

EXPLORING THE ECOLOGY OF COMPLEX MICROBIAL COMMUNITIES THROUGH
THE COCKROACH GUT MICROBIOME

by

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(Under the Direction of Elizabeth A. Ottesen)

ABSTRACT

Microbes represent the majority of biomass and diversity found on planet earth and are essential to the maintenance of global biochemical processes. However, there is still much that is unknown about what drives the formation and maintenance of complex microbial communities. Here, we explore the ecology of complex microbial communities through an examination of the cockroach gut microbiome.

The cockroach gut microbiota is highly complex and is analogous to the human gut microbiome in structure, function, and overall diversity. Insects in the superorder Dictyoptera include: carnivorous praying mantids, omnivorous cockroaches, and herbivorous termites. We use 16S rRNA amplicon sequencing to survey the structure and diversity across of gut microbiota 237 cockroaches in the Blattodea order. Results show that host species plays a key role in the gut microbiota of cockroaches. This suggests that cockroach host-microbe coevolution preceded the emergence and possibly facilitated the dietary specialization of termites. Previous work suggests that diet is plays an important role in shaping the Blattodea gut microbiome. We conducted a series of dietary perturbations to determine the effect of diet on the structure of the cockroach gut microbiome. We found the cockroach hosts a taxonomically stable gut

microbiome, which may aid the host in survival during low-food and/or starvation events. This stability is highly unusual and has not been found in any other animal that hosts a complex gut microbial community. This suggests that cockroaches have evolved unique mechanisms for establishing and maintaining a diverse and stable core microbiome.

Cockroaches and termites are known for their diverse gut microbiota, however little is known about the praying mantid gut microbiome. In order to better understand the mantid gut microbiome, we conducted a 16S rRNA gene-based study of gut microbiome composition in adults and late-instar larvae of three praying mantis species. We found that few microbial lineages are shared among praying mantids and cockroach relatives. This adds further support for the role of microbes in facilitating dietary specialization among insects in the superorder Dictyoptera and is an important area for future study.

INDEX WORDS: Dictyoptera, Blattodea, cockroach, insect, invertebrate, microbial ecology, environment-host-microbe, host-microbe, gut microbiome, microbiome, 16S rRNA sequencing, metatranscriptomics

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DEDICATION

To my grandfather, for all of the conversations about life and science that we were never able to have. To my father, for his willingness to listen. To my mother, for her unwavering confidence in my integrity and ability. And to my brothers, for their humor and love.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Most humans consider all cockroaches to be vermin. They are known to carry pathogens and can indirectly spread disease while searching for food or water (1-4). A short lifecycle, high fecundity, and overall tolerance for extreme environments allow cockroaches to quickly infest residential or commercial buildings (1, 5). However, despite their status as household pests, no more than thirty among the thousands of cockroach species that have been documented have known human-associations (6-8). Most cockroaches are highly beneficial to the ecosystem. They act as scavengers, consuming detritus and other waste within their native forest, cave, and brush habitats (9-11).

Cockroaches are both efficient scavengers and common pests because they have the ability to survive on virtually any diet (9, 12). They owe this ability, in part, to the microbiota that inhabits their gut. Their gut microbiota assists in breaking down food substrates and provides the cockroach with bioavailable nutrients for absorption (9, 13-29). Emerging work suggests that the gut microbiota in cockroaches and other terrestrial arthropods also play an important role in global nutrient cycling (30). Therefore, it is important to understand both the host-microbe relationship and how these mutualists interact with the broader ecosystems they inhabit.

This review seeks to provide the background necessary to begin to understand these complex ecological relationships. Rather than focus on one specific cockroach species, I provide an overview of the general cockroach lifecycle with an emphasis on how they acquire their gut

microbiota, what is known about cockroach host-microbe interactions, what influences the structure and function of their gut microbiome, and how this might affect the broader ecosystems in which they live.

1.1 Acquisition of gut microbiota

Cockroaches in the Blattodea order employ a variety of reproduction strategies. Most are oviparous or ovoviviparous, however there is one reported viviparous cockroach, *Diploptera punctate* (31, 32). All known cockroaches, except the genus *Nocticola*, host the gram-negative endosymbiont *Blattabacterium* (33). This endosymbiont is vertically transmitted from mother to offspring through endocytosis during embryo development (34). *Blattabacterium* is found in specialized fat body cells, bacteriocytes, and assists the cockroach host with nitrogen recycling (19).

A stable, co-evolutionary relationship with the gut microbiota requires a stable transmission mechanism by which this microbiota is acquired. In contrast to maternally transmitted endosymbionts, cohorts of nymphs emerge from the ootheca with no native gut microbial community (35). Thus, the gut microbiota must be acquired from the environment after hatching. This acquisition is likely mediated in part by cockroach reproductive style. Ovoviviparous and viviparous cockroaches begin life while in direct contact with their mother and are initially exposed to her cockroach-associated microorganisms. In contrast, oviparous cockroaches lack extended maternal contact. Oviparous cockroaches prefer to deposit ootheca in protected locations, such as wall cracks or in storage boxes, near easily accessible food sources (32). Many oviparous cockroaches, including *Periplaneta americana* and *Blattella germanica*, provide additional protection to their ootheca by securing it with a secreted attachment glue (32,

36). Therefore, alternate routes of gut microbiota acquisition must be considered, such as inoculation of ootheca surfaces with maternal gut microbiota prior to or during deposition.

Cockroaches acquire microorganisms through social behavior and environmental contact. In many insect-gut symbiont systems, social contact is essential for the transmission of insect-specific bacteria (21, 37). These microbes are generally transferred through trophallaxis, coprophagy, or exposure in common living areas (18, 38-40). It has long been known that many cockroach species are gregarious and that social isolation can result in abnormal host behavior (5, 27, 40-44). Recent work suggests the cockroach gut microbiome may play a role in cockroach aggregation (27). This may, in part, provide a feedback loop supporting co-evolution between the cockroach and its gut microbiota. In this case, host social behavior would enable transmission of the gut microbiota and, in turn, social behavior (and potentially reproductive success) would depend on the acquisition of a healthy gut microbiome.

In addition to horizontal transfer, cockroaches also routinely acquire microorganisms from the environment. Most of these environmentally acquired microbes are either beneficial or harmless to the cockroach host. However, certain bacteria and fungi, including at least two members of the genus *Metarhizium*, are known cockroach pathogens (45-47). Food and water sources play a major role in the transmission of these microbes, though cockroaches can also pick up and deposit transient microbes from surfaces. This deposition can be particularly problematic for humans, as cockroaches are known reservoirs for environmentally acquired pathogenic microbes such as *Klebsiella pneumoniae* (3, 6-8).

1.2 Host-microbe interactions

Most insects typically host simple gut microbial communities composed of only a few species (21). In contrast, members of the Blattodea order host a highly complex gut microbia

composed of hundreds of unique species (20, 21, 23, 26, 48, 49). The majority of bacteria in the cockroach gut are from the *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* phylum (20, 23, 26). Many of the microbes found in the cockroach gut are also found in the guts of higher omnivores, including humans (50, 51), although most of the cockroach gut microbiota belong to understudied insect-specific lineages (48).

While its high complexity makes it difficult to determine the functional role of specific microbial species, we do know that the cockroach gut microbiota plays an important role in the acquisition of nutrients for the host (9, 18, 37). Food consumed by the cockroach passes through the fore- and mid- sections of the gut. Once in the hindgut, the gut microbiota break down any remaining recalcitrant dietary components through fermentation, supplying the cockroach with volatile fatty acids (VFAs) and other nutrients (13-15, 18). If the host diet is lacking in a particular essential nutrient, the *Blattabacterium* symbiont produces and transports it to the cockroach host using a yet unidentified mechanism (19).

In addition to helping with the digestion process, a healthy gut microbiota protects the gut from colonization by pathogens or from developing dysbiosis (37). Although no direct work has been published on the dysbiotic cockroach gut microbiota, a 1978 study by Bracke et al. demonstrated that using antibiotics to reduce the number of cockroach gut microbiota results in stunted nymphs (52). This reduced growth rate could be a consequence of dysbiosis of the cockroach gut microbiome. Similar work conducted in the honeybee and mosquito support the idea that gut dysbiosis is harmful to insects and generally results in reduced fitness and/or increased mortality (53-55). However mosquitoes and honeybees host much simpler gut microbial communities, composed of no more than 10 unique microbes, than the cockroach (56-62).

Therefore, it is impossible to draw firm conclusions about the cockroach gut microbiota from work in these insects.

