96E-05	0	0	0.000185816	2.52E-05	0	0	0	0.0000311236
k__Bacteria;p__Proteobacteria;c__TA18;o__PHOS-HD29;f__;g__	0.000914077	5.71298E-05	0	0	0	0	0	0	0	0	0.000914077	0	0	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__EB1017;g__	0.000884832	5.5302E-05	8.79E-05	1.67E-05	5.82E-05	3.15E-05	1.96E-05	0	0	0	0	0.000343776	3.10E-05	0	7.25E-05	0	6.82E-05	0.0000155618
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptomycetaceae;g__Streptomyces	0.000881316	5.50822E-05	0.00014649	5.01E-05	0	0	5.88E-05	4.77E-05	0.00013097	1.96E-05	0	2.15E-05	6.19E-05	2.52E-05	7.25E-05	2.28E-05	6.82E-05	0.0000155618
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Ewingella	0.00087994	5.49963E-05	0	0	0	0	0	0	0	0	0	0	0	0.000857157	0	2.28E-05	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Dermabacteraceae;g__Brachybacterium	0.00087453	5.46581E-05	0	0.000817853	0	3.15E-05	0	0	0	0	0	0	0	2.52E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Devosia	0.000869183	5.4324E-05	2.93E-05	0	0	6.29E-05	0	4.77E-05	0	0	0.000152346	0.000472691	3.10E-05	5.04E-05	0	2.28E-05	0	0

Supplemental table 1.2. Continued.

k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__	0.00086236	5.38975E-05	0	5.01E-05	0	0	0	0	0	1.96E-05	0.000761731	0	3.10E-05	0	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Segetibacter	0.000838227	5.23892E-05	0	1.67E-05	0.000261788	3.15E-05	1.96E-05	0	0	0	0	2.15E-05	3.10E-05	0	0.000145001	0	0	0.000311236
k__Bacteria;p__Acidobacteria;c__Acidobacteria-5;o__;f__;g__	0.000837854	5.23659E-05	8.79E-05	0	5.82E-05	0	1.96E-05	0	4.37E-05	7.83E-05	0.000152346	6.45E-05	0.000123877	5.04E-05	0	2.28E-05	0.000136305	0
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__C111;g__	0.00083553	5.22207E-05	2.93E-05	3.34E-05	0	0	1.96E-05	0	0	1.96E-05	0.000304692	8.59E-05	0.000247755	0	7.25E-05	2.28E-05	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Enhydrobacter	0.000826525	5.16578E-05	0	0	2.91E-05	0	7.84E-05	4.77E-05	0	3.92E-05	0.000609385	0	0	0	0	2.28E-05	0	0
k__Bacteria;p__Acidobacteria;c__Acidobacteriia;o__Acidobacteriales;f__Koribacteraceae;g__Candidatus Koribacter	0.000825274	5.15796E-05	0	0	2.91E-05	0	0	0	0	0	0	0.000709037	6.19E-05	2.52E-05	0	0	0	0
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__Rhabdochlamydiaceae;g__Candidatus Rhabdochlamydia	0.000789906	4.93691E-05	2.93E-05	0	5.82E-05	0	0	0	4.37E-05	5.88E-05	0	2.15E-05	9.29E-05	7.56E-05	7.25E-05	4.56E-05	0.000136305	0.000155618
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;Other	0.000761731	4.76082E-05	0	0	0	0	0	0	0	0	0.000761731	0	0	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__Iamiaceae;g__Iamia	0.000760973	4.75608E-05	5.86E-05	0	0	3.15E-05	1.96E-05	9.55E-05	0	0	0.000152346	2.15E-05	3.10E-05	5.04E-05	0.000145001	0	0	0.000155618
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__	0.000753892	4.71182E-05	0	0	0	0.000125869	0	4.77E-05	0	0	0	4.30E-05	9.29E-05	7.56E-05	0.000145001	0	6.82E-05	0.000155618
k__Bacteria;p__Acidobacteria;c__Sva0725;o__Sva0725;f__;g__	0.000753365	4.70853E-05	5.86E-05	0	0	3.15E-05	3.92E-05	0	0	3.92E-05	0.000304692	2.15E-05	9.29E-05	2.52E-05	7.25E-05	0	6.82E-05	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae;g__Terracoccus	0.000721451	4.50907E-05	0	0	0.000639926	0	0	0	0	1.96E-05	0	0	6.19E-05	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Salinibacterium	0.000710005	4.43753E-05	8.79E-05	1.67E-05	0	3.15E-05	5.88E-05	9.55E-05	0	1.96E-05	0.000304692	6.45E-05	3.10E-05	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__;g__	0.000705901	4.41188E-05	0.000117192	3.34E-05	0	3.15E-05	0	4.77E-05	0	0	0	0.000236346	3.10E-05	0	7.25E-05	0	0.000136305	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Methylophilales;f__Methylophilaceae;g__Methylotenera	0.000691685	4.32303E-05	0	3.34E-05	5.82E-05	3.15E-05	1.96E-05	0	4.37E-05	1.96E-05	0.000304692	2.15E-05	6.19E-05	2.52E-05	7.25E-05	0	0	0
k__Bacteria;p__Chloroflexi;c__S085;o__;f__;g__	0.000688805	4.30503E-05	8.79E-05	0	0	6.29E-05	0	0	0	0	0.000304692	2.15E-05	3.10E-05	2.52E-05	0	0	0	0.000155618
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;g__Enterococcus	0.000666782	4.16738E-05	0.000263682	0	0	3.15E-05	0	0	0	0	0	0	0.000371632	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Paenibacillus	0.000660331	4.12707E-05	8.79E-05	0	0	9.44E-05	5.88E-05	0	4.37E-05	3.92E-05	0	2.15E-05	0.000123877	5.04E-05	7.25E-05	0	6.82E-05	0
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella	0.000658565	4.11603E-05	0	0	0	0	3.92E-05	0	0	0	0	0	0.000619387	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;Other	0.000646641	4.04151E-05	2.93E-05	0	2.91E-05	3.15E-05	5.88E-05	0	0	1.96E-05	0	0.00021486	0	5.04E-05	0.000145001	0	6.82E-05	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Kineosporiaceae;g__Kineococcus	0.000641493	4.00933E-05	2.93E-05	1.67E-05	2.91E-05	0	1.96E-05	0	0	0	0	2.15E-05	9.29E-05	7.56E-05	0	4.56E-05	0	0.000311236
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae;g__Rhodococcus	0.000630028	3.93768E-05	0	3.34E-05	5.82E-05	0	0.000430958	0	4.37E-05	1.96E-05	0	2.15E-05	0	0	0	2.28E-05	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Geodermatophilaceae;g__	0.000626285	3.91428E-05	2.93E-05	0.000350508	0	3.15E-05	1.96E-05	0	0	0	0	0	0	5.04E-05	0.000145001	0	0	0
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__;g__	0.000621065	3.88166E-05	0	1.67E-05	0	0	7.84E-05	0	0	1.96E-05	0.000152346	4.30E-05	9.29E-05	5.04E-05	0.000145001	2.28E-05	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Gemellales;f__Gemellaceae;Other	0.000609601	3.81001E-05	2.93E-05	1.67E-05	0	6.29E-05	0	0	0	0	0.000152346	0.000128916	0	0.000151263	0	0	6.82E-05	0
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Stramenopiles;f__;g__	0.000609157	3.80723E-05	0	0	0	0	0.000587671	0	0	0	0	2.15E-05	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__[Marinicellales];f__[Marinicellaceae];g__	0.000592219	3.70137E-05	0.000117192	1.67E-05	2.91E-05	9.44E-05	1.96E-05	0	0	1.96E-05	0	2.15E-05	0	0.000201684	7.25E-05	0	0	0
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__	0.000589911	3.68695E-05	0	0	0	9.44E-05	0	0	0	0	0	0	0.000495509	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae;g__Carnobacterium	0.000588417	3.67761E-05	0	0	0	0	0	0	0	0	0	0	0.000588417	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__	0.00058572	3.66075E-05	8.79E-05	3.34E-05	0	0	1.96E-05	0	0	0	0.000152346	6.45E-05	0.000154847	5.04E-05	0	2.28E-05	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Aliivibrio	0.000579842	3.62401E-05	0	0	0	0	0	0	0	0	0	0	0	0.000579842	0	0	0	0
k__Bacteria;p__Chlorobi;c__SJA-28;o__;f__;g__	0.000575458	3.59661E-05	5.86E-05	0	0	3.15E-05	0	4.77E-05	0	0	0.000152346	2.15E-05	9.29E-05	7.56E-05	7.25E-05	2.28E-05	0	0
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Conexibacteraceae;g__	0.00056809	3.55056E-05	5.86E-05	0	0	0	0	0	0	0	0.000457038	2.15E-05	3.10E-05	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Dokdonella	0.000565617	3.5351E-05	2.93E-05	0	5.82E-05	0	0	4.77E-05	4.37E-05	0	0	0.000386747	0	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__;g__	0.00055636	3.47725E-05	2.93E-05	1.67E-05	0	9.44E-05	0	0	0	0	0.000152346	0	6.19E-05	0.000201684	0	0	0	0

Supplemental table 1.2. Continued.

k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Dyadobacter	0.000552146	3.45091E-05	2.93E-05	3.34E-05	2.91E-05	0	7.84E-05	0	0	5.88E-05	0.000152346	0	0	7.56E-05	7.25E-05	2.28E-05	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae;g__Neisseria	0.000538623	3.3664E-05	0	0	0.000407225	0	1.96E-05	0	4.37E-05	0	0	0	0	0	0	0	6.82E-05	0
k__Bacteria;p__Elusimicrobia;c__Elusimicrobia;o__FAC88;f__;g__	0.000534192	3.3387E-05	2.93E-05	3.34E-05	0	0.000440542	0	0	0	0	0	0	3.10E-05	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;g__	0.000525045	3.28153E-05	0	0	0	0	1.96E-05	0	0	1.96E-05	0	0.000386747	3.10E-05	0	0	0	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__;f__;g__	0.000524485	3.27803E-05	8.79E-05	0	5.82E-05	0	3.92E-05	0	0	1.96E-05	0.000152346	4.30E-05	3.10E-05	2.52E-05	0	0	6.82E-05	0
k__Bacteria;p__Acidobacteria;c__Acidobacteriia;o__Acidobacteriales;f__Acidobacteriaceae;g__	0.000519207	3.24504E-05	5.86E-05	0	2.91E-05	0.000125869	1.96E-05	0	0	3.92E-05	0	6.45E-05	6.19E-05	2.52E-05	7.25E-05	2.28E-05	0	0
k__Bacteria;p__[Thermi];c__Deinococci;o__Thermales;f__Thermaceae;g__Thermus	0.000515663	3.2229E-05	0	0	0	0	0	0	0	0	0	0.000515663	0	0	0	0	0	0
k__Bacteria;p__TM7;c__TM7-3;o__EW055;f__;g__	0.000500731	3.12957E-05	0.00014649	0	0	3.15E-05	5.88E-05	0	4.37E-05	0	0	4.30E-05	3.10E-05	0.000100842	0	4.56E-05	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__	0.000486691	3.04182E-05	2.93E-05	0	0	0	0	4.77E-05	0	0	0	0.00010743	6.19E-05	0	0.000217502	2.28E-05	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Methylophilales;f__Methylophilaceae;Other	0.000485515	3.03447E-05	5.86E-05	3.34E-05	2.91E-05	0	1.96E-05	0	0	0	0.000152346	4.30E-05	3.10E-05	5.04E-05	0	0	6.82E-05	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Agrococcus	0.000485068	3.03167E-05	0	0	0	0	0	0	0	0	0.000152346	2.15E-05	0	0	0	0	0	0.000031236
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Adhaeribacter	0.000481759	3.011E-05	2.93E-05	3.34E-05	0	3.15E-05	3.92E-05	4.77E-05	4.37E-05	3.92E-05	0	2.15E-05	0.000123877	0	7.25E-05	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Agromyces	0.000476758	2.97974E-05	5.86E-05	0	2.91E-05	9.44E-05	1.96E-05	4.77E-05	0	1.96E-05	0	2.15E-05	9.29E-05	2.52E-05	0	0	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;Other	0.000471998	2.94999E-05	5.86E-05	1.67E-05	2.91E-05	9.44E-05	1.96E-05	0	0	1.96E-05	0	4.30E-05	0	5.04E-05	7.25E-05	0	6.82E-05	0
k__Bacteria;p__Acidobacteria;c__RB25;o__;f__;g__	0.000467305	2.92066E-05	8.79E-05	0	0	6.29E-05	0	0	0	3.92E-05	0.000152346	2.15E-05	3.10E-05	0	7.25E-05	0	0	0
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Leptotrichiaceae;g__Leptotrichia	0.000460689	2.8793E-05	0	0	0	0	0	0	0	0	0	0.000429719	3.10E-05	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Pseudoxanthomonas	0.000455465	2.84666E-05	0.000175788	0	0	3.15E-05	0	0	0	0	0	0	0.000154847	2.52E-05	0	0	6.82E-05	0
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__AKIW874;g__	0.000451205	2.82003E-05	0	0	0	0	0	0	0	0	0	0.000451205	0	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae;g__Pseudonocardia	0.000449858	2.81161E-05	2.93E-05	1.67E-05	0	3.15E-05	3.92E-05	4.77E-05	0	0	0	6.45E-05	6.19E-05	0	0	2.28E-05	0.000136305	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Oceanospirillaceae;Other	0.000441505	2.7594E-05	0	0	0	0	0	0	0	0	0	0	0.000340663	0.000100842	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Thermomonosporaceae;Other	0.000440123	2.75077E-05	0.000117192	3.34E-05	0	3.15E-05	3.92E-05	4.77E-05	0	1.96E-05	0	2.15E-05	6.19E-05	0	0	0	6.82E-05	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Cellulomonadaceae;g__Cellulomonas	0.000433444	2.70902E-05	0	0	0	0	0	0	0	0	0	0.000408233	0	2.52E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bdellovibrionaceae;g__Bdellovibrio	0.00042857	2.67856E-05	8.79E-05	1.67E-05	2.91E-05	0.000220271	0	0	4.37E-05	0	0	0	3.10E-05	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Moraxella	0.000425385	2.65865E-05	0	0	0	0	0	0	0	0	0	0	0.000402601	0	0	2.28E-05	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;Other	0.000420302	2.62689E-05	2.93E-05	0	0	3.15E-05	0	0	0	0	0	2.15E-05	6.19E-05	2.52E-05	7.25E-05	2.28E-05	0	0.000015618
k__Bacteria;p__Armatimonadetes;c__[Fimbriimonadia];o__[Fimbriimonadales];f__[Fimbriimonadaceae];g__Fimbriimonas	0.000405013	2.53133E-05	5.86E-05	1.67E-05	0	0	1.96E-05	0	0	0	0.000152346	2.15E-05	0	0	0	0	0.000136305	0
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;Other;Other	0.000404317	2.52698E-05	2.93E-05	0	0	6.29E-05	1.96E-05	0	0	0	0	0.000193374	3.10E-05	0	0	0	6.82E-05	0
k__Bacteria;p__Acidobacteria;c__DA052;o__Ellin6513;f__;g__	0.000396847	2.48029E-05	8.79E-05	0	0	0	0	4.77E-05	4.37E-05	0	0	0	6.19E-05	0	0	0	0	0.000015618
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Aeromonadaceae;g__	0.000391955	2.44972E-05	0	0	8.73E-05	0	0	0	0	0	0.000304692	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__;g__	0.000390473	2.44045E-05	0.000117192	3.34E-05	0	0	1.96E-05	0	0	3.92E-05	0	2.15E-05	6.19E-05	2.52E-05	7.25E-05	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Bradyrhizobium	0.000378191	2.36369E-05	2.93E-05	1.67E-05	2.91E-05	0	3.92E-05	0	0	0	0	2.15E-05	0.000123877	5.04E-05	0	0	6.82E-05	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;Other;Other	0.000377647	2.36029E-05	2.93E-05	0	0	0	0	0	4.37E-05	0	0.000304692	0	0	0	0	0	0	0
k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__[Bryobacteraceae];g__	0.000372846	2.33029E-05	0	0	0	0	0	0	0	1.96E-05	0	0.00032229	3.10E-05	0	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Porphyromonas	0.000371632	2.3227E-05	0	0	0	0	0	0	0	0	0	0	0.000371632	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Erythrobacteraceae;g__	0.000366799	2.29249E-05	2.93E-05	3.34E-05	0	0	0	0	0	0	0	4.30E-05	0	2.52E-05	0.000145001	2.28E-05	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__[Entotheonellales];f__[Entotheonellaceae];g__	0.000356208	2.2263E-05	0	0	5.82E-05	9.44E-05	0	4.77E-05	0	1.96E-05	0	0	0	0	0	0	0.000136305	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhodobiaceae;g__Afifella	0.000351002	2.19376E-05	5.86E-05	0	0	0	0	0	0	0	0.000152346	2.15E-05	0	5.04E-05	0	0	6.82E-05	0

Supplemental table 1.2. Continued.