1.3 Influences on the gut microbiome

As cockroach nymphs grow towards adulthood they molt anywhere from a few to over a dozen times, depending on the species (42). The molting process allows insects to shed their exoskeleton, including the lining of the foregut and midgut, in order to grow larger. Therefore, during the molting process most or all of the host-associated microbiota are lost or severely disrupted (21). After molting the cockroach gut microbiota takes several days to return to the original density, only to be lost again during the next molt (21, 22). Each molting event presents an opportunity for the cockroach microbiota to be substantially altered. Even once the cockroach has completed its final molt and reached adulthood, the cockroach microbiome could still be influenced by host-mediated or environmental factors.

Recent work by Carrasco et al. demonstrates that the *Blattella germanica* gut has different bacterial compositions and loads at each stage (22). This suggests that there may be some host-mediated mechanism that selects for certain microbiota. Further support for this hypothesis was provided by Mikaelyan et al., who found that xenobiotic *Shelfordella lateralis* exposed to diverse populations of microorganisms consistently assemble gut microbiota that resemble those found in conventional cockroaches (25).

The cockroach and its *Blattabacterium* endosymbiont have a coevolutionary relationship (19, 63); it is possible that deterministic assembly of cockroach gut microbiota may be a product of coevolution as well. Hongoh et al. demonstrated that the termite and its gut microbiota have a coevolutionary relationship (64). More recent work has shown that changes in the cockroach gut microbiota broadly match with evolutionary events that led to the emergence of termites (49).

This demonstrates that host phylogeny and the gut microbiome are linked, although future work is needed in order to unravel this relationship.

While host phylogeny has been linked with the structure and function of the cockroach gut microbiome, only a few studies have been conducted that explicitly examine the effect of environmental factors. Of the studies that have been published, all but two focus on diet. In the first, Vicente et al. demonstrated that exposure to a parasitic nematode increases microbial diversity in the hindguts of both *Periplaneta fuliginosa* and *P. americana* (65). In the second, work with gnotobiotic *Shelfordella lateralis* revealed that oxygen level has an effect on the colonization and metabolic activities of gut microbiota (44). Although more studies have been published examining the effect of diet on the cockroach gut microbiome there is still no clear consensus how, or even if, it is influenced by diet (20, 23, 26).

Design differences in the diet-based studies contribute to these ambiguous conclusions. Schauer et al. found that *Shelfordella lateralis* has a stable core gut microbiome, Bertino-Grimaldi et al. reported small but significant changes in the *P. americana* gut microbiome after dietary shifts, and Pérez-Cobas et al. found that the *B. germanica* gut microbiome is highly responsive to changes in diet. However, each study used different cockroach species as well as primers that target different variable regions. Even more importantly, each study had drastically different dietary treatments. Schauer et al. and Bertino-Grimaldi et al. tested only fiber diets while Pérez-Cobas focused on protein diets (20, 23, 26). These contradictory results demonstrate the need for comprehensive studies that examine the effect of a wide range of environmental factors, especially diet, on the cockroach gut microbiome.

1.4 Zooming Out: Cockroaches in the Ecosystem

Although there have been few explicit studies that examine how cockroach microbiota affect the broader ecosystems they inhabit, we know that influence of the cockroach gut microbiota extends beyond the individual insect. In their natural environment, the cockroach is a scavenger, surviving on detritus or other waste (9, 12, 18). As the food is broken down through microbial fermentation several key products are made including carbon dioxide, methane, and VFAs (9, 12, 14, 18). Some of these products remain in the gut of the host, although some amount is excreted in fecal waste or through gas (16, 27, 30, 66). Alluded to in previous sections, these products are known to mediate insect-insect communication and contribute to the global atmospheric cycle (27, 30).

The VFAs present in cockroach feces acts as an aggregation agent. The specific VFA profile produced by an individual cockroach is highly plastic and depends on the composition of the gut microbiome as well as the diet consumed by the host. Thus, each colony of cockroaches exhibits a unique pheromone “signal” that indicates their territory and encourages colony members to interact and horizontally transfer insect-associated microorganisms (27). Additionally, the carbon dioxide and methane produced by cockroaches and other terrestrial arthropods play an important role in global nutrient cycling (30). It is impossible to calculate their exact contribution due to the overall lack of knowledge about the insect biome (67). However, it has been demonstrated that altering the cockroach gut microbiome affects the amount of carbon dioxide and methane released by these insects (16, 17).

1.5 Addressing the gaps: Objectives

Cockroaches are robust insects found in nearly every habitat. Their ability to survive in such variable, and potentially harsh, conditions is due to their microbial-associations. The

cockroach microbiota assists with the digestion process and contribute to the host's overall health. Recent work has shown that these microbial associations not only affect the health and survival of the individual cockroach host, but also influence the broader ecosystems these cockroaches inhabit. Before we can begin to understand the global contributions of the cockroaches, we must have a firm understanding of the cockroach gut microbiome.

My dissertation seeks to provide an understanding of the cockroach gut microbiome. First, I explore the role of host phylogeny on the gut microbiome by using 16S rRNA gene amplicon sequencing to complete a comprehensive survey of gut microbiota from cockroach species across the Blattodea order. Next, I complete a series of dietary manipulations in order to determine how environmental factors may affect the structure of the cockroach gut microbiome. Finally, I begin to consider the role of the cockroach within the broader insect biome by examining the relationship between the cockroach and its close relative and predator, the praying mantis.

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CHAPTER 2
HOST PHYLOGENY PLAYS A KEY ROLE IN SHAPING
THE COCKROACH GUT MICROBIOME¹

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2.1 Abstract

The Blattodea order contains omnivorous cockroaches and termites, eusocial cockroaches that consume specialized lignocellulose-based diets. Previous work suggests that this specialized diet is the primary factor that shapes the gut microbiota of termites. However, less is known about what shapes the gut microbiota of omnivorous cockroaches. We conducted a comprehensive 16S-rRNA gene amplicon-based survey of the gut microbiome from cockroaches across the Blattodea order. A total of 237 insects were surveyed from 19 species representing 5 cockroach families. Our work demonstrates that gut microbiota is highly correlated with host species among omnivorous cockroaches. These differences include changes in abundance among predominant bacterial phyla among cockroach species as well as the gain/loss of low-abundance, insect-associated microbial lineages among cockroach families. This raises new questions about host-microbe evolution and the events leading to the specialization of termites.

2.2 Introduction

Insects in the Blattodea order host complex gut microbiomes composed of hundreds of unique microbes (1-6). This is atypical of most insects, which generally host gut communities composed of only a few microbial species (7-13). These microbes are environmentally acquired through surface contact or the consumption of food and water (2, 14, 15). Additionally, the cockroach gut microbiota are known to facilitate insect-insect communication and are often transmitted through social contact and/or coprophagy (16). Recent evidence demonstrates that assembly of the cockroach gut microbiota is deterministic (17), which implies that selective pressures play a key role in shaping the gut microbiome.

There are two pressures that could mediate deterministic assembly: host physiology and/or phylosymbiosis (18-20). Phylosymbiosis refers to the phenomenon that genetic

differences among host lineages mirror differences in the composition of the gut microbiota among host lineages. This congruence demonstrates a relationship between host-phylogeny and gut microbiota and indicates that certain microbial strains may have coevolved with the host. Several studies have documented a phylogenetic link between the gut microbiota and their host of insects in the Blattodea order. However, these studies primarily focus on termites, which are eusocial cockroaches that consume specialized diets (15, 21, 22). One notable exception is a 2014 study by Dietrich et al. which reports the gut microbiota associated with 36 individual insect hosts, 15 cockroaches and 19 termites, from across the Blattodea order (15). They found that the Blattodea gut microbiome broadly reflects major evolutionary events within the Blattodea order (15). However, it was impossible to determine whether host dietary requirements or phylogeny was the primary driver in these compositional differences.

In order to clarify the host-gut microbiota relationship, we used high-throughput 16S rRNA gene amplicon sequencing to conduct a survey of the gut microbiota from 237 insects from 19 species representing 4 cockroach families in the Blattodea order (Table 2.1). Only 3 of our 19 host species were included in the Dietrich et al. study (15). Additionally, due to sampling limitations with the 2014 study, no calculations could be made to quantify the amount of individual-to-individual variation among and between species. Thus, our work represents the first comprehensive survey of omnivorous cockroaches in the Blattodea order and acts as a complement to the extensive body of work on the termite gut microbiota.

2.3 Methods

Insects

Insects were provided by the University of Georgia's entomology department, collected in the wild on the University of Georgia's campus (6), or purchased from Roach Crossing (Table

2.1). Cockroaches provided by the University of Georgia were maintained in single-species, mixed-age, and mixed-sex colonies in aquarium tanks on a diet of dog food (Pet Pride Chunk Style Complete Nutrition Dog Food [Pet Pride], composed of 21% protein, 9% fat, and 4% fiber) *ad libitum*. Wild-caught cockroaches were placed in aquarium tanks within 24-hours of capture and were given a two-week recovery period under laboratory conditions. Cockroaches received from Roach Crossing were shipped in single-species plastic containers and were allowed a 24 hour-recovery period during which they had *ad libitum* access to dog food [Pet Pride]. Only adult cockroaches were used in this study.

Sample Collection and DNA Extraction

Cockroaches were placed in CO₂ chambers or on ice in sterile culture plates. Once sufficiently torpid, insects were dissected. The whole gut was removed and washed with 1XPBS diluted from 20X stock at pH 7.5 (Amresco, Solon, OH). Any visible debris, including fat bodies or exoskeleton, was removed with forceps. Whole guts from cockroaches were placed on a sterile surface. The hindgut was separated from the rest of the gut with a scalpel, placed in 1XTE Buffer, and stored at -80 °C (SI Table 2.1).

DNA was extracted from the stored gut sample using a modified version of the EZNA Bacteria kit (Omega Biotek, Norcross, GA). Preserved frozen hindgut samples were thawed on ice. After thawing, they were pulverized with a sterile microcentrifuge pestle and centrifuged for 10 min at 5,000 g. The supernatant was discarded and the pellet was resuspended in 100 µL of 1XTE Buffer. 10 µL lysozyme (as supplied by kit) was added to the sample before incubation at 37°C for 30 min. After incubation, approximately 25 mg of glass beads (as supplied by kit) was added and the samples were bead beaten for 5 min at 3,000 rpm using a vortex mixer with a horizontal adapter. 100 µL BTL buffer and 20 µL proteinase K solution (as supplied by the kit)

were added to each sample. Samples were incubated at 55°C while shaking at 600 rpm for 1 h. Next, the manufacturer's protocol (June 2014 version) was followed beginning at step 11. Samples were eluted in 50 µL preheated elution buffer after a 5 min incubation at 65°C. The final DNA concentrations (anywhere from 2 to 800 ng/µL) and A260/A280 were measured using a NanoDrop Lite spectrophotometer (Thermo Scientific, Wilmington, DE).

Insect Phylogeny and COII Sequencing

Cockroaches were visually identified by morphology. These identifications were confirmed by sequencing the CO-II gene (CO-II F: AGAGCWTCACCTATTATAGAAC; R: GTARWACRTCTGCTGCTGTTAC or modified A-tLeu/B-tLys F: CAGATAAGTGCATTGGATTT; R: GTTTAAGAGACCAGTACTTG) from a representative sample from each insect species (23-25) (SI Table 2.1). For PCR amplification, a 25 µL master mix composed of 1X Standard Taq Buffer (New England BioLabs [NEB], Ipswich, MA), 200 µM dNTPS, 0.2 µM forward primer, 0.2 µM reverse primer, variable DNA template, and 0.025 U/µL Taq DNA polymerase (NEB) was prepared. PCR conditions were 95°C for 30 s for initial denaturation followed by 35 cycles at 95°C for 30 s, 41.1°C (CO-II) or 48°C (modified A-tLeu/B-tLys) for 30 s, and 68°C for 1 min (SI Table 2.1). The final extension was 68°C for 5 min. Resulting amplicons were visualized on a 2% agarose gel, purified using the EZ Cycle Pure Kit (Omega Biotek), and submitted to EuroFins Genomics for Sanger Sequencing.

16S rRNA Library Preparation and Sequencing

The V4 region of the 16S rRNA gene for each gut microbiota sample was amplified using a previously described two-step PCR method (6). The initial reaction amplified extracted DNA using 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) primers. The initial reaction contained 1X Q5 buffer (NEB),

200 μM dNTPS, 0.5 μM 515F, 0.5 μM 806R, 2 ng template DNA, and 0.02 U/ μL Q5 Hot Start High-Fidelity DNA polymerase (NEB) for a total reaction volume of 10 μL . PCR conditions were 98°C for 30 s for denaturation followed by 15 cycles at 98°C for 10 s, 52°C for 30 s, and 72°C for 30 s. The final extension step was 72°C for 2 min.

Immediately after amplification, 9 μL of the initial reaction product was reamplified using 515F and 806R primers that contained unique double Hamming barcodes (6) (SI Table 2.2). The second reaction master mix contained 1X Q5 buffer (NEB), 200 μM dNTPS, 0.5 μM 515F, 0.5 μM 806R, and 0.02 U/ μL Hot Start High-Fidelity DNA polymerase (NEB) at a volume of 21 μL . PCR conditions were for 98°C for 30 s for initial denaturation; 4 cycles at 98°C for 10 s, 52°C for 10 s, and 72°C for 30 s; 6 cycles at 98°C for 10 s and 72°C for 1 min; and the final extension step at 72°C for 2 min.

The resulting PCR product was visualized on a 2% w/v agarose gel. Failed PCRs were redone with an additional 5 cycles during the initial reaction (SI Table 2.1). After confirmation of amplification, all samples were purified using the EZ Cycle Pure Kit (Omega Biotek). Purified samples were quantified using a NanoDrop Lite spectrophotometer (Thermo Scientific), normalized, and pooled to a concentration of 10 nM on the basis of a predicted total product size of ~400bp. The pooled library was submitted to the Georgia Genomics facility for sequencing (Illumina MiSeq 250x250 bp; Illumina, Inc., San Diego, CA)

Data Analysis

16S rRNA gene sequences were analyzed using Mothur (26). The Miseq standard operating protocol (27, 28) was followed with the following modifications: after sequence assembly, any sequence that had ambiguous bases or was longer than 275 base pairs was removed; remaining sequences were aligned to the Silva reference database (Release 128) (29-

31); aligned sequences that contained homopolymers of 8 or more base pairs were removed; chimeras were identified via UCHIME and removed (32); sequences were classified using the Greengenes reference database (August 2013 Version) (33); any sequences that were unclassified or identified as chloroplasts, mitochondria, Eukaryota, or *Blattabacterium* (cockroach endosymbiont found in fat body cells) were removed; remaining sequences were clustered using the OptiClust (34) method into OTUs based on 97% or greater sequence identity.

Accession Numbers

16S rRNA gene sequences were deposited in the NCBI Sequence Read Archive and are available under accession number SRP132948.

2.4 Results

Insect Phylogeny

We examined the relationship between host phylogeny and gut microbiota among insects in the Blattodea order. Insects were initially identified by morphological characteristics (Table 2.1). This identification was later confirmed by comparing mitochondrial COII gene sequences from host cockroaches with known insect phylogenies (Figure 2.1). Phylogeny from the sequencing data supported morphology-based identification, confirming that host insects represented 19 unique species belonging to four Blattodea families: Blaberidae, Blattidae, Corydiidae, and Ectobiidae (Table 2.1).

The Blaberidae family contained ten host insect species, most of which were direct matches to sequences from classified insect species deposited in GenBank. Bb-G was initially classified as *Panchlora nivea*, although after sequencing it was reclassified as *Panchlora viridis*. Bb-F and Bb-H were initially classified as *Oxyhaloa deusta* and *Paraplecta sp.* “Kenya” using morphological characteristics. There were no close matches to sequencing data from Bb-F and

Bb-H, however sequence-based identification did confirm Bb-F and Bb-H were members of the Blaberidae family. Two host insect species, *Periplaneta americana* (Bt-A) and *Periplaneta fuliginosa* (Bt-B), were from the Blattidae family. *P. americana* and *P. fuliginosa* are well characterized, therefore our morphological- and sequencing-identifications were in direct agreement. Three of the four Corydiidae species were also in agreement, however the sequence data from C-A was only resolved to the genus level as no sequences from classified *Eragula pilosa* were available for comparison. Finally, sequences from two host species in the Ectobiidae family matched well-classified insects. *Parcoblatta fulvescens* (E-B) could not be fully resolved, however sequencing data indicated E-B is related to members of the *Ischnoptera* genus. The *Parcoblatta* and *Ischnoptera* genera are closely related; therefore it is likely the initial morphological identification is correct.

Hindgut Microbial Diversity

A 16S rRNA sequence library was prepared from hindgut microbiota from 237 unique insects representing 19 species from 4 families in the Blattodea order (Table 2.1, Figure 2.1). 16S amplicons sequencing resulted in a total of 21,406,687 sequences with 14,408,721 remaining after quality filtering. Although Firmicutes, Proteobacteria, and Bacteroidetes were the predominant phyla present in the hindgut of all Blattodea insects, there are clear phylum-level differences in abundance among cockroach species. This is consistent with previously published studies (1-6, 15). For example, normalized and pooled hindguts of *Therea olegrandjeani* (C-D) are composed of 55.8% Firmicutes, 11.6% Proteobacteria, and 18.2% Bacteroidetes, while *Panchlora nivea* (Bb-G) have 20.2% Firmicutes, 37.6% Proteobacteria, and 18.6% Bacteroidetes (SI Figure 2.1). Blattodea hindguts also contained a high proportion of microbes from other, less abundant phyla including Actinobacteria and Fusobacteria. Across all

normalized and pooled Blattodea hindguts Actinobacteria and Fusobacteria compose an average of 2.4 and 2.2%, respectively. Within host Blattodea species, Actinobacteria abundance ranged from 0.1-5.8%, and average Fusobacteria abundance ranged from 0.2-6.5% (SI Figure 2.1).