k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__;g__	0.000334269	2.08918E-05	2.93E-05	0	5.82E-05	0	0	0	0	1.96E-05	0	2.15E-05	6.19E-05	7.56E-05	0	0	6.82E-05	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;g__Pilimelia	0.00033426	2.08913E-05	0	0	0	0.000314673	0	0	0	1.96E-05	0	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Pelomonas	0.00033399	2.08744E-05	2.93E-05	0	0	0	0	0	0	0	0.000304692	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;Other;Other	0.000330712	2.06695E-05	0	3.34E-05	2.91E-05	0	3.92E-05	0	0	1.96E-05	0	2.15E-05	6.19E-05	0.000126053	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Vibrio	0.000320353	2.00221E-05	0.000117192	0	0	6.29E-05	1.96E-05	0	0	0	0	6.45E-05	3.10E-05	2.52E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Rubrivivax	0.00031646	1.97787E-05	5.86E-05	0	0	0	0	0	0	0	0	0	3.10E-05	0.000226895	0	0	0	0
k__Bacteria;p__Acidobacteria;c__iii1-8;o__DS-18;f__;g__	0.000314464	1.9654E-05	2.93E-05	1.67E-05	0	3.15E-05	0	0	0	0	0	0	3.10E-05	5.04E-05	0	0	0	0.000155618
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;Other	0.000312954	1.95597E-05	0	0	0	0.000157337	0	0	0	0	0	0	0	0	0	0	0	0.000155618
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Dietziaceae;g__Dietzia	0.000304692	1.90433E-05	0	0	0	0	0	0	0	0	0.000304692	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Ferrimonadaceae;g__Ferrimonas	0.000304692	1.90433E-05	0	0	0	0	0	0	0	0	0.000304692	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__	0.000304692	1.90433E-05	0	0	0	0	0	0	0	0	0.000304692	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Balneimonas	0.000304264	1.90165E-05	2.93E-05	0	0	0	0	0	4.37E-05	0	0.000152346	0	3.10E-05	2.52E-05	0	2.28E-05	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;Other	0.000303586	1.89741E-05	0	1.67E-05	2.91E-05	0	3.92E-05	0	0	1.96E-05	0.000152346	2.15E-05	0	2.52E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium	0.000302735	1.89209E-05	5.86E-05	0	2.91E-05	3.15E-05	1.96E-05	0	0	1.96E-05	0	2.15E-05	0	5.04E-05	7.25E-05	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae;g__	0.000299674	1.87296E-05	0	0	0	0	0	0	0.000218283	0	0	0	3.10E-05	5.04E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__A21b;Other;Other	0.000299166	1.86979E-05	5.86E-05	0	2.91E-05	3.15E-05	1.96E-05	0	4.37E-05	0	0	2.15E-05	0	0	7.25E-05	2.28E-05	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae;Other	0.000297599	1.85999E-05	0	0	0.000203613	0	0	0	0	0	0	2.15E-05	0	0	7.25E-05	0	0	0
k__Bacteria;Other;Other;Other;Other;Other	0.000297591	1.85995E-05	0	0	0	0	0.000117534	0	0	0	0	0	0.000154847	2.52E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Geobacteraceae;g__Geobacter	0.000296965	1.85603E-05	2.93E-05	1.67E-05	0	0	3.92E-05	0	0	0	0	0	3.10E-05	2.52E-05	0	0	0	0.000155618
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;Other;Other	0.000296406	1.85254E-05	0	3.34E-05	0	3.15E-05	0	0	0	0	0	2.15E-05	6.19E-05	7.56E-05	7.25E-05	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__	0.000295844	1.84903E-05	2.93E-05	0	0	6.29E-05	0	0	0	0	0	0	0	2.52E-05	0	2.28E-05	0	0.000155618
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Kineosporiaceae;g__	0.000294956	1.84347E-05	0.00014649	0	0	3.15E-05	1.96E-05	0	4.37E-05	0	0	0	3.10E-05	0	0	2.28E-05	0	0
k__Bacteria;p__Actinobacteria;c__MB-A2-108;o__0319-7L14;f__;g__	0.000287188	1.79492E-05	0	0	0	6.29E-05	0	0	0	0	0.000152346	2.15E-05	0	5.04E-05	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae;g__	0.00028255	1.76594E-05	0	0	2.91E-05	0	3.92E-05	0	0	0	0.000152346	0	6.19E-05	0	0	0	0	0
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__Parachlamydiaceae;g__Candidatus Protochlamydia	0.000279189	1.74493E-05	0	0	5.82E-05	0	0	0	0	3.92E-05	0	2.15E-05	6.19E-05	7.56E-05	0	2.28E-05	0	0
k__Bacteria;p__Chloroflexi;c__C0119;o__;f__;g__	0.000277672	1.73545E-05	0	1.67E-05	0	0	0	0	0	0	0.000152346	2.15E-05	6.19E-05	2.52E-05	0	0	0	0
k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__Ellin517;g__	0.000272009	1.70006E-05	0	0	0	6.29E-05	1.96E-05	0	0	1.96E-05	0	0	3.10E-05	2.52E-05	0	4.56E-05	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;Other;Other;Other	0.00026654	1.66587E-05	5.86E-05	1.67E-05	0	3.15E-05	3.92E-05	0	0	0	0	2.15E-05	3.10E-05	0	0	0	6.82E-05	0
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__;g__	0.000263892	1.64932E-05	0	3.34E-05	0	3.15E-05	0	0	0	0	0.000152346	2.15E-05	0	2.52E-05	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae];g__	0.000263682	1.64801E-05	0.000263682	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Chitinophaga	0.00026028	1.62675E-05	5.86E-05	0	0	0	0	0	0	0	0	0	0	0.000201684	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;Other	0.000253178	1.58236E-05	0	0	0	0	0	0	0	1.96E-05	0	4.30E-05	9.29E-05	2.52E-05	7.25E-05	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;Other;Other	0.000252105	1.57566E-05	0	0	0	0	0	0	0	0	0	0	0	0.000252105	0	0	0	0
k__Bacteria;p__Chloroflexi;c__TK17;o__mle1-48;f__;g__	0.000251578	1.57236E-05	0	0	0	0	0	0	0.00013097	0	0	2.15E-05	3.10E-05	0	0	0	6.82E-05	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__	0.000245254	1.53284E-05	0	0	0	0	0	0	0	0	0.000152346	0	9.29E-05	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Achromobacter	0.000239819	1.49887E-05	2.93E-05	0	5.82E-05	0	0	0	0	0	0.000152346	0	0	0	0	0	0	0

Supplemental table 1.2. Continued.

k__Bacteria;p__Acidobacteria;c__Acidobacteriia;o__Acidobacteriales;f__Acidobacteriaceae;g__Edaphobacter	0.000228893	1.43058E-05	2.93E-05	0	0	0	1.96E-05	0	0	0	0	0	6.19E-05	0	7.25E-05	4.56E-05	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;Other	0.000224654	1.40409E-05	0.00014649	0	0	3.15E-05	0	0	0	0	0	2.15E-05	0	2.52E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Luteimonas	0.000224389	1.40243E-05	0	0	0	0	0	0	0	1.96E-05	0.000152346	2.15E-05	3.10E-05	0	0	0	0	0
k__Bacteria;p__Chlorobi;c__;o__;f__;g__	0.000224253	1.40158E-05	0	0	0	0	0	0	0	0	0.000152346	2.15E-05	0	5.04E-05	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;Other;Other	0.00022377	1.39856E-05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.82E-05	0.000155618
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae;g__Steroidobacter	0.000222667	1.39167E-05	0	0	0	0	0	0	0	0	0	2.15E-05	3.10E-05	2.52E-05	0.000145001	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;Other	0.000221187	1.38242E-05	0	0	0	3.15E-05	0	4.77E-05	0	0	0	2.15E-05	0	2.52E-05	7.25E-05	2.28E-05	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__Legionella	0.000218584	1.36615E-05	0	0	0	0	0	0	0	0	0	0.000193374	0	2.52E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;Other	0.00021486	1.34287E-05	0	0	0	0	0	0	0	0	0	0.00021486	0	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Solirubrobacteraceae;Other	0.000208073	1.30046E-05	0	0	0	0	0	0	0	0	0	2.15E-05	3.10E-05	0	0	0	0	0.000155618
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Flectobacillus	0.000207489	1.2968E-05	0	0	2.91E-05	0	0	0	0	0	0	0	0	0	0	2.28E-05	0	0.000155618
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae;Other	0.000206751	1.29219E-05	5.86E-05	0	0	9.44E-05	0	0	0	0	0	0	3.10E-05	0	0	2.28E-05	0	0
k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__RB41;f__;g__	0.00019644	1.22775E-05	5.86E-05	5.01E-05	0	0	1.96E-05	0	0	0	0	4.30E-05	0	2.52E-05	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Dermabacteraceae;Other	0.00019589	1.22431E-05	0	0	0	0	0.00019589	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Gemellales;f__Gemellaceae;g__Gemella	0.000191206	1.19504E-05	5.86E-05	0	0	0	0	0	0	0	0	6.45E-05	0	0	0	0	6.82E-05	0
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;Other	0.000191061	1.19413E-05	2.93E-05	0	0	0	0	9.55E-05	0	1.96E-05	0	2.15E-05	0	2.52E-05	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Rudanella	0.000189258	1.18286E-05	0	0	0	3.15E-05	0	0	0	0	0	2.15E-05	0	0	0	0	0.000136305	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinospicaceae;g__	0.000189007	1.18129E-05	2.93E-05	3.34E-05	5.82E-05	0	0	0	0	0	0	0	0	0	0	0	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingopyxis	0.000186587	1.16617E-05	0	0	0	0	0	0	0	0	0	0	3.10E-05	0	0	0	0	0.000155618
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Oceanospirillaceae;g__Marinobacterium	0.000185816	1.16135E-05	0	0	0	0	0	0	0	0	0	0	0.000185816	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;Other	0.000181644	1.13528E-05	2.93E-05	0	0	0	0	0	0	0	0.000152346	0	0	0	0	0	0	0
k__Bacteria;p__TM7;c__MJK10;o__;f__;g__	0.000181644	1.13528E-05	2.93E-05	0	0	0	0	0	0	0	0.000152346	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__mitochondria;g__Plantago	0.000181424	1.1339E-05	2.93E-05	1.67E-05	0	6.29E-05	0	0	0	0	0	0	0	0	7.25E-05	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;Other;Other	0.000176474	1.10296E-05	0	0	0	0	0	0	0	0	0	0	0	0.000176474	0	0	0	0
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae];g__	0.000176301	1.10188E-05	0	0	0	0	0.000176301	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__;g__	0.000174525	1.09078E-05	0	0	0.000174525	0	0	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__Ellin5290;f__;g__	0.000171888	1.0743E-05	0	0	0	0	0	0	0	0	0	0.000171888	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__NB1-j;f__;g__	0.000169037	1.05648E-05	0	1.67E-05	0	0	0	0	0	0	0.000152346	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__Uliginosibacterium	0.000166361	1.03975E-05	2.93E-05	0	0	3.15E-05	0	0	4.37E-05	0	0	0	6.19E-05	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__	0.000161992	1.01245E-05	0.000117192	0	0	0	1.96E-05	0	0	0	0	0	0	2.52E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__	0.000155351	9.70941E-06	2.93E-05	0	0	0	0	0	0	0	0	0	0	0.000126053	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__;f__;g__	0.000150475	9.40469E-06	2.93E-05	0	2.91E-05	0	1.96E-05	0	0	0	0	0	0	0	7.25E-05	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__IS-44;f__;g__	0.000149936	9.37099E-06	2.93E-05	0	0	0	0	0	0	0	0	6.45E-05	3.10E-05	2.52E-05	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__At12OctB3;o__;f__;g__	0.00014908	9.3175E-06	2.93E-05	0	0	0	0	4.77E-05	0	1.96E-05	0	2.15E-05	3.10E-05	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Haliangiaceae;g__	0.00014168	8.85502E-06	2.93E-05	0	0	0	3.92E-05	0	0	0	0	0	0	5.04E-05	0	2.28E-05	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Aeromicrobium	0.000139928	8.74552E-06	2.93E-05	0	5.82E-05	0	0	0	0	0	0	2.15E-05	3.10E-05	0	0	0	0	0

Supplemental table 1.2. Continued.

k__Bacteria;p__[Thermi];c__Deinococci;o__Deinococcales;f__Deinococcaceae;g__Deinococcus	0.000137123	8.5702E-06	0	0	0	0	0.000137123	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Saprospiraceae;g__Haliscomenobacter	0.00012483	7.80189E-06	0	0	0	3.15E-05	0	0	0	0	0	0	0	2.52E-05	0	0	6.82E-05	0
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__	0.000123557	7.72231E-06	0	0	0	3.15E-05	1.96E-05	0	0	0	0	0	0	0	7.25E-05	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Hyphomonadaceae;g__	0.000120608	7.53798E-06	0	0	0	0	0	0	0	0	0	2.15E-05	3.10E-05	0	0	0	6.82E-05	0
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cyclobacteriaceae;g__	0.000120264	7.51648E-06	2.93E-05	0	0	0	0	0	0	0	0	4.30E-05	0	2.52E-05	0	2.28E-05	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Kribbella	0.000119784	7.48649E-06	0	0	0	0	1.96E-05	4.77E-05	0	0	0	2.15E-05	3.10E-05	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Turicibacterales;f__Turicibacteraceae;g__Turicibacter	0.000118711	7.41942E-06	0	0	0	0	1.96E-05	0	0	0	0	0	3.10E-05	0	0	0	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Cupriavidus	0.000117192	7.3245E-06	0.000117192	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Acidobacteria;c__PAUC37f;o__;f__;g__	0.000113238	7.07738E-06	2.93E-05	3.34E-05	0	0	1.96E-05	0	0	0	0	0	3.10E-05	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Phyllobacterium	0.000111322	6.95761E-06	2.93E-05	0	0	3.15E-05	0	0	0	1.96E-05	0	0	3.10E-05	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptomycetaceae;g__	0.000103968	6.49799E-06	0	0	0	3.15E-05	0	0	0	0	0	0	0	0	7.25E-05	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;g__Actinoplanes	0.000103634	6.47713E-06	0	0	0	0	1.96E-05	0	0	1.96E-05	0	6.45E-05	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Polyangiaceae;g__	0.00010347	6.46687E-06	0	0	0	0	0	0	0	0	0	0	3.10E-05	0	7.25E-05	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;Other	0.000101588	6.34925E-06	0	0	2.91E-05	0	0	0	0	0	0	0	0	0	7.25E-05	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;Other;Other;Other	9.96691E-05	6.22932E-06	5.86E-05	0	0	0	0	0	0	1.96E-05	0	2.15E-05	0	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Cellulomonadaceae;g__Demequina	9.91217E-05	6.19511E-06	0	0	0	0	0	0	0	0	0	0	3.10E-05	0	0	0	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;Other;Other	9.91217E-05	6.19511E-06	0	0	0	0	0	0	0	0	0	0	3.10E-05	0	0	0	6.82E-05	0
k__Bacteria;p__Bacteroidetes;c__VC2_1_Bac22;o__;f__;g__	9.8313E-05	6.14456E-06	0	0	2.91E-05	0	0	4.77E-05	0	0	0	2.15E-05	0	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__WAL_1855D	9.79451E-05	6.12157E-06	0	0	0	0	9.79E-05	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Alcanivoracaceae;g__Alcanivorax	9.74504E-05	6.09065E-06	2.93E-05	0	0	0	0	0	0	0	0	0	0	0	0	0	6.82E-05	0
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Sporocytophaga	9.39865E-05	5.87416E-06	0	0	0	0	0	0	0	0	0	2.15E-05	0	0	7.25E-05	0	0	0
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__Peptostreptococcus	9.2908E-05	5.80675E-06	0	0	0	0	0	0	0	0	0	0	9.29E-05	0	0	0	0	0
k__Bacteria;p__Chloroflexi;c__TK10;o__;f__;g__	9.26854E-05	5.79284E-06	2.93E-05	1.67E-05	0	0	0	0	0	0	0	2.15E-05	0	2.52E-05	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Cryomorphaceae;g__Fluviicola	9.17347E-05	5.73342E-06	2.93E-05	0	0	3.15E-05	0	0	0	0	0	0	3.10E-05	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Dermabacteraceae;g__Dermabacter	9.11328E-05	5.6958E-06	0	0	0	0	0	0	0	0	0	0	0	0	0	9.11E-05	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae;Other	8.88145E-05	5.55091E-06	0	0	0	0	1.96E-05	4.77E-05	0	0	0	2.15E-05	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Lysobacter	8.77395E-05	5.48372E-06	0	0	0	0	0	0	0	1.96E-05	0	0	0	0	0	0	6.82E-05	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Yersinia	8.73134E-05	5.45709E-06	0	0	0	0	0	0	8.73E-05	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira	8.54779E-05	5.34237E-06	2.93E-05	0	0	0	0	0	0	0	0	0	3.10E-05	2.52E-05	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Cryomorphaceae;g__Crocinitomix	8.38065E-05	5.23791E-06	5.86E-05	0	0	0	0	0	0	0	0	0	0	2.52E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;Other	8.25237E-05	5.15773E-06	0	0	0	6.29E-05	1.96E-05	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__	7.92518E-05	4.95324E-06	0	1.67E-05	0	0	1.96E-05	0	0	0	0	4.30E-05	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;Other;Other;Other	7.92069E-05	4.95043E-06	0	0	0	3.15E-05	0	4.77E-05	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Dermacoccaceae;g__Dermacoccus	7.83561E-05	4.89726E-06	0	0	0	0	7.84E-05	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Thiotrichales;f__Piscirickettsiaceae;g__	7.5784E-05	4.7365E-06	0	0	2.91E-05	0	0	0	0	0	0	2.15E-05	0	2.52E-05	0	0	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Brevibacillus	7.44393E-05	4.65245E-06	0	0	0	3.15E-05	0	0	0	0	0	4.30E-05	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Nannocystaceae;g__Plesiocystis	7.1907E-05	4.49419E-06	0	0	0	0	0	0	0	0	0	2.15E-05	0	5.04E-05	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae;g__	7.11994E-05	4.44996E-06	2.93E-05	1.67E-05	0	0	0	0	0	0	0	0	0	2.52E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Kaistia	6.71119E-05	4.19449E-06	0	1.67E-05	0	0	0	0	0	0	0	0	0	5.04E-05	0	0	0	0

Supplemental table 1.2. Continued.

k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__Zoogloea	6.29346E-05	3.93342E-06	0	0	0	6.29E-05	0	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales];f__[Chthoniobacteraceae];g__Chthoniobacter	6.24367E-05	3.90229E-06	0	0	0	3.15E-05	0	0	0	0	0	0	3.10E-05	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__Rummeliibacillus	6.19387E-05	3.87117E-06	0	0	0	0	0	0	0	0	0	0	6.19E-05	0	0	0	0	0
k__Bacteria;p__Proteobacteria;Other;Other;Other;Other	6.19387E-05	3.87117E-06	0	0	0	0	0	0	0	0	0	0	6.19E-05	0	0	0	0	0
k__Bacteria;p__TM7;Other;Other;Other;Other	6.07653E-05	3.79783E-06	2.93E-05	0	0	3.15E-05	0	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Leptothrix	6.06621E-05	3.79138E-06	0	0	0	0	1.96E-05	0	0	1.96E-05	0	2.15E-05	0	0	0	0	0	0
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;Other;Other	6.02674E-05	3.76671E-06	2.93E-05	0	0	0	0	0	0	0	0	0	3.10E-05	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__	6.00569E-05	3.75355E-06	0	0	2.91E-05	0	0	0	0	0	0	0	3.10E-05	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Oleiphilaceae;g__	5.87671E-05	3.67294E-06	0	0	0	0	5.88E-05	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Mesorhizobium	5.8596E-05	3.66225E-06	5.86E-05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;Other	5.8596E-05	3.66225E-06	5.86E-05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__Anaerococcus	5.8175E-05	3.63594E-06	0	0	5.82E-05	0	0	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae;Other	5.66778E-05	3.54236E-06	0	0	0	3.15E-05	0	0	0	0	0	0	0	2.52E-05	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Bogoriellaceae;g__Georgenia	5.61798E-05	3.51124E-06	0	0	0	0	0	0	0	0	0	0	3.10E-05	2.52E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;g__	5.61798E-05	3.51124E-06	0	0	0	0	0	0	0	0	0	0	3.10E-05	2.52E-05	0	0	0	0
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Aerococcaceae;Other	5.45085E-05	3.40678E-06	2.93E-05	0	0	0	0	0	0	0	0	0	0	2.52E-05	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae;g__Phycicoccus	5.37525E-05	3.35953E-06	0	0	0	0	0	0	0	0	0	0	3.10E-05	0	0	2.28E-05	0	0
k__Bacteria;p__Gemmatimonadetes;c__;o__;f__;g__	5.24553E-05	3.27846E-06	0	0	0	0	0	0	0	0	0	2.15E-05	3.10E-05	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__TA18;o__CV90;f__;g__	5.24553E-05	3.27846E-06	0	0	0	0	0	0	0	0	0	2.15E-05	3.10E-05	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptosporangiaceae;Other	5.20812E-05	3.25508E-06	2.93E-05	0	0	0	0	0	0	0	0	0	0	0	0	2.28E-05	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__	5.10563E-05	3.19102E-06	0	0	0	3.15E-05	1.96E-05	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Patulibacteraceae;g__Patulibacter	5.10563E-05	3.19102E-06	0	0	0	3.15E-05	1.96E-05	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Armatimonadetes;c__Armatimonadia;o__FW68;f__;g__	5.10544E-05	3.1909E-06	0	0	0	3.15E-05	0	0	0	1.96E-05	0	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Hydrogenophaga	5.0421E-05	3.15131E-06	0	0	0	0	0	0	0	0	0	0	0	5.04E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Halomonadaceae;Other	5.0421E-05	3.15131E-06	0	0	0	0	0	0	0	0	0	0	0	5.04E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Enterovibrio	5.0421E-05	3.15131E-06	0	0	0	0	0	0	0	0	0	0	0	5.04E-05	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Hydrogenophilales;f__Hydrogenophilaceae;g__Thiobacillus	4.88851E-05	3.05532E-06	2.93E-05	0	0	0	0	0	0	1.96E-05	0	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Nitrosomonadales;f__Nitrosomonadaceae;g__	4.79937E-05	2.99961E-06	0	0	0	0	0	0	0	0	0	0	0	2.52E-05	0	2.28E-05	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Frankiaceae;g__Actinomycetales	4.29719E-05	2.68575E-06	0	0	0	0	0	0	0	0	0	4.30E-05	0	0	0	0	0	0
k__Bacteria;p__Chloroflexi;c__Chloroflexi;o__Herpetosiphonales;f__;g__	4.29719E-05	2.68575E-06	0	0	0	0	0	0	0	0	0	4.30E-05	0	0	0	0	0	0
k__Bacteria;p__AD3;c__ABS-6;o__;f__;g__	4.10731E-05	2.56707E-06	0	0	0	0	0	0	0	1.96E-05	0	2.15E-05	0	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nakamurellaceae;g__	3.9178E-05	2.44863E-06	0	0	0	0	3.92E-05	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae;g__Nocardia	3.9178E-05	2.44863E-06	0	0	0	0	3.92E-05	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Sanguibacteraceae;g__Sanguibacter	3.9178E-05	2.44863E-06	0	0	0	0	3.92E-05	0	0	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__;g__	3.6278E-05	2.26737E-06	0	1.67E-05	0	0	0	0	0	1.96E-05	0	0	0	0	0	0	0	0
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Streptophyta;f__;g__	3.33817E-05	2.08636E-06	0	3.34E-05	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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CHAPTER 2

Endosymbiont interference and microbial diversity of the Pacific coast tick, *Dermacentor occidentalis*, in San Diego County, California.

Abstract

The Pacific coast tick, *Dermacentor occidentalis* Marx, can carry agents that cause human diseases such as anaplasmosis, ehrlichiosis, tularemia, Rocky Mountain spotted fever and rickettsiosis 364D. Studies of other tick species have demonstrated that non-infectious endosymbiotic *Rickettsia* species and other endosymbiotic bacteria can interfere with other bacteria from co-infecting ticks. We hypothesized that similar patterns of interference exist within *D. occidentalis* ticks infected with Spotted Fever Group *Rickettsia* (SFGR). Specifically, we used PCR amplification and sequencing of the *romp*A gene and intergenic region to determine if ticks were infected with SFGR. We then amplified a partial segment of the 16S rRNA gene and used next-generation sequencing to determine whether the microbiomes of SFGR-infected ticks were significantly different than ticks lacking SFGR and if this microbial diversity was consistent with a hypothesis of competitive exclusion. *D. occidentalis* ticks were collected from four different hiking areas representing three different watersheds in San Diego County. Analysis of the 16S rRNA gene libraries generated from individual ticks revealed significant differences between SFGR-infected and non-SFGR-infected ticks and that male ticks had a greater diversity of bacteria than female ticks. Most strikingly, there was an inverse relationship between infection with *Francisella*-like

endosymbionts (FLE) and *Rickettsia*. Overall microbial diversity, excluding the endosymbionts *Rickettsia* and FLE*,* had a small but significant difference among collection locales. We also found a pattern of geographic isolation by distance of tick microbiomes and that tick microbiomes from ticks that weren't infected with SFGR had components of a canine skin microbiome. Our findings suggest that FLEs and to a lesser extent, other bacteria, negatively affect the ability of *D. occidentalis* to be infected with certain SFGR.

Introduction

The Pacific Coast tick, *Dermacentor occidentalis* Marx (henceforth *D. occidentalis*) is the most widely distributed tick in California and is found in chaparral and shrubland areas from northern Baja California, through to California and Oregon (Furman and Loomis 1984). It is a 3-host tick that feeds on a variety of animals such as rodents, rabbits, cattle, deer, horses and humans. Surveys of this tick have uncovered human pathogens such as *Francisella tularensis* (tularemia), *Coxiella burnetii* (Q fever), *Anaplasma phagocytophilum* (human granulocytic anaplasmosis), *Ehrlichia chaffeensis* (human monocytic ehrlichiosis), *Rickettsia rickettsii* (Rocky Mountain spotted fever, RMSF) and *Rickettsia philipii* 364D (hereafter *R. philipii*) as well as the non-pathogenic spotted fever group *Rickettsia, R. rhipicephali* (Cox, 1940, Holden K, Boothby J T, Anand S, 2003, Lane, Emmons, Dondero, & Nelson, 1981;

Parker R R, Brooks C S, 1929; Shapiro et al., 2010; Wikswo et al., 2008). *Rickettsia philipii*, was originally described as an unclassified *Rickettsia* found by Bell in *D. occidentalis* from California (Philip et al. 1978). It is closely related to *Rickettsia rickettsii* but can be serologically and genetically distinguished (Karpathy, Dasch, and Eremeeva 2007; Philip, Lane, and Casper 1981). Although discovered in 1966, and long suspected of being able to cause disease, it was only recently confirmed to be associated with eschars and lymphadenopathy in people at the site of a tick bite (Johnston et al. 2013; Lane et al. 1981; Shapiro et al. 2010).

Francisella-like endosymbiotic bacteria (FLEs) have also been detected in *Dermacentor occidentalis* as well as other tick species (Willy Burgdorfer, Brinton, and Hughes 1973; Kugeler et al. 2005; Noda, Munderloh, and Kurtti 1997; Scoles 2004). FLEs share 16S rRNA gene homology with *Francisella* bacteria, are vertically transmitted, have been observed within tick ovaries and Malpighian tubules, and vary by tick species (Rounds et al. 2012). Although Burgdorfer *et al*. demonstrated pathogenicity of a *Francisella* endosymbiont derived from *Dermacentor andersoni* Stiles ticks (previously categorized as *Wolbachia persica,* Forsman, Sandström, & Sjöstedt, 1994) to guinea pigs and hamsters via injection, most FLEs are not transmitted by tick bites and are considered non-pathogenic (Willy Burgdorfer, Brinton, and Hughes 1973; Mark L Niebylski et al. 1997).

Interestingly, "interference" between different endosymbiotic *Rickettsia* species co-infecting ticks has been demonstrated. Early studies seeking to understand the epidemiology of RMSF in the Bitterroot Valley in Montana demonstrated that the non-pathogenic tick endosymbiont *Rickettsia peacockii* (East side agent) colonized the ovaries of *D. andersoni* ticks and excluded pathogenic *Rickettsia rickettsii* from infecting the ovaries and being transmitted to eggs (Willy Burgdorfer, Hayes, and Mavros 1981). Similarly, studies of *Dermacentor variabilis* (Say) infected with *R. montanensis* or *R. rhipicephali* demonstrated resistance to transovarial transmission of the reciprocal *Rickettsia* in challenge experiments (Macaluso et al. 2002). Competition between co-infecting species of *Rickettsia* has been suggested in other vectors as well (Azad and Beard 1998).

The use of next generation sequencing has allowed further exploration into endosymbionts and complex bacterial communities that colonize different tick species (Nakao et al. 2013), their organs (Budachetri et al. 2014; Qiu et al. 2014), different life stages (Carpi et al. 2011) and different states of nutrition (Menchaca et al. 2013; Zhang et al. 2014). Attention to the microbiome of ticks was driven, in part, by the fact that ticks can transmit the broadest range of diseases, including new and emerging diseases, of any arthropod and the recognition that tick co-infections can have

dramatic consequences on the tick host and human patient (Clay and Fuqua 2010). Microbiome studies using next generation sequencing techniques have demonstrated that each species of tick harbors its own unique bacterial community often dominated by Proteobacteria and one or two endosymbionts (Clay and Fuqua 2010; Ponnusamy et al. 2014; Hawlena et al. 2012; van Treuren et al. 2015; Narasimhan and Fikrig 2015). Given these findings, we hypothesized that *Dermacentor occidentalis* ticks would demonstrate patterns of competitive exclusion within their microbiomes that would be associated with the carriage of pathogenic or non-pathogenic bacteria. We used culture-independent PCR amplification of the 16S rRNA gene and next-generation sequencing (NGS) to determine whether the microbiomes of SFGR-infected ticks differed from non-SFGR-infected ticks and if this microbial diversity was consistent with a hypothesis of competitive exclusion. We also examined the microbiome in the context of infection and geography. Our results reveal patterns consistent with competitive exclusion between SFGR and other bacteria and an association of non-endosymbiotic bacteria with geographic locale. In addition, male ticks exhibited greater microbiome diversity than female ticks. Furthermore, the historical blood meal hosts of the ticks were implicated by the make up of bacterial communities within the ticks and were correlated with SFGR infection.

Materials and Methods

Sample Collection. Ticks were collected from February to May 2014 from 4 different areas of San Diego County: Escondido Creek, Los Peñasquitos Canyon, Lopez Canyon and Mission Trails Regional Park by dragging a 1 m^2 piece of canvas over grass and chaparral and then capturing the ticks with forceps and placing them in individual sterile microfuge tubes. The ticks were transported live back to the Vector Disease and Diagnostic Laboratory at the San Diego County Operations Center where, by visual examination, their species and sex were determined and cataloged before freezing them at -80 °C.

DNA Extraction, PCR Amplification and Next Generation Sequencing. The ticks were thawed and washed sequentially in 3% hydrogen peroxide, 100% isopropanol, and sterile distilled water for 1 minute in each solution. The final distilled water wash was aspirated from the ticks and then the ticks were sectioned sagittally at midline with a sterile scalpel. Half of the tick was saved at -80 °C; the other half was used for DNA extraction. Briefly, 180 µl of ATL buffer (Qiagen, Valencia, CA) and 20 µl of proteinase K were added to each tick and the ticks lysed overnight at 37 °C in an Eppendorf Thermomixer (Hauppauge, NY) with agitation at 1400 rpm for 15 s every 15 min, before centrifuging the lysate for 3 min at 18,400 x g. The supernatant was transferred into a sterile microfuge tube and DNA extracted using a Qiagen DNeasy Blood and Tissue kit in a Qiacube using the DNeasy Blood and Tissue protocol for Tissue and Rodent Tails (Qiagen, Valencia, CA). Negative extraction controls

consisted of sterile water processed via the same washing, chopping and extraction procedure used on the ticks.

The ticks were screened for spotted fever group rickettsia using a Power SYBR Green PCR Mastermix kit (Life Technologies, Carlsbad, CA) and primers for the *romp*A gene (Eremeeva et al., 2003). Reactions were carried out in a total volume of 20 µL composed of 10 µL Power SYBR Green Mastermix, 0.125 µL each of primers RR190.547F (20 µM) and RR190.701R (20 µM), 7.75 µL of nuclease-free water, and 2 µL of template DNA (Eremeeva et al., 2003; Wikswo et al., 2008). PCR cycling conditions were: 3 min at 95 °C; 40 cycles of: 20 s at 95 °C, 30 s at 57 °C, 30 s at 65 °C; a holding cycle of 5 min at 72 °C; and a continuous cycle of: 15 s at 95 °C, 1 min at 55 °C, 30 s at 95 °C, 10 s at 55 °C; and a final holding temperature of 4 °C.

DNA from ticks that screened positive for SFGR were subjected to semi-nested PCR amplification of *romp*A using primers Rr190-70, Rr190-701, and Rr190-602 and the intergenic region (IGR) using primary and nested primers RR0155-*rpm*B (Eremeeva et al., 2006; Shapiro et al., 2010; Wikswo et al., 2008). Briefly, 20 µL of 2X Taq Master Mix (Qiagen, Valencia, CA), 2 µL of forward primer Rr190-70 (20 mM), 2 µL of reverse primer Rr190-701/Rr190-602 (20 mM), 14 µL of nuclease-free H_2O, and 2 µL of DNA was amplified using PCR cycling conditions of 95 °C for 3

min followed by 35 cycles of 95 °C for 20 s, 57 °C for 30 s, and 68 °C for 2 min and then 72 °C for 5 min before holding the products at 4 °C. For the IGR PCR amplification, 20 µL of 2X Taq Master Mix (Qiagen, Valencia, CA), 1 µL of forward primer RR 0155 PF (20 mM), 1 µL of reverse primer 0155 PR (20 mM), 16 µL of nuclease-free H_2O, and 2 µL of DNA was amplified using PCR cycling conditions of 95 °C for 5 minutes followed by 35 cycles of 95 °C for 30 s, 50 °C for 30 s, and 68 °C for 1 min and then 72 °C for 7 min before holding the products at 4 °C.

Amplification products were visualized in a 1% agarose gel stained with ethidium bromide on a UV illuminator and subsequently purified using the PureLink PCR Purification Kit, following the manufacturer's protocol (Life Technologies, Carlsbad, CA). Products were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit and purified using the BigDye XTerminator Purification Kit following the manufacturer's protocols on an AB 3500xL Genetic Analyzer (Applied Biosystems, Grand Island, NY). Ticks were also tested for the presence of *Francisella tularensis* using a multi-target real time PCR test employing primers IS*Ftu*2, *iglC* and *tul4* (Kugeler et al. 2005).

PCR amplification of the cytochrome b gene was used to query the DNA from the ticks for determining the hosts of their prior blood meals using the primers

UNFOR403 and UNREV1025 (Kent and Norris 2005; Lah et al. 2015). PCR reactions were conducted using 2X Taq PCR Master Mix (Qiagen, Valencia, CA) with primer concentrations at 0.2 µM, 8 µL of template per reaction and a total reaction volume of 40 µL. PCR cycling conditions were: denaturation at 94 °C for 3 min followed by 35 cycles of 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 1 min; then final extension at 72 °C for 7 min before holding the PCR products at 4 °C.

For the bacterial community analysis, a segment of the conserved bacterial 16S rRNA gene was amplified using universal primers 515F and 806R that flank the V4 region (Caporaso et al. 2012). The 806R primers also contained a unique 12-nucleotide Golay ‘‘barcode’’ for each sample that allowed us to pool the PCR products from all the samples into one Illumina MiSeq sequencing run. PCR reactions were conducted in a total volume of 40 µL using Taq98® Hot Start 2X Master Mix (Lucigen, Middleton WI) with primer concentrations at 0.2 µM. PCR cycling conditions were: denaturation at 98 °C for 2 min followed by 35 cycles of 98 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; then final extension at 72 °C for 10 min before holding the PCR products at 4 °C. The PCR products were visualized under UV light on 1% agarose gels stained with ethidium bromide before being normalized and sequenced on an Illumina MiSeq instrument by The Scripps Research Institute DNA Array Core Facility using their standard protocols (TSRI, San Diego, CA).