Predominant microbial families were considered to be any microbial family that composed >5% of the total hindgut sequences for any one host species. As with microbial phyla, the most predominant microbial families are present across all Blattodea species, although there are family-level differences in abundance among cockroach species (Figure 2.2, SI Figure 2). Of the 29 predominant families, 6 were unclassified at the family-level, 1 at the order-level, 3 at the class-level, and 1 at the phylum level. These 11 families represented anywhere from 14.9-98.7% of total bacteria in any individual cockroach (SI Figure 2).

Host phylogeny shapes the cockroach gut microbiome

We completed an ordination analysis in order to gain insight into the factors contributing to differences in the gut microbiota among the 237 cockroaches. The ordination analysis revealed a strong relationship between host phylogeny and gut microbial community composition at the 97% OTU level (Figure 2.3, SI Figure 3). This relationship was present in both classification-independent (Figure 2.3) and phylogeny-based approaches (SI Figures 3). In order to determine the strength of this relationship, we examined the host phylogeny distance and dissimilarity between pairs of hosts (Figure 2.4). There was a general linear trend, with the gut microbiota from closely related host insects having higher similarity than the gut microbiota from distantly related host insects. This trend was not especially strong, with the Pearson correlation ranging from 0.12-0.29 (Figure 2.4). This is likely an artifact of our phylogenetic tree, which contains only the cockroach hosts sampled as well as their close relatives. This resulted in cockroaches within the same family appearing more distant to one other than cockroaches in different

families. For example, within the Corydiidae family *Eragula pilosa* (C-A) is a distance of 0.38126 from *Polyphaga aegyptiaca* (C-C). However, there is only a distance of 0.35393 between *Ergaula capucina* (C-B) and *Luchihormetica verrucosa* (Bb-A), which is in the Blaberidae family (Figure 2.1).

A hierarchical clustering analysis also revealed a relationship between host phylogeny and gut microbial community composition, with host species clustering together (Figure 2.5). Host families tended to cluster together, however there were several exceptions. *Blattella germanica* (E-A) did not cluster with the other members of the Ectobiidae family. Additionally, *Luchihormetica verrucosa* (Bb-A) and *Pycnoscelus surinamensis* (Bb-I) clustered with the Corydiidae family, rather than with other members of the Blaberidae family. Analysis of similarities (ANOSIM) supported these findings. There was a significant difference between the gut microbiota hosted by both host species and families ($p < 0.001$). However species-level clustering was stronger than family-level clustering, with R-values of 0.8469 and 0.3005, respectively (SI Table 2.3).

Although there were clear differences in the gut microbiota among host species, all host species and families had similar alpha-diversity levels (Figures 2.6 and 2.7). Weighted beta-diversity metrics revealed variable levels of individual-to-individual variation among different host species. Unweighted beta-diversity metrics were also variable, with *Oxyhaloa deusta* (Bb-F), *Panchlora nivea* (Bb-G), and *Paraplecta sp.* “Kenya” (Bb-H) demonstrating significantly more individual-to-individual variation than other species (Figure 2.3, SI Figure 3). Although *Oxyhaloa deusta* (Bb-F), *Panchlora nivea* (Bb-G), and *Paraplecta sp.* “Kenya” (Bb-H) are all members of the Blaberidae family, they are not close relatives (Figure 2.1) and they did not cluster together in our hierarchical clustering analysis (Figure 2.5).

The number of species sampled within each family influences dissimilarity among Blattodea families. Therefore, when examined by family rather than species, beta-diversity was highest among insects in the Blaberidae family followed by the Corydiidae, Ectobidae, and then Blattidae families. Interestingly, weighted between-group dissimilarity was equally high among all cockroach families. However, unweighted between-group dissimilarity was variable (Figure 2.7).

2.5 Discussion

Cockroaches can subsist on a wide range of dietary substrates, from household food items to leaves and other detritus (14). This diversity is reflected in the cockroach gut microbiome, which is composed of hundreds of unique microbial species. The exact drivers behind the formation and maintenance of the complex cockroach gut microbiota are unknown; therefore our goal was to determine the extent to which host phylogeny influences the gut microbiota of omnivorous cockroaches. Our results demonstrate that adult cockroaches across the Blattodea order host hindgut microbiota primarily composed of bacterial lineages from the Firmicutes, Proteobacteria, and Bacteroidetes families. However, the Blattodea hindgut also contains highly diverse, low-abundance microbial strains that are from uncharacterized, insect-associated lineages. These results are consistent with previously published work in cockroaches and termites (1, 3, 5, 6, 35). We also demonstrate the host species plays a key role in shaping the cockroach hindgut microbiota.

We found that hindgut microbiota hosted by cockroaches within the same species are significantly more similar to one another than those hosted by different cockroach species. However, the relationship between host-family and gut microbiota is less strong. Although weighted beta-diversity measurements are equal between host families, unweighted

measurements reveal that genetically close Blattodea families host gut microbiota that more similar to one another than those from distant Blattodea families. One explanation for this phenomenon is that a subset of cockroach gut microbiota coevolved with their insect host, such that the oldest Blattodea families harbor low-abundant, highly conserved microbial lineages that emerging Blattodea families lost and/or replaced with new microbial lineages.

This explanation is consistent with the pattern observed in termites (4, 21, 22, 36, 37). The termite family gained protists during the transition to a specialized wood-based diet, evolving into the lower termites. During the subsequent transition to a general lignocellulose diet, the protists were lost and higher termites emerged (4, 15, 36-43). Previous studies, including the 2014 study by Dietrich et al., suggest that microbial lineages coevolved with the host during the transition to a specialized diet (4, 15, 37, 40, 42, 43). However, our examination of the gut microbiota from omnivorous cockroaches across the Blattodea reveals that this relationship between host phylogeny and gut microbiota existed before the emergence of termites and wood-eating cockroaches. Further work should be conducted in order to identify how strongly insect-associated lineages are conserved among cockroaches and if these lineages are especially efficient at breaking-down certain nutrient classes.

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2.8 Tables

Table 2.1. Morphological Identifications of Cockroach Hosts

Species Id.	Host species	Total no. of samples
Blaberidae		
Bb-A	<i>Lucihormetica verrucosa</i> ^R	12
Bb-B	<i>Blaberus craniifer</i> ^G	11
Bb-C	<i>Diploptera punctata</i> ^G	15
Bb-D	<i>Gromphadorhina portentosa</i> ^G	12
Bb-E	<i>Nauphoeta cinerea</i> ^G	14
Bb-F	<i>Oxyhaloa deusta</i> ^R	12
Bb-G	<i>Panchlora nivea</i> ^R	12
Bb-H	<i>Paraplecta</i> sp. "Kenya" ^R	12
Bb-I	<i>Pycnoscelus surinamensis</i> ^R	12
Bb-J	<i>Schultesia lampyridiformis</i> ^G	15
Blattidae		
Bt-A	<i>Periplaneta americana</i> ^G	15
Bt-B	<i>Periplaneta fuliginosa</i> ^W	15
Corydiidae		
C-A	<i>Eragula pilosa</i> ^R	8
C-B	<i>Ergaula capucina</i> ^R	12
C-C	<i>Polyphaga aegyptiaca</i> ^R	11
C-D	<i>Therea olegrandjeani</i> ^R	12
Ectobiidae		
E-A	<i>Blattella germanica</i> ^G	15
E-B	<i>Parcoblatta fulvescens</i> ^R	11
E-C	<i>Symphloe pallens</i> ^R	11

^G provided by the University of Georgia's Entomology Department

^R purchased from roachcrossing.com

^W field-collected on the University of Georgia's campus

2.9 Figures

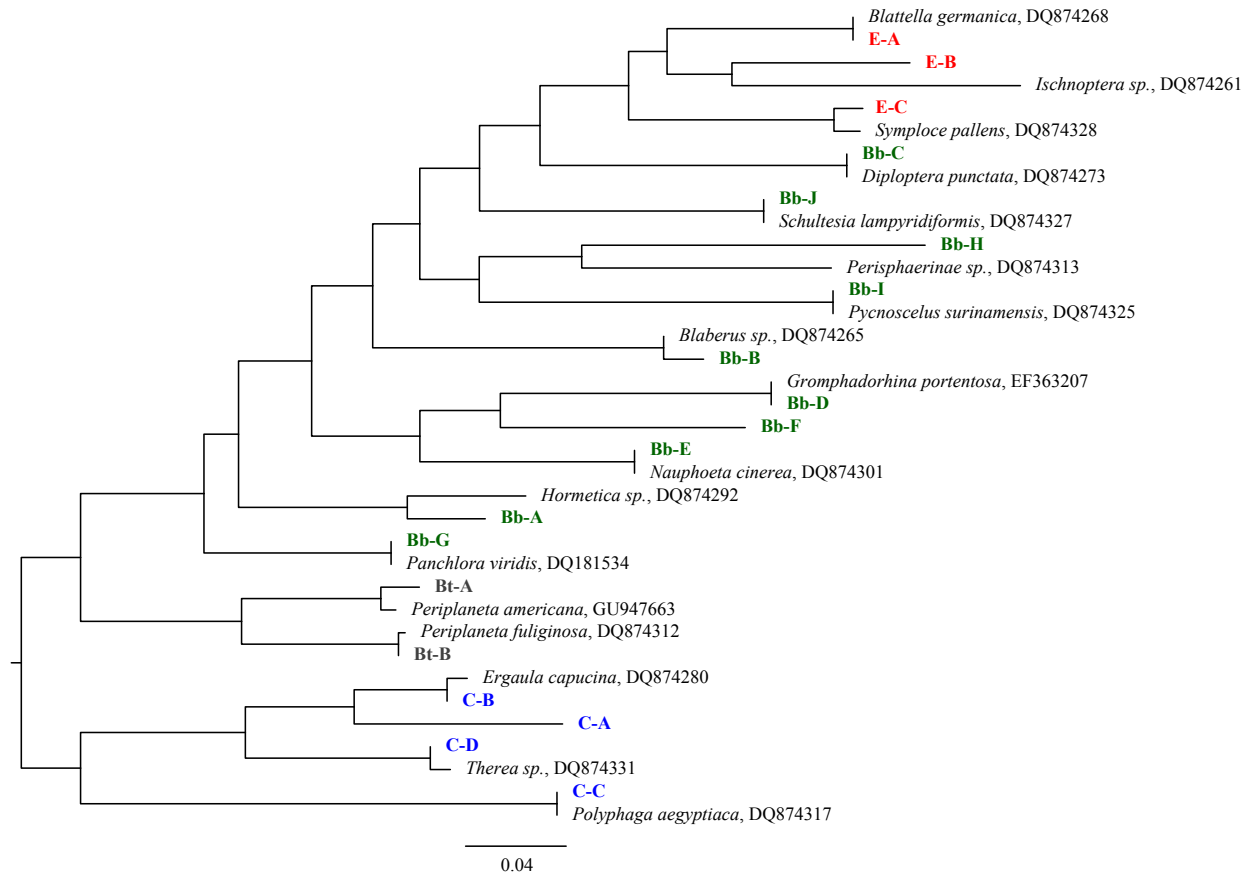


Figure 2.1 Phylogenetic tree of host Blattodea species sampled.

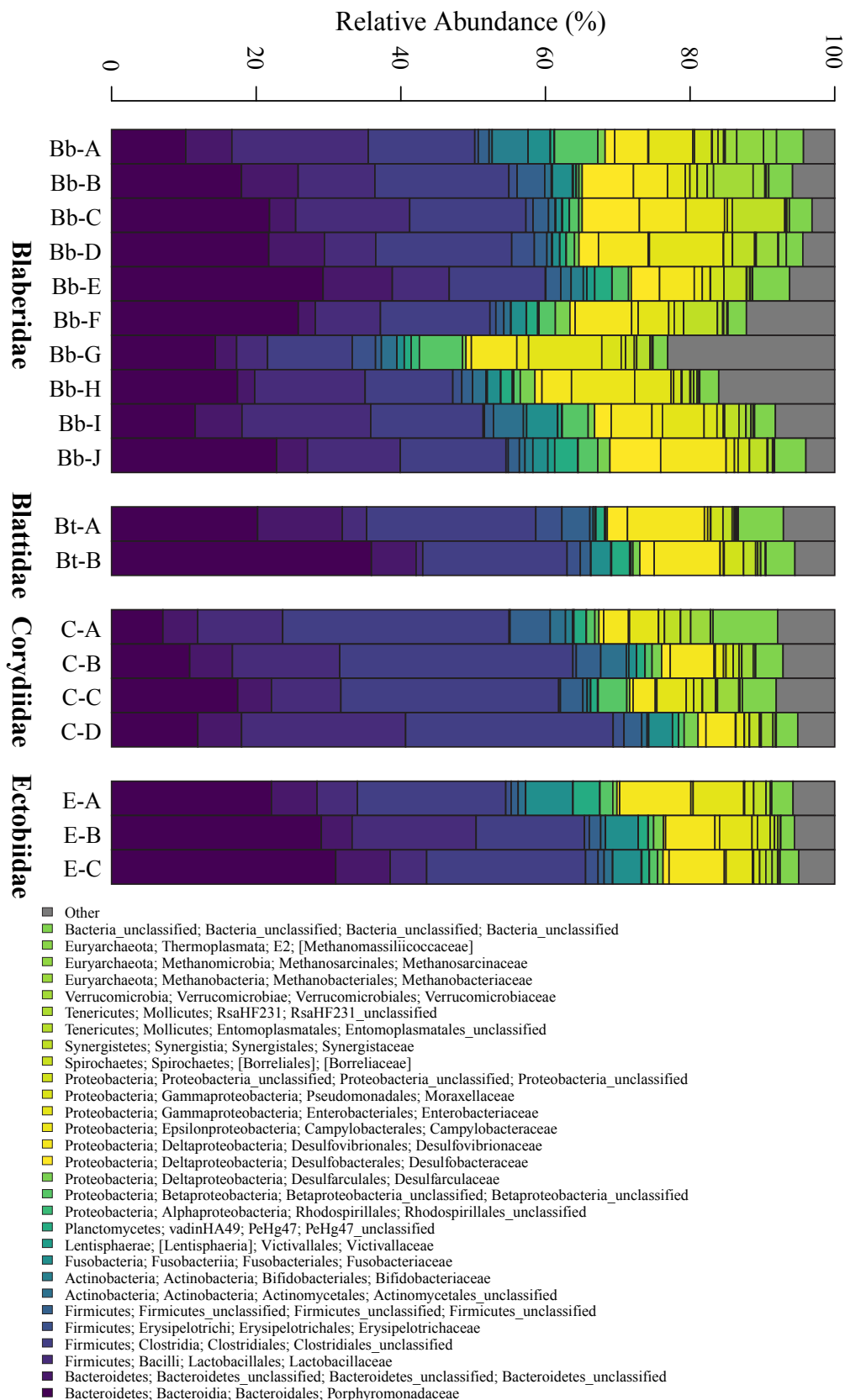


Figure 2.2 Relative abundances of microbial families from the gut of host Blattodea species.

Relative abundance of microbial families across each Blattodea species sampled. Each bar contains the pooled normalized data from all individual insect guts for the indicated species. All families that represent $\geq 5\%$ from any one host Blattodea species are shown.