Computational and statistical analyses. The sequence data was analyzed using the QIIME (Quantitative Insights Into Microbial Ecology) version 1.8.0 software program (Caporaso, Kuczynski, et al. 2010). Raw sequence data was demultiplexed into samples by barcode and filtered by mean quality score below 25, homopolymers greater than 6, uncorrected barcodes, barcodes not found in the mapping file, chimeric sequences and mismatched primers. Sequences were grouped into operational taxonomic units (OTUs) at the 97% sequence similarity level using UCLUST (Edgar 2010) and a consensus taxonomic classification was assigned to each representative OTU using the UCLUST classifier with a Greengenes 13_8 reference database (DeSantis et al. 2006) in which at least 90% of the sequences within the OTU matched the consensus taxonomic classification 16S rRNA gene. Sequences were aligned using PyNAST (Caporaso, Bittinger, et al. 2010) against the Greengenes 13_8 reference core set and a phylogenetic tree of the OTUs inferred using FastTree (Price, Dehal, and Arkin 2010). In order to remove spurious OTU's and samples with low numbers of sequences, OTU's that occurred only once in the data and samples with less than 150 OTUs were removed. *Rickettsia, Francisella* and other selected taxonomic sequence identifications were crosschecked against the NCBI nucleotide database using BLASTn. Sequence, OTU table and map files can be downloaded from Figshare: 10.6084/m9.figshare.2056275, 10.6084/m9.figshare.2068644, and 10.6084/m9.figshare.2056272, respectively.

The OTU dataset was rarefied to an even sampling depth of 150 and weighted and unweighted UniFrac distance measures between all pairs of microbial communities were calculated and visualized by principal coordinate analyses (PCoA) (Lozupone and Knight 2005). Rarefaction at 1500 even sampling depth resulted in similar results. The *Rickettsia* and *Francisella* populations were compared to the non-*Rickettsia* non-*Francisella* bacterial populations using a Procrustes analysis (Gower 1975) in QIIME to determine dependencies of the populations. The Pearson product-moment correlation coefficient (PPMC) between *Rickettsia* and *Francisella* was calculated using Social Science Statistics calculator (http://www.socscistatistics.com/tests/Default.aspx). Random forests supervised learning was performed in QIIME using 1000 trees and 10 times cross validation to determine if microbial populations were associated with the presence of spotted fever group *Rickettsia*. Genetic isolation by distance of the microbiomes was determined using the isolation by distance web service http://ibdws.sdsu.edu/~ibdws/distances.html (Jensen, Bohonak, and Kelley 2005). In order to determine which of the abundant genera were responsible for differences in UniFrac measures between locations, OTUs that occurred in less than 10% of the samples were removed and the null hypothesis that abundances of OTUs were the same for all locations was tested using a Kruskal-Wallis H test in QIIME. Similarly, non-*Rickettsia* non-*Francisella* genera within the tick microbiomes that were

associated with high *Rickettsia* to *Francisella* ratios (>5), even *Rickettsia* to *Francisella* ratios (0.2-5) and low *Rickettsia* to *Francisella* ratios (<5) were determined via a Kruskal-Wallis H test.

SourceTracker was used to compare the tick microbial profiles to microbiome datasets of dog, fish, iguana, human, pigeon, rat, and soil. SourceTracker is a tool that uses Bayesian methods to predict the source(s) of microbial communities in a set of samples (sink) (Knights et al. 2011). To test for sources of the tick microbiomes (sink), microbial source tracking was performed on the merged sink and source OTU file. SourceTracker version 1.0 was implemented in QIIME (version 1.9.1) with default settings. As source datasets, we used publicly available sequence data in QIITA (https://qiita.ucsd.edu/) that included 16S rRNA data from a wide range of samples such as canine skin, mouth, and feces (Study ID 1684), human skin, mouth and stool (Study ID 1684), soil (Study ID 1684, 10363), fish, frog, iguana, pigeon, and rat skin (Study ID 1748) and negative water controls (Study ID 10363) as sources. All source and sink samples were sequenced using Illumina and the same 16S rRNA V4 region primers.

Results

Four hundred seventy four ticks were collected. No ticks were positive for *Francisella tularensis,* however, 39 ticks (8.2%) were positive for *R. rhipicephali* and 12 (2.3%) were positive for *R. philipii* 364D as identified by sequencing of the *romp*A gene and IGR. No significant difference in infection rate between male and female ticks by *R. rhipicephali* and *R. philipii* was observed (Fisher's exact test; P=0.47). From this group, one hundred two ticks were selected for Illumina sequencing: 44 *Rickettsia*-positive ticks and 58 *Rickettsia*-negative ticks (forty-five male and fifty-seven female) from the four locations (Table 2.1). Amplification and gel electrophoresis of the V4 segment of the 16S rRNA gene produced visible PCR products of the expected 300 bp size from all ticks, while negative PCR and DNA extraction controls yielded no visible bands. After quality filtering, the total number of sequences was 6,799,927 with sample depths ranging from 2013 to 250403 (Supplemental table 2.1). Clustering sequences at the 97% level of similarity and discarding OTUs that occurred only once yielded 105,174 different OTUs and 535 different taxa including one unassigned taxon. *Rickettsia* and *Francisella* genera were the most prevalent genera present in the ticks, representing 46.8% and 41.4%% of all genera, respectively. The next most frequently occurring genera were *Sphingomonas* (3%), *Methylobacterium* (1%) and *Hymenobacter* (0.4%) (Figure 2.1).

Female ticks had significantly less microbial diversity (alpha diversity) than male ticks (Faith's PD, two sample t-test; t=3.63, P<0.01; Figure 2.2.A). There was no

significant difference in the amount of *Francisella* and *Rickettsia* in male versus female ticks. Escondido Canyon had lower average alpha diversity than Lopez and Peñasquitos canyons, P=0.05 (Figure 2.2.B). Beta diversities of unweighted and weighted tick microbiomes had small but statistically significant associations with location as measured by analysis of similarity (ANOSIM) of UniFrac distances and visualized on Principal coordinate analysis (PCoA) (Figures 2.3.A and 2.3.B). When microbiomes consisting of only *Rickettsia* and *Francisella* genera were assessed for association with location, ANOSIM results were not statistically significant (ANOSIM, unweighted UniFrac; R=-0.06, P=0.92; ANOSIM, weighted UniFrac R=0.02, P=0.13). However, when tick microbiomes excluding *Rickettsia* and *Francisella* were evaluated, association with location was higher and statistically significant (ANOSIM, unweighted UniFrac; R=0.20, P<0.01; ANOSIM, weighted UniFrac; R=0.28, P<0.01). Procrustes least squares orthogonal mapping analysis also demonstrated that *Rickettsia* and *Francisella* did not share the same association with location as seen with microbiomes excluding the main *Rickettsia* and *Francisella* endosymbionts (error, M^2=0.91, P<0.01). Isolation by distance (IBD) analysis of pairwise microbiome unweighted UniFrac distances with geographic distances revealed little geographic IBD (Mantel test, unweighted UniFrac, R=0.09, $P < 0.01$) whereas IBD of microbiomes excluding *Rickettsia* and *Francisella* was greater (Mantel test, unweighted UniFrac, R=0.14, P<0.01). IBD of *Rickettsia* and *Francisella* only was not significant and the null hypothesis was not rejected (Mantel test,

unweighted UniFrac, R=-0.03, *P*=0.74). After removing *Rickettsia* and *Francisella* genera from the dataset, significant differences in the abundances of *Nevskia*, *Curtobacterium* and *Sphingomonas* were observed between locations (Kruskal-Wallis H=25.7, 24.2, 22.9; Bonferroni corrected *P*<0.01, respectively) with Peñasquitos and Lopez Canyons having higher abundances of *Nevskia* than Mission Trails and; Peñasquitos and Lopez Canyons having more *Curtobacterium* and *Sphingomonas* than Escondido Creek and Mission Trails (Table 2.2).

One *Rickettsia* sp. (OTU 83718) accounted for 89% of all *Rickettsia* OTUs and matched 100% to *R. rhipicephali* (GenBank accessions CP013133.1, NR_074473.1, CP003342.1, NR_025921.1, and U11019.1). The next closest matches were to *Rickettsia* sp. Tenjiku01 (GenBank acc. LC089861.1) and several uncultured *Rickettsia* partial 16S rRNA gene sequences (GenBank accs. KF981787.1, KF981786.1) as well as other *Rickettsia* species *Rickettsia aeschlimannii* (GenBank acc. KT318741.1), *R. prowazekii* (GenBank acc. CP004888.1), *R. felis* (GenBank acc. NR_074483.1) and others. The second most abundant OTU (553807) accounted for 0.7% of all Rickettsia OTUs and matched most closely with several different *R. rickettsii* strains including *R. philipii* str. 364D (GenBank NR 074470.1) and other strains of *R. rickettsii* (including GenBank accs. CP006010.1, NR_102941.1, and CP003311.1). All other *Rickettsia* OTUs comprised less than 0.09% of total *Rickettsia* OTUs. OTU 840032 comprised 87.4% of all *Francisella* OTUs and matched 100%

with *Francisella*-like endosymbiont (FLE) of *D. occidentalis* (GenBank accs. AY805304.1, and AY375402.1). The next closest matches were *Francisella* endosymbionts of other tick species *D. albipictus*, *D. andersoni* and *D. variabilis* (GenBank accs. GU968868.1, FJ468434.1, and AY805307.1, respectively). The next most abundant OTU (399541) (GenBank acc. KU355875.1, this paper) accounted for 3.1% of all *Francisella* OTUs and matched 97% with gene sequences of endosymbionts previously determined from a spectrum of *Dermacentor* species including *Dermacentor occidentalis (*AY375403.1*), D. albipictus (*GU968868.1), *D. variabilis (*AY805307.1), *D. nitens (*AY375401.1*) and D. andersoni (*AY375398.1). All other *Francisella* OTUs accounted for less than 0.4% of the total *Francisella* OTUs.

Interestingly, *Rickettsia* and *Francisella* were negatively correlated in the ticks (Pearson's product moment correlation; R=-0.44, $P<0.01$; Figure 2.4). In order to assess whether the tick microbiomes were predictive of infection with spotted fever group *Rickettsia*, a random forests supervised learning analysis using 1000 trees and 10x cross validation was performed on the OTU dataset minus *Rickettsiaceae* and *Rickettsia* OTUs. The ratio of baseline error to the estimated generalization error was 8.8, which indicates that the classifier was greater than eight times more predictive than random chance. A ratio greater than 2 is accepted as a good classifier result (http://qiime.org/tutorials/running_supervised_learning.html, (Knights, Costello, and

Knight 2011). The most predictive OTU was the FLE OTU 840032 and it accounted for 13% of the model. OTUs 866436 and 639277 each accounted for 3% of the model and the closest database matches to it were the Firmicutes *Geobacillus* and *Aeribacillus (Geobacillus),* respectively (Miñana-Galbis, Pinzón, Lorén, Manresa, & Oliart-Ros, 2010). Non-*Rickettsia* non-*Francisella* bacteria associated with *Francisella* to *Rickettsia* >2 (range 2.4-119.0) were *Planococcaceae* and *Geobacillus* (Kruskal-Wallis test; H=23.8, 14.2, Bonferroni $P<0.001$ and $P=0.011$, respectively).

Amplification of vertebrate cytochrome b gene was attempted to determine the origin of the ticks' host blood meals. Although positive and negative controls worked, no cytochrome b was amplified from the ticks, which is not surprising since the ticks collected were questing in search of a blood meal (data not shown). However, SourceTracker analysis revealed that 31% of ticks had microbiomes that were 1.1-27.4% similar to dog skin microbiomes (Supplemental table 2.2). Ticks negative for *R. philipii* or *R. rhipicephali* were more likely to have microbiomes similar to dog skin than ticks that were infected with *R. philipii* or *R. rhipicephali* (Student's *T*-test; $t=2.90$, $P<0.01$). *Sphingomonadaceae*, *Oxalobacteraceae*, and *Comamonadaceae* were the most abundant families of bacteria shared between tick and dog skin microbiomes. *Geobacillus* (OTU 4414596) and *Planococcaceae* (OTU 219154) were also present in both microbiomes. The tick microbiomes were less than 1% similar to

microbiomes of the skins of fish, iguana, pigeon, rat, and human, as well as human oral, plant and soil microbiomes (Supplemental table 2.2).

Discussion

This is the first study of the microbiome of *D. occidentalis* ticks using next generation sequencing techniques to examine geographical associations and pathogen interference within the tick microbiome. *D. occidentalis* is one of the most common tick species found in San Diego and is a vector of human pathogens including *Francisella tularensis* and *Rickettsia philipii*. Although *Francisella tularensis* has been detected previously in ticks in San Diego (Kugeler et al. 2005), none of the ticks harbored this bacterium or genera of other recognized zoonotic tick-borne pathogens such as *Borrelia*, *Anaplasma*, *Ehrlichia*, *Babesia* or *Bartonella*; however, a low percentage of the ticks were infected with spotted fever group *Rickettsia*: 2.5% with *R. philipii* and 8.2% with *R. rhipicephali*. This is a slightly lower prevalence of *R. philipii* than surveys of ticks performed in Orange, Riverside, Los Angeles, Santa Barbara and Ventura counties north of San Diego, that reported an overall 7.5% prevalence of *R. philipii* (Wikswo et al. 2008) but is within the range of *R. philipii* prevalence reported from northern California of 0.4-5.1% (Lane et al. 1981; Philip, Lane, and Casper 1981). Similar to other tick species, the microbiome of *D. occidentalis* was dominated

by Proteobacteria, primarily *Rickettsia* or *Francisella,* with much lesser amounts of *Sphingomonas*, *Methylobacterium* and *Hymenobacter* (*Bacteroidetes*). These last three genera are all decomposer microbes found in the soil and except for *Hymenobacter*, have been detected in other tick microbiome studies. Even though the ticks were washed multiple times before DNA extraction, the possibility that some of these represent surface bacteria cannot be completely excluded.

Although 58 of the ticks were negative for SFGR by real-time PCR assays, all of the ticks contained OTUs whose partial 16S rRNA gene segments aligned with SFGR in GenBank. The cause of this discrepancy may be due to the increased sensitivity of the Illumina sequencing platform compared to real-time PCR of *romp*A sequences and/or the presence of other *Rickettsia* spp. with highly conserved 16S rRNA genes but that lack *romp*A sequences complementary to the PCR primers used. Analysis of other genes would be required to resolve them at the species level (Regnery, Spruil, and Plikaytis 1991; Eremeeva, Yu, and Raoult 1994). Additional data support that more than two different *Rickettsia* species were present within the tick population tested. *R. rhipicephali* was detected by real-time PCR of the *romp*A gene in ticks that had OTU 837189 counts greater than 5900/tick, except for two ticks, T14-0667 and T14-0769 that had high OTU 837189 counts of 73,527 and 53,714, respectively, but were negative for *R. rhipicephali*. Similarly, *R. philipii* was detected in ticks with OTU 553807 counts ranging from 11 to 2158, except for one sample,

T14-0667, that had 884 counts of OTU 553807 yet was negative for *R. philipii* by real-time PCR of *romp*A gene. These findings are consistent with the presence of species of *Rickettsia* different from *R. rhipicephali* and *R. philipii* that could not be discriminated by the partial 16S rRNA gene or *romp*A sequences.

The two most abundant *Francisella* OTUs, 840032 and 399541, accounted for over 90% of all *Francisella* OTUs and were 100% identical to *Francisella*-like endosymbionts (FLE) of *D. occidentalis* (GenBank accs. AY805304 and AY375402 for OTU 840032, and KU355875 for OTU 399541). Taken as a whole, these results are consistent with tick co-infection with a mixture of *Rickettsias* and FLEs.

Similar to *Ixodes scapularis* and *Amblyomma americanum* ticks, female *D. occidentalis* ticks harbored a less diverse array of bacteria than males (Figure 2.2.A) (Ponnusamy et al. 2014; van Treuren et al. 2015). Although previous studies attributed the difference to the higher abundance of *Rickettsia* in females than males, in *D. occidentalis*, the amounts of *Rickettsia* or *Francisella* did not differ significantly between male and female ticks. Endosymbionts belonging to *Rickettsia*, *Coxiella*, *Francisella* and *Arsenophous* genera have been found in different tick species and are thought to interfere and partially exclude other bacteria and pathogenic forms of closely related organisms from transovarial transmission (Burgdorfer and Brinton

1975; Macaluso et al. 2002; Niebylski, Peacock, and Schwan 1999; Noda, Munderloh, and Kurtti 1997; Reinhardt, Aeschlimann, and Hecker 1972; Telford III 2009). However, factors causing the difference in alpha diversity between male and female ticks do not appear to be attributed to *Rickettsia* or FLE in *D. occidentalis*.

The beta diversity of the endosymbiont and non-endosymbiont compartments differed. Although the non-endosymbiont compartment demonstrated a small association with location, geographic association was not observed by the *Rickettsia* and *Francisella* endosymbionts. In addition, Procrustes analysis results demonstrated non-concordance between *Rickettsia* and FLE presence and the remaining microbiome components with respect to geographical location, illustrating that different factors shape these components of the *D. occidentalis* microbiomes. One factor that appeared to contribute to the geographical differences in the non-endosymbiont microbiome was isolation by distance. Differential geographic localization of *Nevskia*, *Curtobacterium* and *Sphingomonas*, genera that are associated with environmental sources such as the air-water interface (*Nevskia*) and soils (*Curtobacterium* and *Sphingomonas*), may be the result of differences in soil microbial ecology at each location. Alternatively, non-endosymbiont microbiome differences could be the result of different populations of ticks at each location. In contrast, the dependency of *Rickettsia* and *Francisella* endosymbionts on their *D. occidentalis* host likely restricted the degree of variation that population separation could impart upon these

endosymbionts.