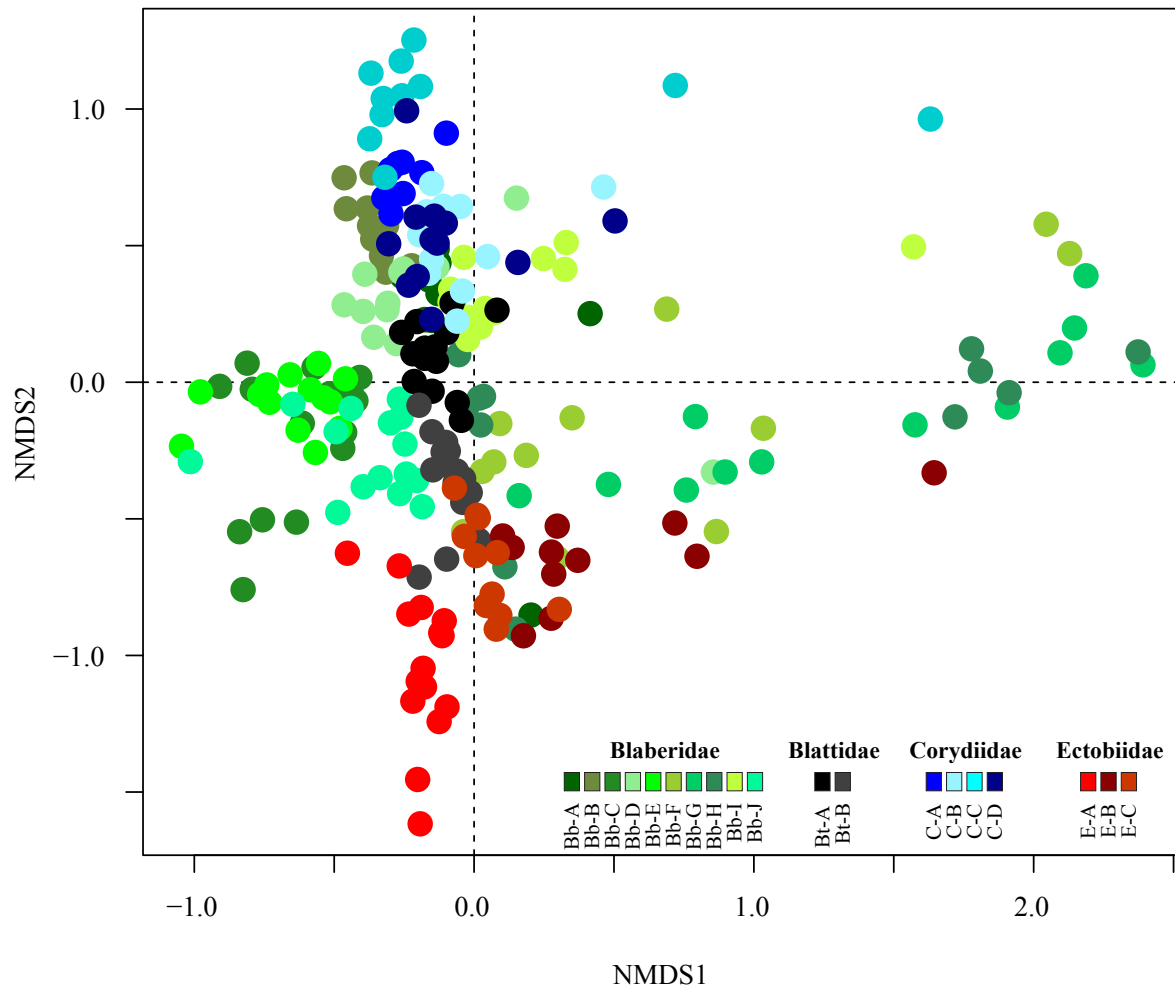


Figure 2.3 Nonmetric multidimensional scaling (NMDS) plot of Blattodea hindgut microbiota. Plot was constructed with weighted Bray-Curtis metrics based on the distribution of OTUs (97% sequence identity). Libraries were resampled to a depth of the sample with the fewest sequences (4365). Clustering by host family ($R=0.30$, $P<0.001$) and species ($R=0.85$, $P<0.001$) was supported by ANOSIM.

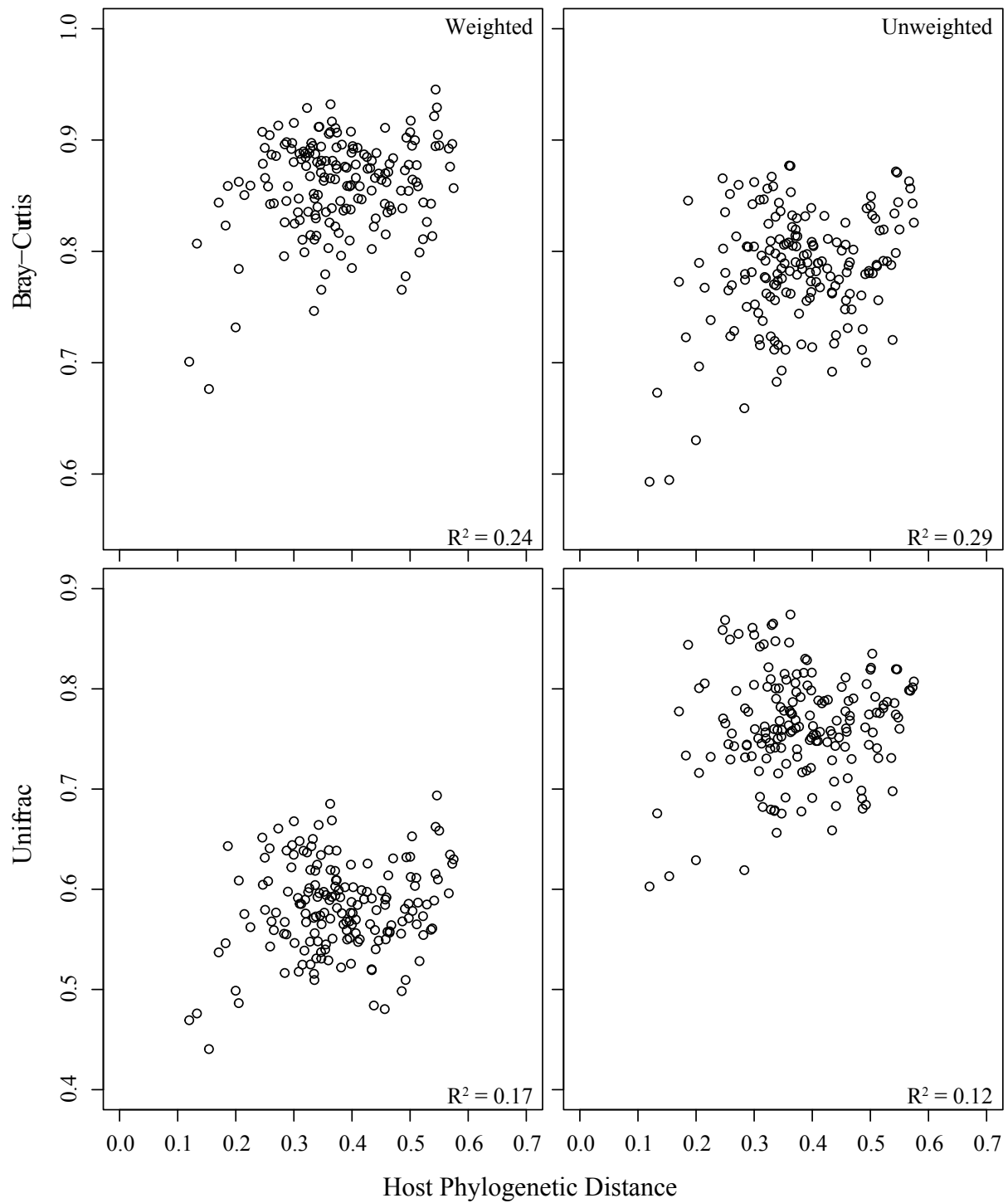


Figure 2.4 Weighted (left) and unweighted (right) Bray-Curtis dissimilarity (top) and Unifrac (bottom) measurements versus phylogenetic distance between host species.

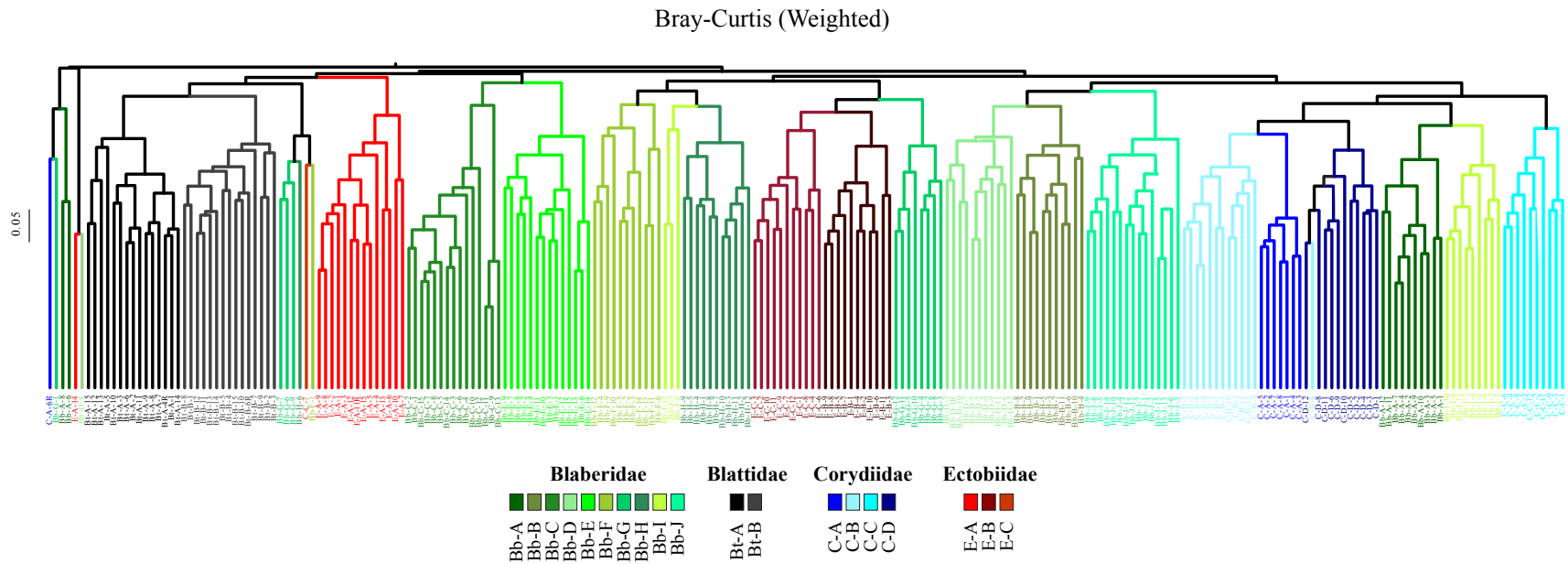


Figure 2.5 Dendrogram of hierarchical cluster analysis using Bray-Curtis dissimilarity (weighted) measurements calculated from OTU (97% sequence identity) measurements

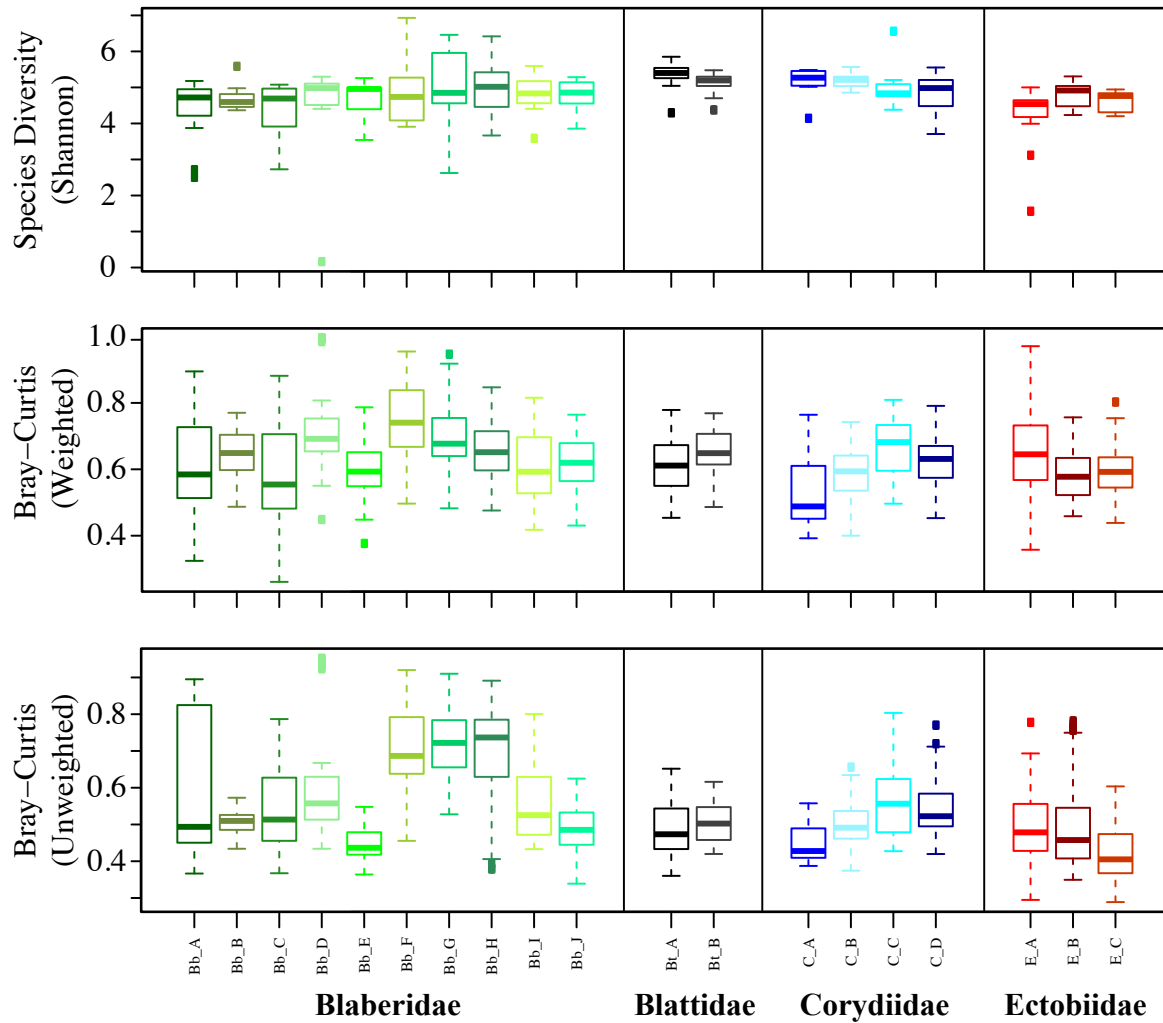


Figure 2.6 Box-plots showing and beta diversity within species. For each box, the bars delineate the median, the hinges represent the lower and upper quartiles, the whiskers extend to the most extreme values (which are no more than 1.5 times the interquartile range from the box), and outliers are plotted, if present.

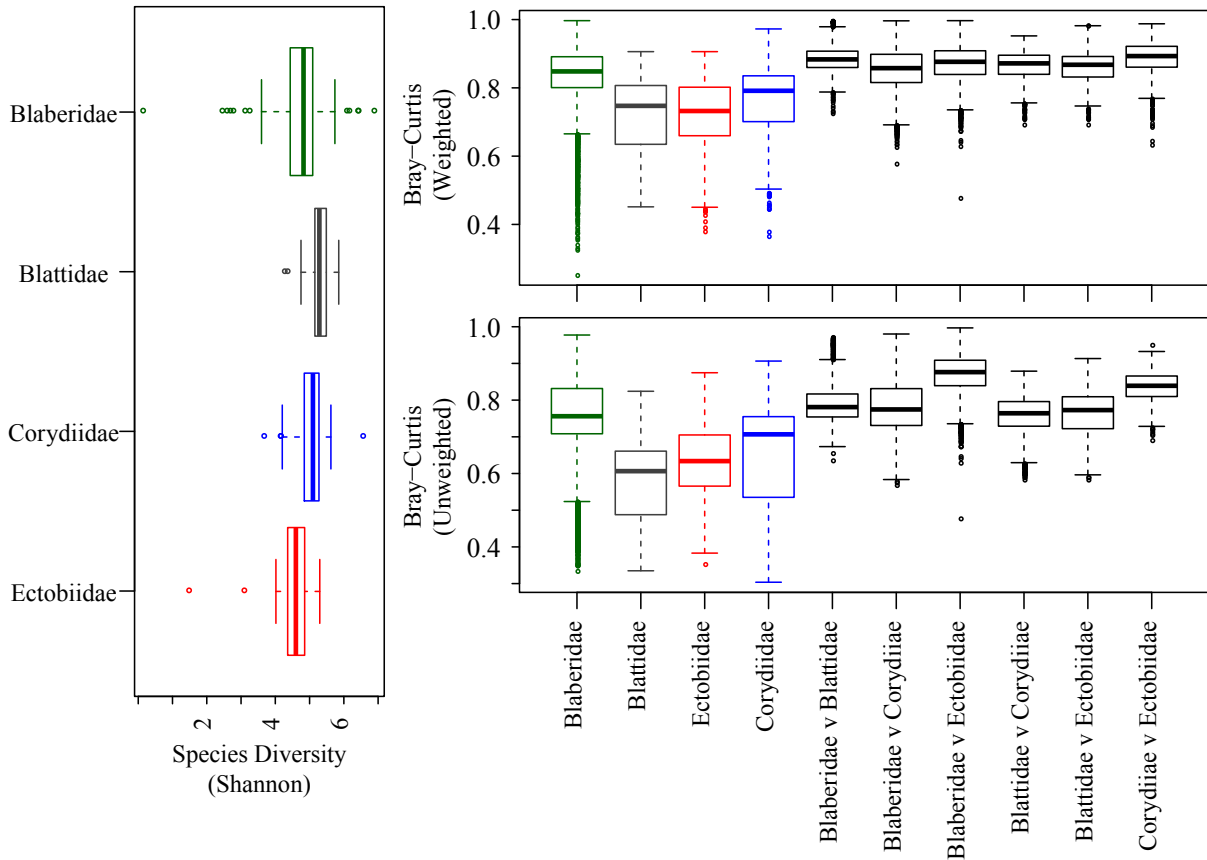


Figure 2.7 Boxplots showing alpha and beta diversity within and between families. For each box, the bars delineate the median, the hinges represent the lower and upper quartiles, the whiskers extend to the most extreme values (which are no more than 1.5 times the interquartile range from the box), and outliers are plotted, if present.

CHAPTER 3

THE CORE GUT MICROBIOME OF THE AMERICAN COCKROACH, PERIPLANETA AMERICANA, IS STABLE AND RESILIENT TO DIETARY SHIFTS¹

¹ Tinker KA and Ottesen EA. 2016. *Appl Environ Microbiol* 82:6603-6610.