One of the primary hypotheses of this study was to determine if bacteria competitively excluded one another within ticks especially as it relates to pathogens. Indeed, a strong inverse relationship was observed between *Rickettsia* and FLE infection (Pearson's product moment correlation $R=-0.44$, $p<0.01$, Figure 2.4) and a Random Forests supervised learning model successfully predicted the absence of SFGR within the ticks (baseline error:observed error=8.8; an error ratio≥2 is significant). Not surprisingly, FLE OTU 840032 contributed most to the model. FLE and different uncategorized *Rickettsia* co-infection in ticks has been previously observed but not enumerated (Niebylski et al. 1997; Scoles 2004) and partial interference between co-infection by different *Rickettsia* species has been demonstrated (Burgdorfer, Hayes, and Mavros 1981; Macaluso et al. 2002). However, the quantitative data of FLE and *Rickettsia* co-infection in this study are the first suggestion that competitive exclusion between FLE and *Rickettsias* may occur.

The mechanisms by which *Rickettsia* and *Francisella* interfere with each other in co-infections are not known. Although the localization of *R. rhipicephali* and *R. philipii* within ticks has not been determined, FLEs have been found in female tick reproductive tissues and hemolymph (Scoles 2004; Goethert and Telford 2005). In

addition, non-*Francisella* microbes were also associated with low *Rickettsia* to *Francisella* ratios. *Planococcaceae* and *Geobacillus* were associated with greater abundance of *Francisella* relative to *Rickettsia* within the ticks (Kruskal-Wallis H=23.8, 14.2; $P<0.001$, 0.011, respectively). Interestingly, although blood meals of the ticks could not be detected by amplification of vertebrate cytochrome b gene from the ticks, 31% of the tick microbiomes had microbiome components similar to canine skin (which may be similar to coyote skin) and suggests the source of a prior blood meal if they incorporated some of the skin flora into their own microbiome. In addition, ticks with dog skin microbiome components were less likely to be infected with *R. philipii* or *R. rhipicephali* which is consistent with *R. rhipicephali* and *R. philipii* being endosymbionts without a canine host. Both *Geobacillus* and *Planococaccaceae* were present in dog microbiomes as well. *Geobacillus* also demonstrated a negative association with *Rickettsia* infection in the Random Forests model. *Geobacillus* are thermophilic gram-positive bacteria and have been explored for use in biofuel synthesis due to their ability to catabolize hemicellulose and starch (Hussein, Lisowska, and Leak 2015). The *Planococcaceae* family belongs to the *Firmicute* phylum and consists of 14 soil dwelling genera, some of which have been proposed to have possible applications in bioremediation (Shivaji, Srnivas, and Reddy 2013). Their interactions with endosymbionts, much less *Rickettsia* or *Francisella,* have not been described, thus, how they might influence *Rickettsia* or *Francisella* co-infection is unknown. Unfortunately, a microbiome dataset of another common tick

blood meal host, i.e. deer, was not available for comparison.

Conclusion

The presence of an endosymbiont and other bacteria that may exclude pathogenic bacteria from a tick vector is an exciting prospect. In many cases, chemical control of ticks with organophosphates, pyrethrins or pyrethroids in the environment is neither feasible nor desired due to deleterious affects on other insects and wildlife. Although antibiotic treatment of ticks was demonstrated to reduce fitness in some ticks or their ability to vector pathogens (Narasimhan et al. 2014; Zhong, Jasinskas, and Barbour 2007) their use as a means of chemical tick control does not appear to be a feasible alternative due to the obvious risks of promoting antibiotic resistance. A biological control of tick's ability to transmit pathogens, however, could prove to be a more sustainable solution and be less intrusive to the environment. This technique has been employed for mosquito control by releasing *Wolbachia*-infected mosquitoes to reduce mosquito abundance and vectoral capacity (Iturbe-Ormaetxe, Walker, and O' Neill 2011). Whether, FLEs can be shown to inhibit *Rickettsia* co-infection in the laboratory and could be propagated through a tick population as a means to render the ticks unable to vector pathogenic *Rickettsia* or other pathogens is an intriguing possibility that warrants further exploration.

Acknowledgement

Chapter 2 was prepared for submission for publication regarding endosymbiotic bacterial phenomena in *Dermacentor occidentalis* ticks. The dissertation author was the primary investigator and author of this material. Its coauthors were Drs. Saran Grewal and Lynnie Cua from the Department of Environmental Health, County of San Diego, San Diego, CA USA, and Pedro Torres and Professor Scott T. Kelley from the Department of Biology, San Diego State University, San Diego, CA, USA.

Table 2.1. Tick collection locations, number of ticks infected with spotted fever group *Rickettsia*, and number of male and female *D. occidentalis* ticks collected at each location.

Location	GPS Coordinates	*R. rhipicephali*	*R. philipii*	Negative	M/F*
Escondido Creek	33.060700, -117.179500	7	1	9	8/9
Lopez Canyon	32.906776, -117.202964	14	9	22	23/22
Mission Trails	32.834444, -117.045833	4	1	19	7/17
Peñasquitos Canyon	32.938638, -117.130351	7	1	8	7/9

*No statistically significant association between SFGR infection and male versus female, Fisher's exact test; *P*>0.5

Table 2.2. OTUs and genera associated with different locations.

OTU	Genus	H*	*P***	Escondido Creek+	Mission Trails+	Peñasquitos Cyn+	Lopez Cyn+
73481	*Nevskia*	25.7	0.0002	1.59	0.04	2.31	1.09
643513	*Curtobacterium*	24.2	0.0004	0.18	0.25	3.06	1.91
489455	*Sphingomonas*	22.9	0.0007	0.12	0.13	3.69	2.09

* Kruskal-Wallis H value
**Bonferroni correction (Bonferroni correction is used to reduce the chances of obtaining false-positive results (type I errors) when multiple pair wise tests are performed on a single set of data because the probability of identifying at least one significant result due to chance increases as more hypotheses are tested.)
+ Average number of OTU occurrences per sample

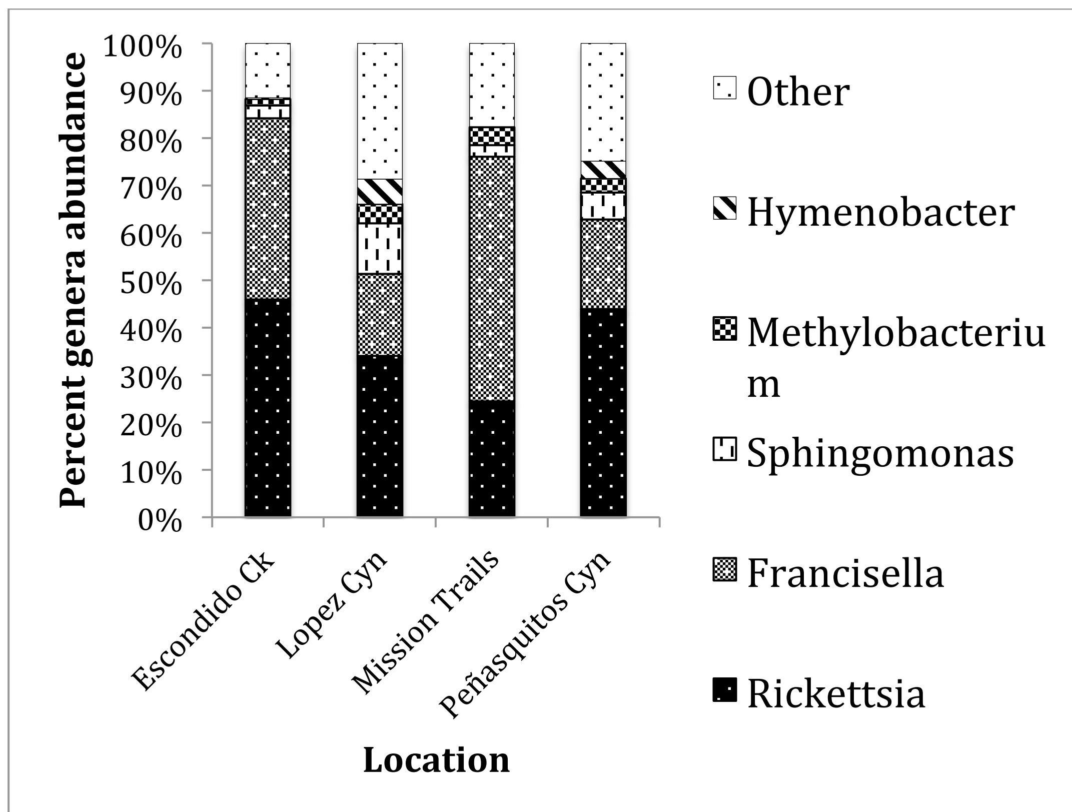


Figure 2.1. Most abundant bacterial genera detected in *D. occidentalis* from four different locations in San Diego County.

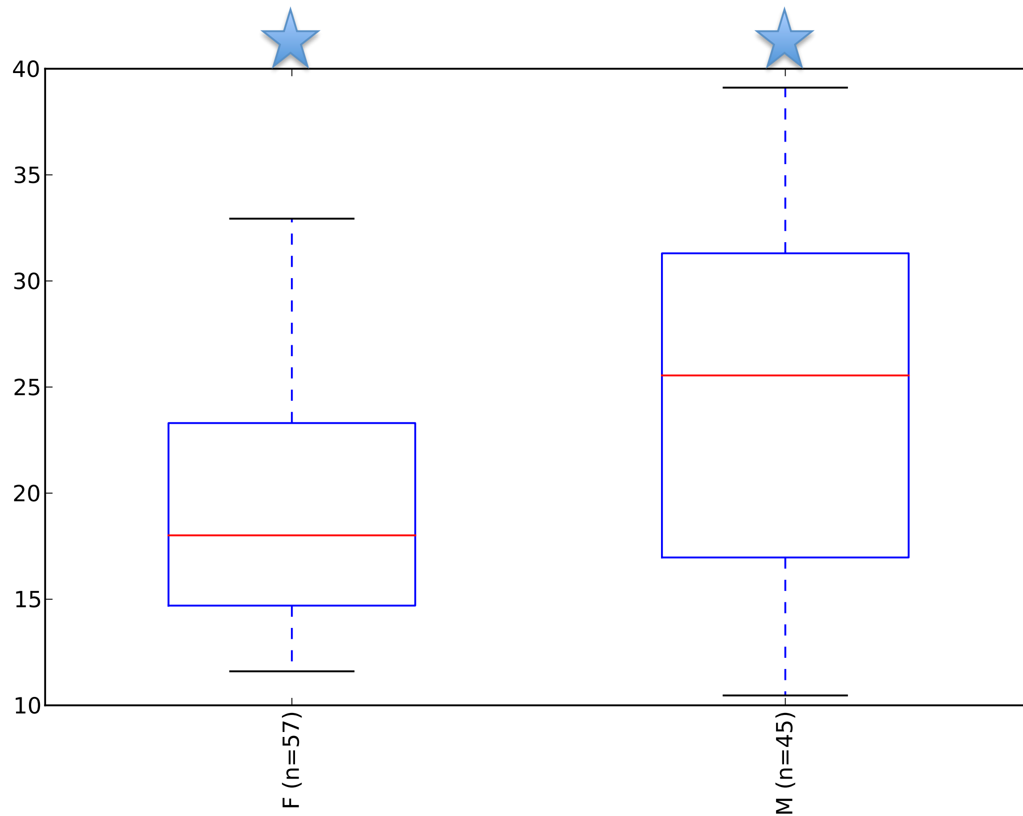


Figure 2.2.A. Boxplot of microbiome alpha diversity in *D. occidentalis* ticks measured by Faith's phylogenetic diversity (PD) whole tree as implemented in QIIME of male and female *D. occidentalis*. Stars indicate statistically significant differences between samples; Faith's PD, two sample *t*-test, male versus female; $t=3.63$, $P<0.01$.

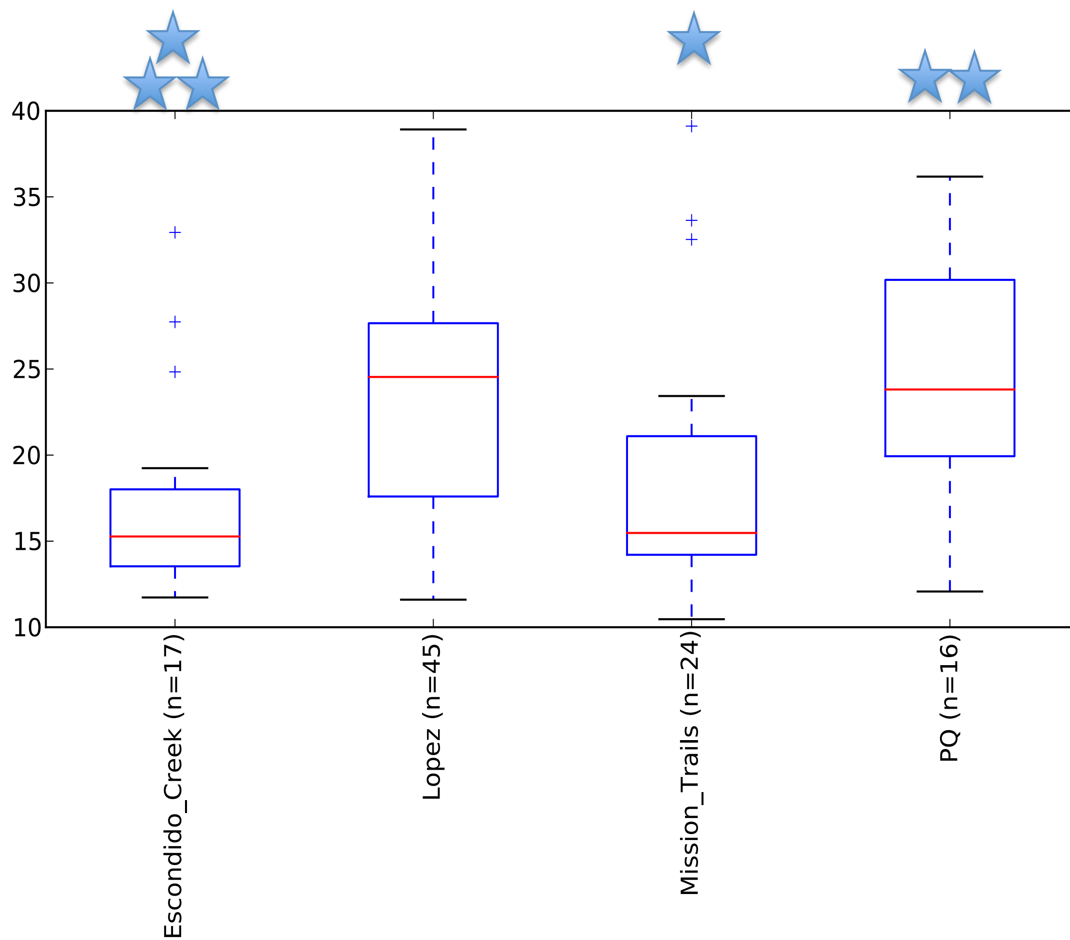


Figure 2.2.B. Boxplot of microbiome alpha diversity in *D. occidentalis* ticks measured by Faith's phylogenetic diversity (PD) whole tree as implemented in QIIME of four different hiking areas in San Diego County. Stars in indicate statistically significant differences between samples; Faith's PD, two sample *t*-test, Escondido Creek versus Lopez Canyon; t=-3.28, *P*=0.02; Escondido Creek versus PQ, t=-3.31; *P*=0.04; other comparisons were not statistically significant. PQ = Peñasquitos Canyon.

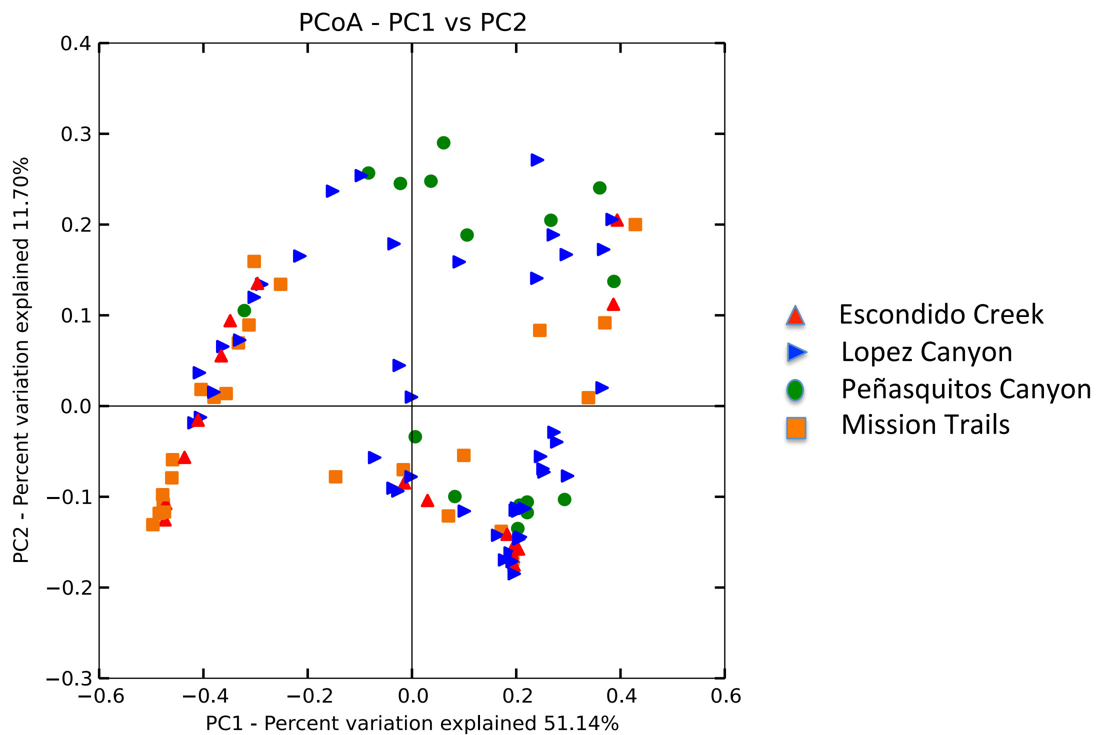
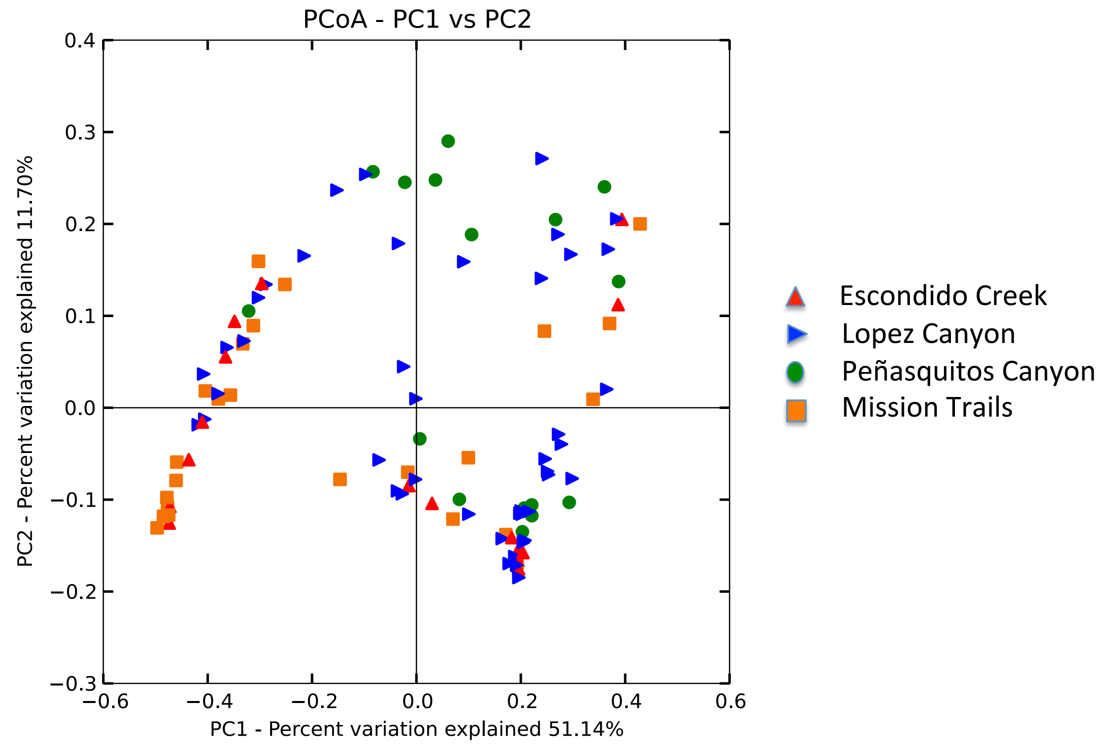


Figure 2.3.A. Unweighted beta diversity of *D. occidentalis* microbiomes at four different locations in San Diego County. ANOSIM, unweighted UniFrac; R=0.14, *P*<0.01.



Figures 2.3.B. Weighted beta diversity of *D. occidentalis* microbiomes at four different locations in San Diego County. ANOSIM, weighted UniFrac; R=0.12, *P*=0.01.

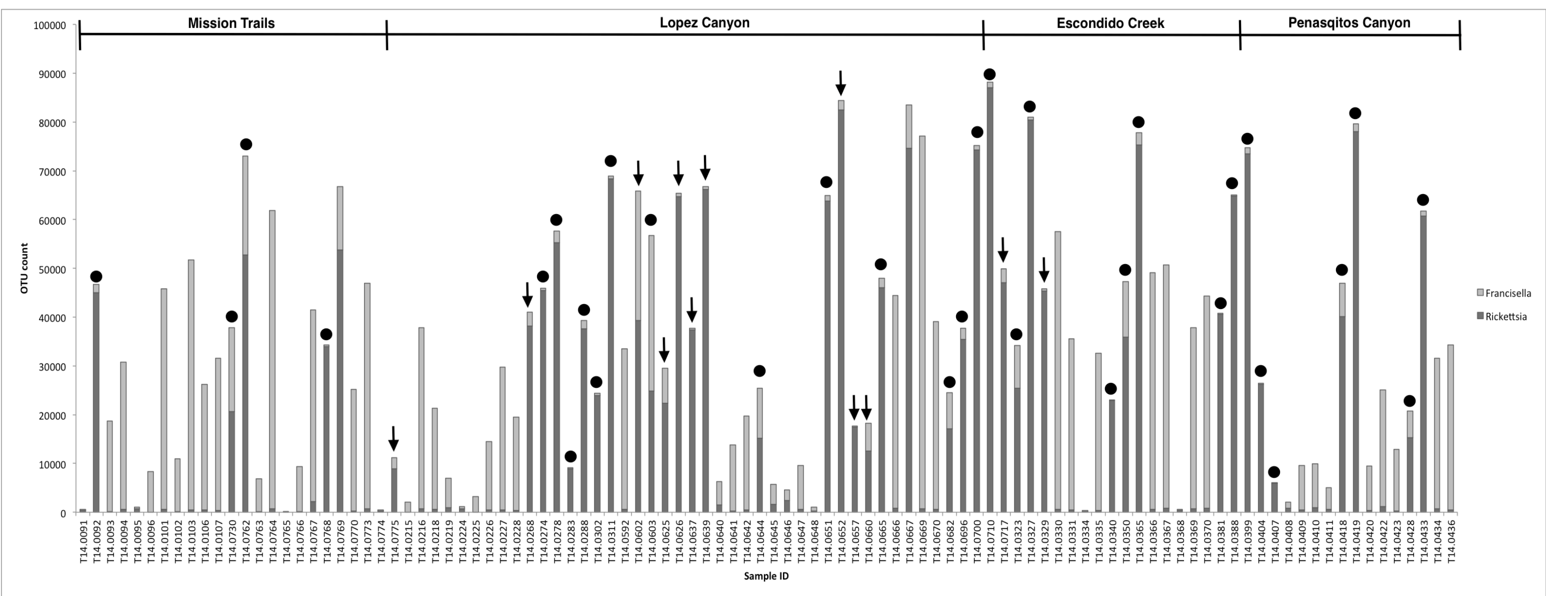


Figure 2.4. *Rickettsia* and *Francisella* OTU abundance in *D. occidentalis* ticks in San Diego County. Circles indicate ticks infected with *R. rhipicephali* and arrows indicate ticks infected with *R. philipii* 364D. Pearson product moment correlation; R=-0.44, *P*<0.01

Supplemental table 2.1. Spotted fever group rickettsia identified and total number of individual 16S rRNA gene sequences from each tick.

Tick	Location	SFGR ID	No. sequences
T14.0091	Mission Trails		48409
T14.0092	Mission Trails	R.rhipicephali	55867
T14.0093	Mission Trails		24444
T14.0094	Mission Trails		66297
T14.0095	Mission Trails		87380
T14.0096	Mission Trails		10803
T14.0101	Mission Trails		58369
T14.0102	Mission Trails		13288
T14.0103	Mission Trails		65361
T14.0106	Mission Trails		41139
T14.0107	Mission Trails		38876
T14.0215	Lopez Canyon		3699
T14.0216	Lopez Canyon		68183
T14.0218	Lopez Canyon		61577
T14.0219	Lopez Canyon		74014
T14.0224	Lopez Canyon		88047
T14.0225	Lopez Canyon		4767
T14.0226	Lopez Canyon		49576
T14.0227	Lopez Canyon		47685
T14.0228	Lopez Canyon		36930
T14.0268	Lopez Canyon	R.philipii	78687
T14.0274	Lopez Canyon	R. rhipicephali	119650
T14.0278	Lopez Canyon	R.rhipicephali	96916
T14.0283	Lopez Canyon	R. rhipicephali	20188
T14.0288	Lopez Canyon	R. rhipicephali	68237
T14.0302	Lopez Canyon	R. rhipicephali	32371
T14.0311	Lopez Canyon	R.rhipicephali	142563
T14.0323	Escondido Creek	R.rhipicephali	41069
T14.0327	Escondido Creek	R.rhipicephali	96110
T14.0329	Escondido Creek	R.philipii	66060
T14.0330	Escondido Creek		73657
T14.0331	Escondido Creek		46351
T14.0334	Escondido Creek		12990
T14.0335	Escondido Creek		42845
T14.0340	Escondido Creek	R.rhipicephali	30688
T14.0350	Escondido Creek	R.rhipicephali	58470
T14.0365	Escondido Creek	R.rhipicephali	97560

Supplemental table 2.1. Continued.

T14.0366	Escondido Creek		88178
T14.0367	Escondido Creek		82780
T14.0368	Escondido Creek		58058
T14.0369	Escondido Creek		57198
T14.0370	Escondido Creek		81748
T14.0381	Escondido Creek	R.rhipicephali	50800
T14.0388	Escondido Creek	R.rhipicephali	82511
T14.0399	Penasquitos Canyon	R.rhipicephali	98911
T14.0404	Penasquitos Canyon	R.rhipicephali	36606
T14.0407	Penasquitos Canyon	R.rhipicephali	8526
T14.0408	Penasquitos Canyon		68734
T14.0409	Penasquitos Canyon		43102
T14.0410	Penasquitos Canyon		113854
T14.0411	Penasquitos Canyon		66105
T14.0418	Penasquitos Canyon	R.rhipicephali	56439
T14.0419	Penasquitos Canyon	R.rhipicephali	125331
T14.0420	Penasquitos Canyon		61818
T14.0422	Penasquitos Canyon		137433
T14.0423	Penasquitos Canyon		82919
T14.0428	Penasquitos Canyon	R.rhipicephali	29414
T14.0433	Penasquitos Canyon	R.rhipicephali	128714
T14.0434	Penasquitos Canyon		88454
T14.0436	Penasquitos Canyon		60751

Supplemental table 2.1. Continued.

T14.0592	Lopez Canyon		68864
T14.0602	Lopez Canyon	R.philipii	102889
T14.0603	Lopez Canyon	R.rhipicephali	108402
T14.0625	Lopez Canyon	R.philipii	43244
T14.0626	Lopez Canyon	R.philipii	97349
T14.0637	Lopez Canyon	R.philipii	62484
T14.0639	Lopez Canyon	R.philipii	93587
T14.0640	Lopez Canyon		138880
T14.0641	Lopez Canyon		42241
T14.0642	Lopez Canyon		91657
T14.0644	Lopez Canyon	R.rhipicephali	35278
T14.0645	Lopez Canyon		95138
T14.0646	Lopez Canyon		250403
T14.0647	Lopez Canyon		67944
T14.0648	Lopez Canyon		43399
T14.0651	Lopez Canyon	R.rhipicephali	79939
T14.0652	Lopez Canyon	R.philipii	102212
T14.0657	Lopez Canyon	R.philipii	24374
T14.0660	Lopez Canyon	R.philipii	22449
T14.0665	Lopez Canyon	R.rhipicephali	57326
T14.0666	Lopez Canyon		87494
T14.0667	Lopez Canyon		105711
T14.0669	Lopez Canyon		106398
T14.0670	Lopez Canyon		58988
T14.0682	Lopez Canyon	R.rhipicephali	34666
T14.0696	Lopez Canyon	R.rhipicephali	97800
T14.0700	Lopez Canyon	R.rhipicephali	106720
T14.0710	Lopez Canyon	R.rhipicephali	106684
T14.0717	Lopez Canyon	R.philipii	69047
T14.0730	Mission Trails	R. rhipicephali	47750
T14.0762	Mission Trails	R. rhipicephali	91329
T14.0763	Mission Trails		14772
T14.0764	Mission Trails		82606
T14.0765	Mission Trails		2013
T14.0766	Mission Trails		15661
T14.0767	Mission Trails		77976
T14.0768	Mission Trails	R.rhipicephali	43455
T14.0769	Mission Trails		93720
T14.0770	Mission Trails		37599

Supplemental table 2.1. Continued.

T14.0773	Mission Trails		70691
T14.0774	Mission Trails		28299
T14.0775	Mission Trails	R.philipii	14981

Supplemental table 2.2. SourceTracker results for *D. occidentalis* microbiomes from San Diego County. EC=Escondido Creek; LC=Lopez Canyon; MT=Mission Trails, PC=Peñasquitos Canyon.

Sample	SFG	Location	Control	Dog skin	Fish skin	Frog skin	Human skin	Iguana skin	Human oral	Pigeon skin	Plant	Rat skin	Soil	Unk
T14.0330	neg	EC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0331	neg	EC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0334	neg	EC	0.0000	0.0610	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.9387
T14.0335	neg	EC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.9997
T14.0366	neg	EC	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9998
T14.0367	neg	EC	0.0000	0.0000	0.0001	0.0000	0.0000	0.0008	0.0000	0.0000	0.0001	0.0000	0.0000	0.9990
T14.0368	neg	EC	0.0000	0.0323	0.0000	0.0000	0.0000	0.0043	0.0000	0.0000	0.0005	0.0000	0.0017	0.9612
T14.0369	neg	EC	0.0000	0.0110	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9888
T14.0370	neg	EC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.9999
T14.0329	R.philipii	EC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0323	R.rhipicephali	EC	0.0000	0.0002	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9997
T14.0327	R.rhipicephali	EC	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999
T14.0340	R.rhipicephali	EC	0.0000	0.0015	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.9983
T14.0350	R.rhipicephali	EC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0365	R.rhipicephali	EC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0381	R.rhipicephali	EC	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999
T14.0388	R.rhipicephali	EC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0215	neg	LC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0216	neg	LC	0.0000	0.0226	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0003	0.9768
T14.0218	neg	LC	0.0000	0.0326	0.0000	0.0000	0.0001	0.0000	0.0000	0.0003	0.0000	0.0000	0.0003	0.9667
T14.0219	neg	LC	0.0000	0.2071	0.0001	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.0005	0.7921
T14.0224	neg	LC	0.0000	0.1967	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.8031
T14.0225	neg	LC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0226	neg	LC	0.0000	0.1256	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.8743
T14.	neg	LC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99

Supplemental table 2.2. Continued.

0227			00	17	00	00	01	00	00	00	00	00	00	82
T14.0228	neg	LC	0.0000	0.0253	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9747
T14.0592	neg	LC	0.0000	0.0437	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.9561
T14.0640	neg	LC	0.0000	0.1252	0.0001	0.0000	0.0000	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.8744
T14.0641	neg	LC	0.0000	0.1105	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.0001	0.0000	0.0000	0.8892
T14.0642	neg	LC	0.0000	0.0698	0.0005	0.0002	0.0005	0.0001	0.0000	0.0000	0.0004	0.0002	0.0001	0.9281
T14.0645	neg	LC	0.0000	0.1831	0.0000	0.0000	0.0003	0.0006	0.0000	0.0000	0.0002	0.0000	0.0000	0.8158
T14.0646	neg	LC	0.0000	0.1442	0.0000	0.0000	0.0018	0.0006	0.0000	0.0000	0.0000	0.0000	0.0000	0.8534
T14.0647	neg	LC	0.0000	0.1037	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.8963
T14.0648	neg	LC	0.0000	0.2927	0.0002	0.0000	0.0000	0.0048	0.0000	0.0000	0.0000	0.0000	0.0000	0.7023
T14.0666	neg	LC	0.0000	0.0480	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.9518
T14.0667	neg	LC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0669	neg	LC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.9992
T14.0670	neg	LC	0.0000	0.0021	0.0000	0.0000	0.0033	0.0003	0.0000	0.0000	0.0010	0.0000	0.0000	0.9933
T14.0268	R.philipii	LC	0.0000	0.0021	0.0000	0.0000	0.0001	0.0002	0.0000	0.0000	0.0002	0.0000	0.0000	0.9974
T14.0602	R.philipii	LC	0.0000	0.0005	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9995
T14.0625	R.philipii	LC	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999
T14.0626	R.philipii	LC	0.0000	0.0001	0.0000	0.0000	0.0004	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.9994
T14.0637	R.philipii	LC	0.0000	0.0001	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.9995
T14.0639	R.philipii	LC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0652	R.philipii	LC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0657	R.philipii	LC	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9998
T14.0660	R.philipii	LC	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999
T14.0717	R.philipii	LC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0274	R.rhipicephali	LC	0.0000	0.1270	0.0000	0.0000	0.0119	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.8611
T14.0278	R.rhipicephali	LC	0.0000	0.0562	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9438
T14.0283	R.rhipicephali	LC	0.0000	0.1027	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0003	0.0000	0.0008	0.8961
T14.0288	R.rhipicephali	LC	0.0000	0.0627	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.9371
T14.0302	R.rhipicephali	LC	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9998

Supplemental table 2.2. Continued.

T14.0311	R.rhipicephali	LC	0.0000	0.0753	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001	0.9244
T14.0603	R.rhipicephali	LC	0.0000	0.0513	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9486
T14.0644	R.rhipicephali	LC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0651	R.rhipicephali	LC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0665	R.rhipicephali	LC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0682	R.rhipicephali	LC	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0696	R.rhipicephali	LC	0.0000	0.0836	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9163
T14.0700	R.rhipicephali	LC	0.0000	0.0221	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.9777
T14.0710	R.rhipicephali	LC	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999
T14.0091	neg	MT	0.0000	0.2995	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001	0.0006	0.6995
T14.0093	neg	MT	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0094	neg	MT	0.0000	0.0007	0.0001	0.0001	0.0000	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.9988
T14.0095	neg	MT	0.0000	0.1547	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.0002	0.0005	0.0005	0.8439
T14.0096	neg	MT	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9999
T14.0101	neg	MT	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0102	neg	MT	0.0000	0.0000	0.0000	0.0000	0.0003	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.9996
T14.0103	neg	MT	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.9999
T14.0106	neg	MT	0.0000	0.0008	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0001	0.0000	0.9990
T14.0107	neg	MT	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0763	neg	MT	0.0000	0.0000	0.0000	0.0000	0.0010	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.9988
T14.0764	neg	MT	0.0000	0.0000	0.0001	0.0000	0.0000	0.0003	0.0000	0.0000	0.0002	0.0000	0.0000	0.9994
T14.0765	neg	MT	0.0000	0.0002	0.0002	0.0004	0.0000	0.0334	0.0000	0.0000	0.0002	0.0000	0.0000	0.9656
T14.0766	neg	MT	0.0000	0.0001	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9998
T14.0767	neg	MT	0.0000	0.0000	0.0005	0.0000	0.0000	0.0019	0.0000	0.0000	0.0000	0.0000	0.0000	0.9976
T14.0769	neg	MT	0.0000	0.0002	0.0000	0.0000	0.0001	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.9995
T14.0770	neg	MT	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999
T14.	neg	MT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Supplemental table 2.2. Continued.

0773			00	00	00	00	00	00	00	00	00	00	00	00
T14.0774	neg	MT	0.0000	0.0429	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.9570
T14.0775	R.philipii	MT	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0092	R.rhipicephali	MT	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0730	R.rhipicephali	MT	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999
T14.0762	R.rhipicephali	MT	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000
T14.0768	R.rhipicephali	MT	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.9999
T14.0408	neg	PC	0.0000	0.2303	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.7697
T14.0409	neg	PC	0.0000	0.0496	0.0000	0.0000	0.0002	0.0007	0.0000	0.0000	0.0001	0.0000	0.0000	0.9494
T14.0410	neg	PC	0.0000	0.1263	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.8736
T14.0411	neg	PC	0.0000	0.3948	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0004	0.0000	0.0002	0.6045
T14.0420	neg	PC	0.0000	0.0437	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9563
T14.0422	neg	PC	0.0000	0.0067	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9932
T14.0423	neg	PC	0.0000	0.1167	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.8832
T14.0434	neg	PC	0.0000	0.0922	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001	0.9076
T14.0436	neg	PC	0.0000	0.0166	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9833
T14.0399	R.rhipicephali	PC	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.9997
T14.0404	R.rhipicephali	PC	0.0000	0.0104	0.0001	0.0000	0.0001	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.9893
T14.0407	R.rhipicephali	PC	0.0000	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9843
T14.0418	R.rhipicephali	PC	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999
T14.0419	R.rhipicephali	PC	0.0000	0.0306	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9693
T14.0428	R.rhipicephali	PC	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999
T14.0433	R.rhipicephali	PC	0.0000	0.1227	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.8772

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CHAPTER 3

Prevalence of Spotted Fever Group *Rickettsia* in *Dermacentor occidentalis* ticks in San Diego County

Abstract

Dermacentor occidentalis Marx, the Pacific Coast tick, is distributed in throughout coastal and mountain areas of California and is a vector of many bacteria, including pathogenic and non-pathogenic spotted fever group *Rickettsia* (SFGR). The prevalence of SFGR in *D. occidentalis* has been determined in other regions of California, but a comprehensive survey has never been performed in San Diego County. From 2011-2014, we collected ticks from four different watershed areas, to identify SFGR species and determine their abundance. Overall, we found a low prevalence of the pathogenic *Rickettsia philipii* strain 364D and greater amounts of non-pathogenic *Rickettsia rhipicephali*. *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever, was detected once. The proportions of the SFGR species varied between locations and from year-to-year and overall frequencies did not track with regional climatic variables.

Introduction

Rickettsiae are small, gram negative, rod-shaped, obligate intracellular alphaproteobacteria that are responsible for several of the oldest zoonoses known to man. Genetically, *Rickettsiae* lie between viruses and bacteria and are phylogenetically related to the ancestral bacteria that gave rise to mitochondria (Andersson et al. 1998). They are vectored by arthropods such as ticks, fleas, lice and mites. Clinical manifestations of the prototypical tick-borne rickettsiosis, Rocky Mountain Spotted Fever (RMSF), include headache, fever, muscle aches and occasionally, a rash, and were first described by Edward E. Maxey in 1899 (Maxey 1899). Howard T. Ricketts, an American pathologist, subsequently elucidated one of its tick vectors, the wood tick *Dermacentor andersoni* (Ricketts 1906a; Ricketts 1906b; Groß and Schäfer 2011). The causative agent, *Rickettsia rickettsii*, is found throughout north, central and south America and can be vectored by a number of different hard shelled ticks, including *Dermacentor andersoni* (wood tick), *Dermacentor occidentalis* (American dog tick) and *Rhipicephalus sanguineus* (brown dog tick) (Burgdorfer et al., 1975; Rotramel, Schwan, & Doty, 1976). Recent outbreaks of RMSF in Mexico and Arizona have claimed the lives of over a dozen adults and children and sickened hundreds (Demma et al. 2005; Sanchez et al. 2009). A study conducted of ticks and dogs in Mexicali, Mexico and the US/Mexico border area of Baja California found 32% of *Rhipicephalus sanguineus* ticks that were parasitizing dogs tested positive for *R. rickettsii* (Eremeeva et al., 2011). *R. sanguineus* prefers to feed on dogs and humans and survives well indoors in cracks and crevices, making it particularly well adapted for transmitting disease between dogs and people.

Interestingly, infection of *R. sanguineus* by *R. rickettsii* in the border region appears to be limited to the Mexican side of the border. Despite a porous border that is frequently crossed by dogs and other animals, two studies of *R. sanguineus* ticks in Imperial and San Diego counties failed to discover any rickettsia-positive ticks on the U.S. side of the border (Fritz et al., 2012; Gurfield et al., unpublished results).

In 2010, a new rickettsial disease was described in humans, which caused an eschar and swelling at the site of a presumed tick bite and enlarged lymph nodes (Shapiro et al. 2010). Genetic analysis of a biopsy from the affected skin revealed the genetic material of a rickettsia called *Rickettsia philipii* strain 364D (here forward *R. philipii*) (Philip et al. 1978). Previous studies of *R. philipii* revealed that it cross reacted serologically with *Rickettsia rickettsii* R-type and a low-virulence *R. rickettsii* found in the rabbit tick *Haemaphysalis leporispalustris* called Hlp (Parker et al. 1951). In contrast to *R. rickettsii*, *Rickettsia philipii* had lower virulence in a guinea pig infection model and was proposed to be responsible for RMSF-like disease in people (Philip, Lane, and Casper, 1981). The pathogenic potential of *R. philipii* also included cytotoxicity to endothelial cells as observed through *in vitro* studies (Eremeeva et al., 2001). Distinguishing *R. philipii* from closely related *Rickettsia* can be accomplished via genetic analysis of the intergenic regions (IGR) and the *romp*A gene (Eremeeva et al., 2003; Karpathy et al., 2007).

A survey conducted in several counties in California revealed that up to 8% of the Pacific Coast tick, *Dermacentor occidentalis,* tested positive for *R. philipii* (Wikswo et al. 2008). Additionally, three pediatric cases of *R. philipii*-induced disease were detected in 2011 (Johnston et al. 2013). In order to assess the potential risk to public health in San Diego, the prevalence of *R. philipii* and other spotted fever group rickettsia (SFGR) in *Dermacentor occidentalis* ticks was assessed from 2011 through 2014 at four different locations in San Diego County. We found a low prevalence of *R. philipii* and a non-pathogenic rickettsia, *R. rhipicephali* throughout the county. In addition, we detected *R. rickettsii* for the first time in ticks in the county.

Materials and Methods

Sample Collection. Ticks were collected from four different tick-infested areas in San Diego County, California, primarily during winter and spring seasons from 2011-2014, by dragging a 1 m^2 section of canvas over vegetation. The areas, Escondido Creek, Los Peñasquitos Canyon, Lopez Canyon and Mission Trails regional park, are frequented by hikers and represent three different watersheds in the county with Lopez and Peñasquitos canyons being adjacent to each other draining the same Peñasquitos watershed. Additionally, in 2011, 2012 and 2014, ticks were collected from the Green Valley Falls fire road in the Cuyamaca Mountains; except where indicated in the Results section, these ticks are not included in the analysis. In 2012, ticks were not

collected from the Escondido Creek location. All ticks were placed into vials and transported live back to the Vector Disease and Diagnostic Laboratory where they were separated into species and sex using standard keys ((Furman and Loomis 1984) and cataloged before freezing at -80 °C in pools of up to 10 ticks per pool during the years 2011-2013. In 2014 the ticks were analyzed individually and not pooled.

DNA Extraction and PCR Amplification. The ticks were thawed, washed and vortexed sequentially in 3% hydrogen peroxide, 100% isopropanol, and sterile distilled water for 1 minute in each solution in order to remove surface contaminants. The final distilled water wash was aspirated from the ticks and then the ticks were diced with a sterile razor blade. One hundred eighty microliters of ATL buffer (Qiagen, Valencia, CA) and 20 μL of proteinase K were added to each tick and the ticks lysed overnight at 56°C in an Eppendorf Thermomixer (Hauppauge, N.Y.) with agitation at 1400 rpm for 15 s every 10 min, before centrifuging the lysate for 3 min at 18,400 x g. The supernatant was transferred into a sterile microfuge tube and DNA extracted using a Qiagen DNeasy Blood and Tissue kit in a Qiacube using the DNeasy Blood and Tissue protocol for Tissue and Rodent Tails (Qiagen, Valencia, CA).

The ticks were screened for spotted fever group rickettsia using a Power SYBR Green PCR Mastermix kit (Life Technologies, Carlsbad, CA) and primers for the *romp*A gene (Eremeeva et al., 2003). Reactions were carried out in a total volume of

20 μL composed of 10 μL Power SYBR Green Mastermix, 0.125 μL each of primers RR190.547F (20 μM) and RR190.701R (20 μM), 7.75 μL of nuclease-free water, and 2 μL of template DNA (Eremeeva et al., 2003; Wikswo et al., 2008). PCR cycling conditions were: 3 min at 95 °C; 40 cycles of: 20 s at 95 °C, 30 s at 57 °C, 30 s at 65 °C; a holding cycle of 5 min at 72 °C; and a continuous cycle of: 15 s at 95 °C, 1 min at 55 °C, 30 s at 95 °C, 10 s at 55 °C; and a final holding temperature of 4 °C.

DNA from ticks that screened positive for SFGR were subjected to semi-nested PCR amplification of *romp*A using primers Rr190-70, Rr190-701, and Rr190-602 and the intergenic region (IGR) using primary and nested primers RR0155-*rpm*B (Eremeeva et al., 2006; Shapiro et al., 2010; Wikswo et al., 2008). Briefly, 20 μL of 2X Taq Master Mix (Qiagen, Valencia, CA), 2 μL of forward primer Rr190-70 (20 mM), 2 μL of reverse primer Rr190-701/Rr190-602 (20 mM), 14 μL of nuclease-free H_2O, and 2 μL of DNA was amplified using PCR cycling conditions of 95 °C for 3 min followed by 35 cycles of 95 °C for 20 s, 57 °C for 30 s, and 68 °C for 2 min and then 72 °C for 5 min before holding the products at 4 °C. For the IGR PCR amplification, 20 μL of 2X Taq Master Mix (Qiagen, Valencia, CA), 1 μL of forward primer RR 0155 PF (20 mM), 1 μL of reverse primer 0155 PR (20 mM), 16 μL of nuclease-free H_2O, and 2 μL of DNA was amplified using PCR cycling conditions of 95 °C for 5 min followed by 35 cycles of 95 °C for 30 s, 50 °C for 30 s, and 68 °C for 1 min and then 72 °C for 7 min before holding the products at 4°C.

Amplification products were visualized under UV light in a 1% agarose gel stained with ethidium bromide and subsequently purified using the PureLink PCR Purification Kit, following the manufacturer's protocol (Life Technologies, Carlsbad, CA). Products were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit and purified using the BigDye XTerminator Purification Kit following the manufacturer's protocols on an AB 3500xL Genetic Analyzer (Applied Biosystems, Grand Island, NY; Life Technologies, Carlsbad, CA). Ticks were also tested for the presence of *Francisella tularensis* using a multi-target real time PCR test employing primers IS*Ftu*2, *iglC* and *tul4* (Kugeler et al. 2005).

Computational analysis. The maximum likelihood estimate of infection of the tick pools was calculated using the MS Excel plugin developed by B. Biggerstaff at the Centers for Disease Control and Prevention, Atlanta, Georgia, (http://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html) (accessed 10 Feb 2016). Botanical, soils, geology, and vegetation at the four sampling locations were evaluated using the San Diego Plant Atlas (http://www.sdplantatlas.org, accessed 3/19/16) and the San Diego Association of Governments SanGIS database (http://www.sangis.org/interactive/index.html, accessed 3/19/16).

Results

Five thousand seven hundred sixty six *D. occidentalis* ticks were collected from 2011-2014 of which 47% were male and 53 % were female. The ticks collected from 2011-2013 were pooled into 276 male and 304 female pools, respectively, containing 1-10 ticks per pool (male ave 9.2, stdev 2.0; female ave 9.3, stdev 1.8). Partial sequences of the *romp*A gene IGR matched 100% to either *R. philipii* 364D (GenBank accession NR 074470.1) or *R. rhipicephali* genotype C strain from California (GenBank accession EU109177).

In addition, out of 34 male ticks obtained from the Green Valley Falls fire road in 2011, one pool of 4 male ticks contained *R. rickettsii*. Ticks collected from this location in 2012 (43 ticks) and 2014 (20 ticks) tested negative for SFGR. The prevalence of *R. philipii* in ticks varied between location and year, ranging from 0% in Peñasquitos Canyon in 2014 to 6.0% in Escondido Creek in 2013 (Table 1). Within the same location of Peñasquitos Canyon, the prevalence of *R. rhipicephali* ranged from 0.6% in 2012 to 15.0% in 2014. In pooled ticks, *R. philipii* was detected in 1.7% (95% CI, 1.2-2.3%) of male ticks and 1.6% (95% CI, 1.1-2.1%) of female ticks and *R. rhipicephali* was detected in 3.3% (95% CI, 2.6-4.1%) and 3.6% (95% CI, 2.9-4.4%) of male and female ticks, respectively (Figure 4). In individually collected male ticks from 2014, percent infection with *R. philipii* and *R. rhipicephali* was 3.8% and 8.1%, respectively. In individually collected female ticks from 2014, percent infection with *R. philipii* and *R. rhipicephali* was 2.2% and 8.8%, respectively. There was no

statistically significant difference in infection prevalence of *R. philipii* or *R. rhipicephali* in male and female tick pools or individual ticks (Chi square P=0.5, Fisher exact test P=0.5, respectively). In 2012, a pool of 10 female ticks collected from Mission Trails and a pool of 10 male ticks collected from Peñasquitos Canyon contained both *R. philipii* and *R. rhipicephali*. No co-infections between *R. philipii* and *R. rhipicephali* were detected in individual ticks from 2014. All tick pools and individual ticks tested negative for *F. tularensis*.

Collectively, the percentage of ticks infected with SFGR ranged from 1.7% in 2012 to 11.5% in 2014 (Figure 1). From 2011-2013, the lowest detection rate of SFGR per pooled tick sample occurred in June at 4.3% per tick pool, and the highest positive rate occurred in ticks pools collected in November at 66.7% (Figure 5). Although the overall percentage of ticks infected with SFGR remained largely the same in Escondido Creek, the ratio of percent ticks infected with *R. philipii* to those infected with *R. rhipicephali* changed over the course of the four years with 2011 and 2014 having a ratio of *R. philipii* to *R. rhipicephali* of 0.1, but 2013 having a ratio of 1.16 (Figure 2). Similarly, in Mission Trails, the ratio of *R. philipii* to *R. rhipicephali* infection was 0.3-0.6 in 2011, 2012 and 2014, but increased to 1.5 in 2013. In contrast, in Lopez Canyon, the highest ratio of *R. philipii* to *R. rhipicephali* occurred in 2011 (1.2) whereas years 2012 to 2014 had lower ratios (0.7, 0.3, 0.6, respectively). Peñasquitos Canyon had a different pattern, yielding the highest ratio in 2012 (1.6), and lesser ratios in 2011 and 2013 (0.2, 0.8, respectively) and zero *R. philipii* found in

2014. Rainfall totals for San Diego, as recorded at Lindberg Field, decreased from 6.3 inches in 2011 to 3.3 inches in 2013 but then increased in 2014 to 6.4 inches (Figure 1) (weatherunderground.com, accessed 3/19/2016). Average temperature and dew point gradually increased from 2011 to 2014 (Figure 3) (weatherunderground.com, accessed 3/19/2016). Plant, soil, geology and vegetation GIS layers from the San Diego Natural History Museum (http://www.sdplantatlas.org, accessed 3/15/2016), San Diego County and San Diego Association of Governments (http://sdgis.sandag.org, accessed 3/15/2016) were analyzed but were not sensitive enough to discern differences between locations (data not shown).

Discussion

An outbreak of Rocky Mountain spotted fever in northern Baja California led us to examine various species of ticks in San Diego County for SFGR. *R. rickettsii* was found once in a pool of ticks from the Cuyamaca Mountains in San Diego in 2011 but not in 2012 or 2014. This is only the second report of *R. rickettsii* being found in *D. occidentalis* ticks and highlights the low frequency of this pathogen in *D. occidentalis* (Wikswo et al., 2008). In contrast, two other SFGR were more commonly found in ticks from the other locations surveyed: the pathogenic *R. philipii* and nonpathogenic *R. rhipicephali*. Overall infection rates with SFGR at the different locations ranged from 1.7-11.5%, which was less than a published account of prevalence in *D. occidentalis* ticks in Orange, Riverside, Los Angeles, Ventura and

Santa Barbara counties, in which 45.6% tested positive (Wikswo et al., 2008). In the study by Wikswo et al. (2008), 375 ticks were collected in contrast to our study in which over five thousand ticks were tested. In the aforementioned study, it is possible that localized foci of high infection prevalence may have elevated the rate of infection since some of the surveyed locations (Hwy 74 rest stop) experienced very high rates of infection, with SFGR as high as 49% of ticks. Another study using an immunofluorescence assay detected *Rickettsia* spp. from the hemolymph of 17.0% of *D. occidentalis* in northern and southern California (Philip, Lane, and Casper 1981). In the Philip study, six out of 11 ticks tested positive for *R. rhipicephali* but negative for *R. philipii* in Torrey Pines, San Diego. The small sample size in San Diego may have precluded the investigators from detecting *R. philipii*. In our study, from 2011-2014, the overall infection rate with SFGR remained relatively constant, with the exception of 2012 in which a decreased prevalence of infection was observed across all areas (Figure 1). The cause of the decrease in 2012 was unknown. Average annual temperature and humidity remained relatively constant throughout the years and 2012 preceded the year with the lowest annual rainfall recorded (Figures 1 and 3). Yearly variations in prevalence of *R. philipii* and *R. rhipicephali* occurred at all locations, which may reflect sampling different tick populations at each location each year, variable microclimatic conditions or other ecological factors that affected endemicity.

Overall, *R. rhipicephali* was at least twice as prevalent in the San Diego tick population as *R. philipii*. This is similar to other studies that have found a higher

prevalence of *R. rhipicephali* than *R. philipii* (Philip, Lane, and Casper, 1981; Wikswo et al., 2008). However, there was no significant difference between *R. philipii* and *R. rhipicephali* infection rate in male and female ticks. A vertebrate reservoir has not been found for either of these rickettsias which is consistent with an endosymbiotic life cycle within ticks that occasionally spills over to humans in the case of *R. philipii*. Although *R. rhipicephali* has not been shown to be pathogenic, over the past 10 years, genetic analysis of *Rickettsiae* found in arthropod vectors, mammal reservoirs and clinical disease specimens has resulted in the proliferation and recognition of new pathogenic and nonpathogenic rickettsial species and a re-evaluation of disease caused by species previously thought to be non-pathogenic (Weinert et al. 2009).

Conclusion

Two known SFGR *Rickettsia* pathogens, *R. rickettsii* and *R. philipii,* and one nonpathogenic SFGR, *R. rhipicephali*, were detected at low prevalence in San Diego County. Although *R. rickettsii* was found only once and in one location, *R. philipii* was detected in all locations over several years. Year-to-year fluctuations of the prevalence of *R. philipii* and *R. rhipicephali* occurred, unrelated to generalized climatic or localized plant, soil or geological factors.

Acknowledgement

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Table 3.1. Tick sampling locations, number of ticks collected and infection rates of *Dermacentor occidentalis* in San Diego County, 2011-2014.

	GIS			2011			2012			2013			2014		
Location	Latitude	Longitude	Elevation (ft)	# of ticks	*R. philipii* % (95% CI)	*R. rhipicephali* % (95% CI)	# of ticks	*R. philipii* % (95% CI)	*R. rhipicephali* % (95% CI)	# of ticks	*R. philipii* % (95% CI)	*R. rhipicephali* % (95% CI)	# of ticks	*R. philipii* % (95% CI)	*R. rhipicephali* %
Escondido Creek	33.06016	-117.1799	253-628	643	1.1 (0.5-2.2)	9.9 (7.3-13.3)	-	-	-	147	6.0 (2.7-12.0)	3.9 (1.5-8.6)	80	1.3	8.8
Lopez Cyn	32.90658	-117.2006	79-293	470	3.4 (2.0-5.6)	2.8 (1.6-4.8)	442	0.7 (0.2-1.9)	0.9 (0.3-2.2)	809	2.0 (1.2-3.2)	6.0 (4.3-8.1)	199	5.0	8.0
Peñasquitos Cyn	32.93703	-117.14681	230-350	536	1.2 (0.5-2.4)	4.7 (3.1-7.1)	497	1.0 (0.4-2.3)	0.6 (0.2-1.7)	126	3.5 (1.2-8.3)	4.4 (1.7-9.5)	44	0.0	15.9
Mission Trails	32.83049	-117.05392	180-500	408	2.1 (1.0-4.0)	5.4 (3.4-8.3)	1024	0.5 (0.2-1.1)	0.8 (0.4-1.5)	251	3.2 (1.4-6.2)	2.2 (0.8-4.8)	90	1.1	5.6
Total				2057	1.8 (1.3-2.5)	5.7 (4.7-7.0)	1963	0.7 (0.4-1.1)	0.8 (0.5-1.3)	1333	2.8 (2.0-3.8)	4.8 (3.7-6.2)	413	2.9	8.5

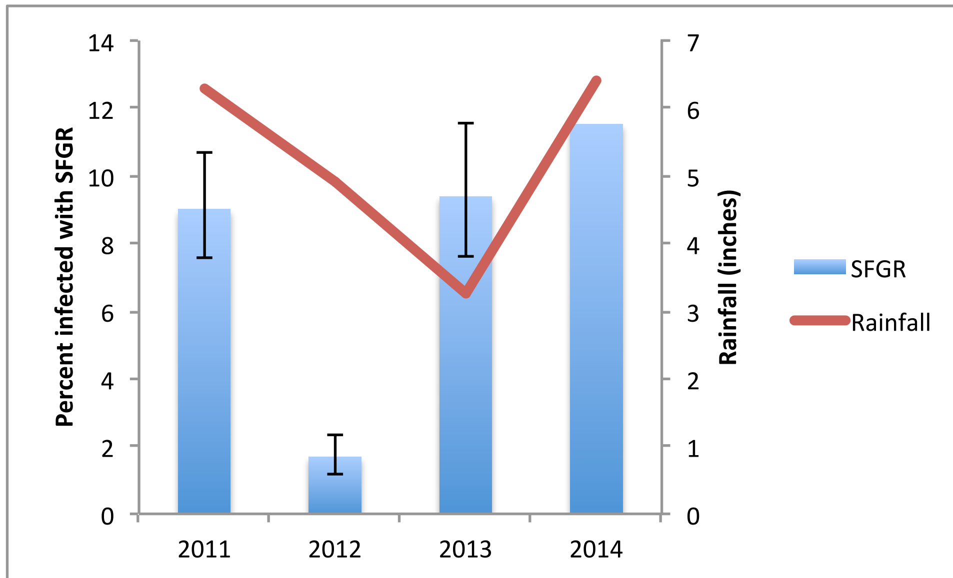

Figure 3.1. Percentage of *D. occidentalis* ticks infected with SFGR each year compared with total annual rainfall in downtown San Diego.

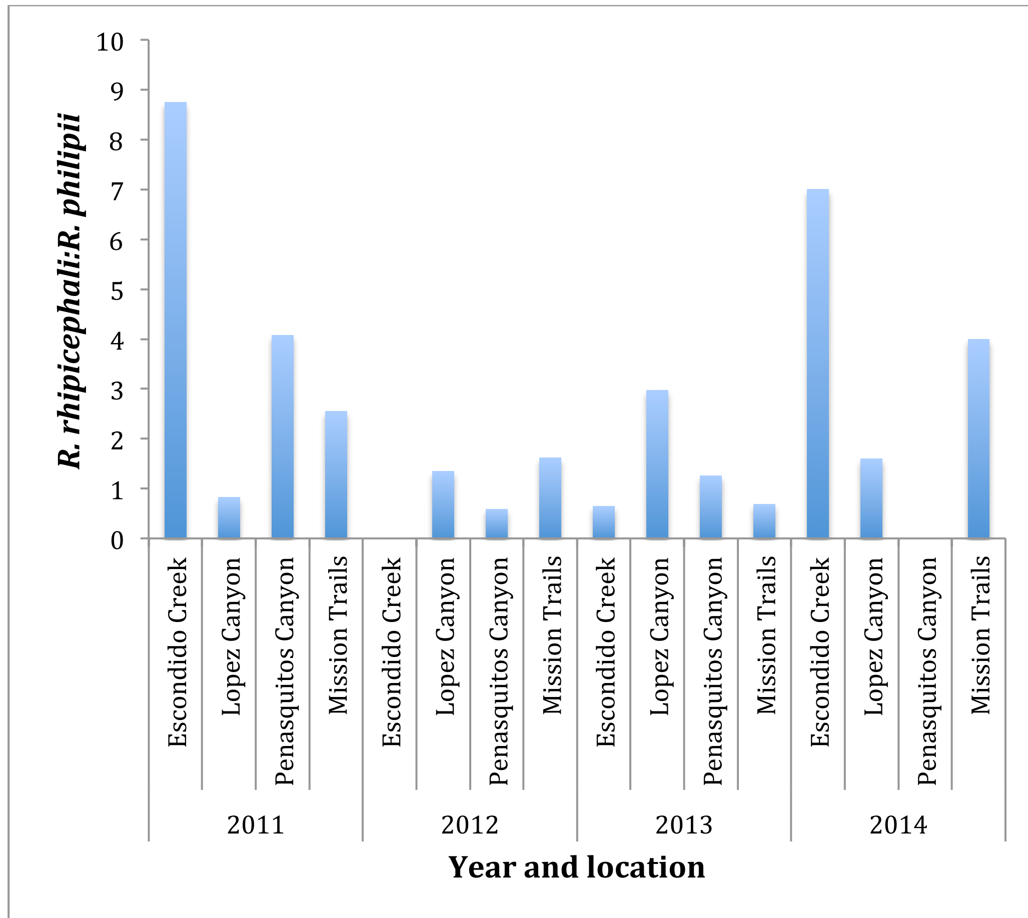

Figure 3.2. Ratio of *R. philipii* to *R. rhipicephali* prevalence in *D. occidentalis* ticks from 2011-2014.

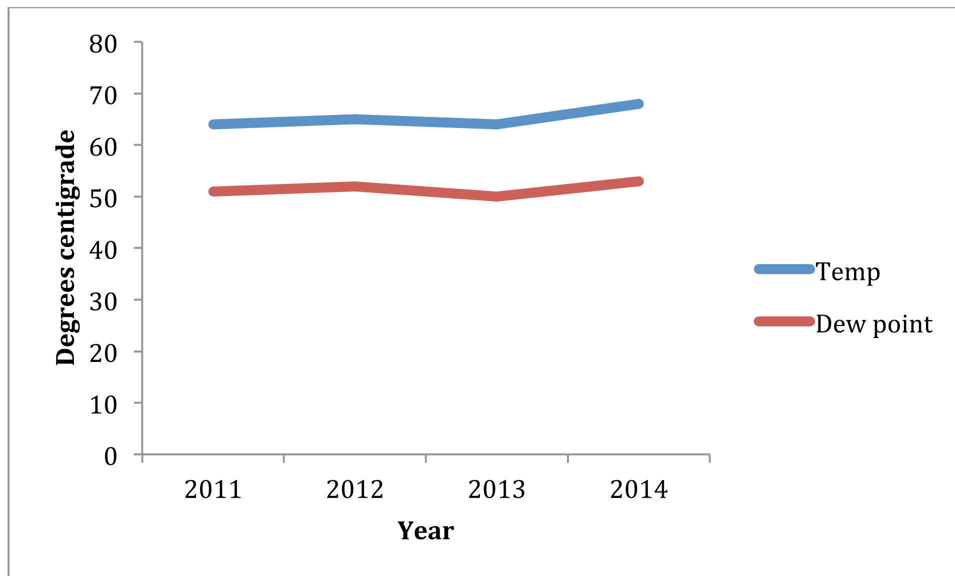

Figure 3.3. Average yearly temperature and dew point at Lindberg Field, San Diego International Airport, 2011-2014.

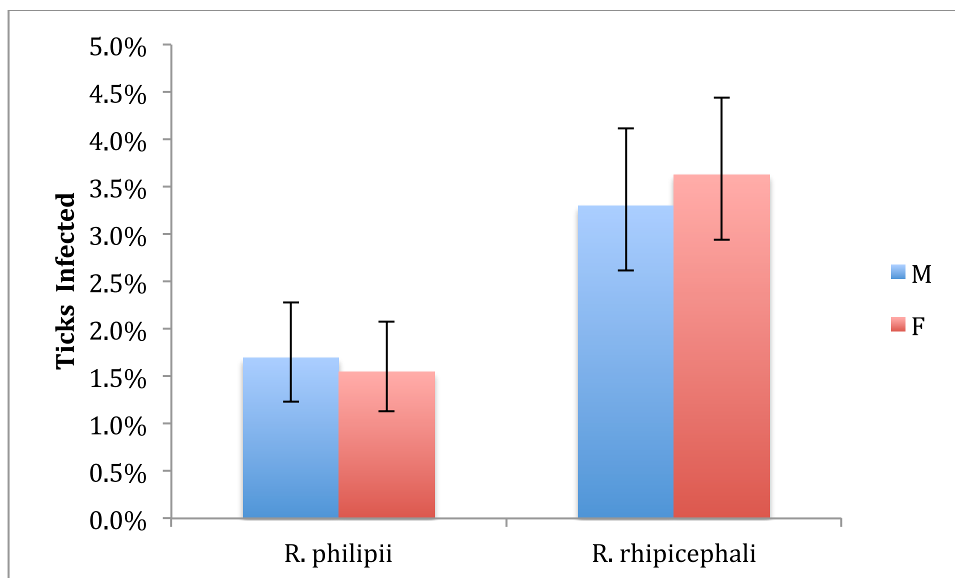

Figure 3.4. Prevalence of *R. philipii* and *R. rhipicephali* in male and female *D. occidentalis* ticks, 2011-2014. Bars represent 95% confidence intervals.

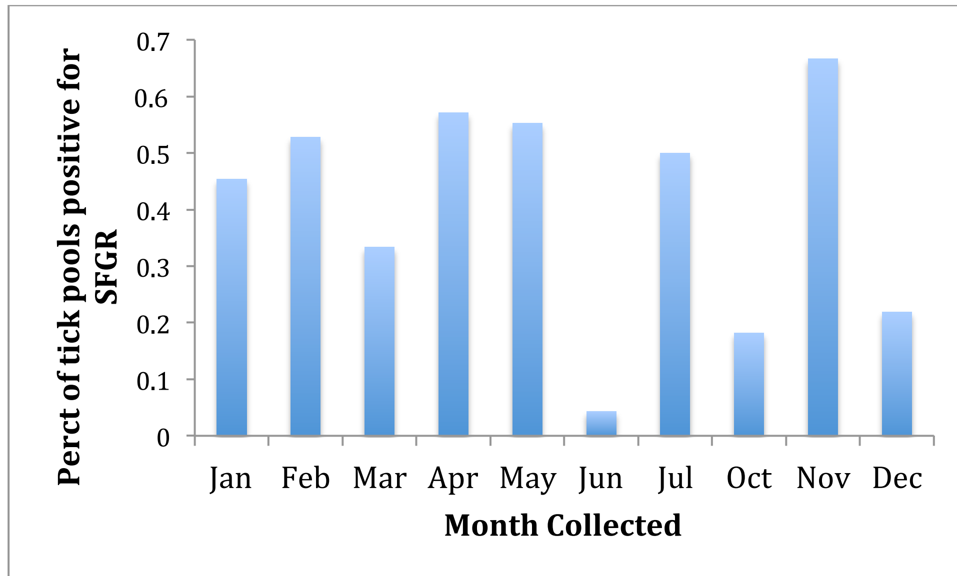

Figure 3.5. Detection rate of SFGR per tick pool by month, 2011-2013. No ticks were collected in August and September during any of these years.

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CONCLUSION OF THE DISSERTATION

These studies revealed that endosymbionts dominate the microbiomes of *Longitarsus luridis*, *L. melanocephalus*, *L. pratensis* and *Dermacentor occidentalis,* and that the composition of the endosymbionts differed between species. In the case of the three *Longitarsus* species, *Rickettsia* and *Wolbachia* were the most abundant endosymbionts and were polyphyletic but they differed in proportion in each *Longitarsus* species. This phenomenon was related to beetle species rather than geographic location or presence of other bacteria. *Wolbachia* influences speciation in some insect species but whether it affects reproduction in *Longitarsus* is unknown.

In contrast, *Dermacentor occidentalis* ticks were infected with different *Rickettsia* and *Francisella*-like endosymbionts (FLE) that were present in inverse proportions within ticks. These findings were consistent with the hypothesis of interference between *Rickettsias* and FLEs co-infecting ticks. Furthermore, ticks infected with a high abundance of FLEs were less likely to be infected with pathogenic *R. philipii* str. 364D. Confirmation of interference between FLEs and *Rickettsia* endosymbionts must be demonstrated in laboratory co-infection studies as well as determining organ distribution, mode of transmission and mechanism of interference. After a more complete understanding of the interference phenomenon is

achieved, exploring endosymbiont propagation and interference as a method to control and mitigate disease transmission would be an exciting novel method for protecting public health from certain tick borne diseases.

Although no vertebrate reservoir for *R. philipii* str. 364D was found, ticks with microbiome components similar to dog skin (or possibly coyote skin), were less likely to be infected with *R. philipii* str. 364D than ticks that did not have microbiomes similar to dog skin microbiomes. Whether this implies a protective role of a dog or coyote blood meal against *R. philipii* 364D infection requires further investigation.

In addition to microbiological effects on SFGR infection, general geographic, rainfall, temperature and moisture indices were examined for association with SFGR incidence. Although no associations for the 2012 decline in SFGR prevalence were found, a more comprehensive climate model could help to predict the geographic range of SFGR and, perhaps, of other microbiome components as well. Determining the microbiome of ticks from a larger and more diverse geographic range (altitude, longitude, latitude) may reveal greater associations between microbiomes, environmental factors and pathogens.