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3.1 Abstract

The omnivorous cockroach *Periplaneta americana* hosts a diverse hindgut microbiota encompassing hundreds of microbial species. In this study, we use 16S rRNA gene sequencing to examine the effect of diet on the composition of the *P. americana* hindgut microbial community. Results show that the hindgut microbiota of *P. americana* exhibits a highly stable core microbial community with low variance in composition between individuals and minimal community change in response to dietary shifts. This core hindgut microbiome is shared between lab-hosted and field-collected individuals, although wild-caught specimens exhibit a higher diversity of low-abundance microbes that are lost following extended cultivation under laboratory conditions. This taxonomic stability strongly contrasts with observations of the gut microbiota of mammals, which have been shown to be highly responsive to dietary change. A comparison of *P. americana* hindgut samples with human fecal samples suggests that the cockroach hindgut community exhibits higher alpha diversity but a substantially lower beta diversity than the human gut microbiome. This suggests that cockroaches have evolved unique mechanisms for establishing and maintaining a diverse and stable core microbiome.

3.2 Importance

The gut microbiome plays an important role in the overall health of its host. Healthy gut microbiota typically assists with defense against pathogens and the digestion and absorption of nutrients from food, while dysbiosis of the gut microbiota has been associated with reduced health. In this study, we examine the composition and stability of the gut microbiota of the omnivorous cockroach *P. americana*. We found *P. americana* hosts a diverse core gut microbiome that remains stable after drastic, long-term changes in diet. While other insects, notably ant and bee species, have evolved mechanisms for maintaining a stable association with

specific gut microbiota, these insects typically host low-diversity gut microbiomes and consume specialized diets. In contrast, *P. americana* host a gut microbiota that is highly species rich and consume a diverse, solid diet, suggesting that cockroaches have evolved unique mechanisms for developing and maintaining a stable gut microbiota.

3.3 Introduction

Most insects host simple gut microbial communities, with only a few unique species represented; the reed beetle, honey bee, fruit fly, and gypsy moth all have fewer than 10 species of bacteria found in the gut (1). The low complexity of these communities have been attributed to selective pressures dictated by host physiology (2) as well as the lack of extensive parental contact with offspring in many insects, which offers few opportunities for vertical and social transmission of gut microbes (1, 3). However, certain social and/or gregarious insect species, including cockroaches and their close relatives, the termites, host complex gut communities comprising hundreds of species (1, 4, 5).

The cockroach gut is composed of three compartments: the foregut, midgut, and hindgut. Of the three, the hindgut has the highest bacterial density and diversity (6). This hindgut microbial community breaks down recalcitrant dietary components from food that has passed through the fore- and midgut, supplying the cockroach with volatile fatty acids such as acetate (7). While this is not thought to be an obligate symbiosis, reducing the gut microbiota in *Periplaneta americana* slows development and results in lowered bodyweight and metabolic activity, suggesting that the gut microbiota plays an important role in the health and fitness of cockroaches (7-9). Recent work also suggests the hindgut microbiota are responsible for producing pheromones, including volatile fatty acids, that promote social behavior among cockroaches (10).

While cockroach gut microbes are most closely related to microbes found in termites and other insects, they share many clades with those found in mammals including humans (4, 11). Mammalian studies have found that diets can have strong impacts on gut microbiome composition (12-14). As a result, we sought to determine the extent to which the response of the cockroach gut microbiota to dietary shifts resembles those identified in mammals.

Several studies have been conducted that examine the effect of diet on various cockroach species (15-19). These studies have found a variety of results, with Schauer *et al.* (18) reporting a highly stable core microbiome in *Shelfordella lateralis*, Bertino-Grimaldi *et al.* (17) reporting a small but significant response to dietary shifts in *P. americana*, and Pérez-Cobas *et al.* (19) reporting a strong response in *Blattella germanica*. However, these studies have typically focused on responses to a limited range of substrates, particularly lignocellulosic materials, and all but Pérez-Cobas (2015; three replicates per treatment) lack replication or characterization of the inter-individual variability in microbiome composition. In this study, we utilize high-throughput 16S rRNA gene sequencing to characterize the hindgut microbiome of *P. americana* and its response to a wide range of dietary compositions, including high-fat, high-carbohydrate, and high-protein diets.

3.4 Methods

Insects

P. americana were provided by the University of Georgia's Entomology Department from a colony that has been maintained in captivity for over 10 years. Cockroaches were maintained in mixed age, mixed sex colonies in aquarium tanks at room temperature on a diet of dog food (Kroger Nutritionally Complete Bite Size Adult Dog Food; composed of 21% protein, 8% fat, and 6% fiber) *ad libitum*. Each tank was provided with corn cob bedding, cardboard

tubes for nesting, and a cellulose sponge saturated with water.

Adult cockroaches were selected, weighed, and marked for later identification. Initial, 14-day experiments used 20 adult cockroaches (5 male, 15 female) per treatment. Later time series experiments used either 43 (26 male, 17 female) or 20 (10 male, 10 female) adult cockroaches per treatment. Each diet group was housed in a single plastic tank that contained pebbles for bedding, PVC tubes for nesting, and food and water in shallow plastic dishes. Food, water, and PVC tubes were changed daily, and any visible debris (or deceased cockroaches) removed. Diet treatments included a diet of bran (Bob's Red Mill Organic High Fiber Oat Bran Hot Cereal), butter (Kroger Unsalted Butter Sticks), filter paper (Whatman Qualitative Filter Paper, Grade 1), honey (Kroger Pure Clover Grade A Honey), tuna (StarKist Selects Low Sodium Chunk Light Tuna in Water), white flour (King Arthur Flour Unbleached Bread Flour), whole wheat flour (King Arthur Flour 100% Whole Grain Whole Wheat Flour), a mixed diet (composed by calorie count of 25% tuna, 25% butter, 16.67% whole wheat flour, 16.67% white flour, and 16.67% honey), and a starvation control (Table 3.1).

For studies of field-collected cockroaches, insects were collected in traps placed outside on the University of Georgia's campus. Traps comprised of glass jars containing glass wool saturated with beer as a lure and with petroleum jelly placed around the jar opening to prevent insects from escaping the jars once entered. Traps were checked daily and any captured adult *P. americana* were either sacrificed immediately or placed in an aquarium tank under laboratory culture conditions (as described above) for 14 days before being sacrificed.

Sample Collection & DNA Extraction

Hindgut sample collection occurred on day 14 of the short-term dietary shift and, as the treatment populations permitted, throughout the long-term dietary shift. For the wild comparison,

hindgut sample collection occurred either within 24 hours of collection or after 14 days in laboratory conditions. Cockroaches were removed from tanks, weighed, and placed on ice in sterile culture plates. After approximately 20 min, or when the cockroaches were sufficiently torpid, cockroaches were dissected and the entire gut removed from the cockroach. Any visible debris, including fat bodies or exoskeleton, was removed with forceps. The hindgut was then separated from the rest of the gut using a scalpel and placed on parafilm. The hindgut was submerged in 100 μ l of RNALater (Ambion, Austin, TX, USA) and a pipette tip was used to break open the gut and disperse the contents into the RNALater (Ambion). The suspended gut lumen was then removed, leaving behind the hindgut wall, and placed in storage at -80°C .

DNA was extracted from an aliquot of the total preserved hindgut sample using a modified version of the EZNA Bacteria Kit (Omega Bio-tek, Norcross, GA, USA). Preserved and frozen hindgut samples were thawed on ice. 30 μ l was removed for extraction while the rest was returned to storage at -80°C for future use. 100 μ l of balanced salt solution (2.5 g K_2HPO_4 , 1 g KH_2PO_4 , 1.6 g KCl, 1.4 g NaCl, and 10 mL of 1M NaHCO_3 per liter, pH 7.2) was added to each sample aliquot, mixed, and centrifuged for 10 min at 5000 x g. After centrifugation, the supernatant was discarded and the pellet was resuspended in 100 μ l TE Buffer (10 nM Tris, 1 mM EDTA, pH 8) and 10 μ l lysozyme (as supplied by kit). The sample was incubated at 37°C for 30 min. Approximately 25 mg of glass beads (as supplied by kit) were added to the sample, which was then bead beaten for 5 min at 3000 rpm using a vortex with a horizontal adaptor. 100 μ l BTL Buffer and 20 μ l Proteinase K solution (as supplied by kit) were added to each sample, which was incubated at 55°C while shaking at 600 rpm for one hour. After this step, the manufacturer's protocol (June 2014 Version) was followed beginning at step 11. Samples were eluted in 50 μ l preheated elution buffer after a 5 min incubation at 65°C . The final DNA

concentrations (typically between 5-50ng/ μ l) and A260/A280 were measured using a NanoDrop Lite spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Library Preparation and Sequencing

The V4 region of the 16S rRNA gene for each gut sample was amplified in replicate using a two-step PCR method based on the work of Caporaso *et al.* (20). The initial PCR reaction used Q5 Hot Start High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA, USA) and 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) primers in a 10 μ l PCR reaction (1X Q5 reaction buffer, 200 μ M dNTPS, 0.5 μ M 515F, 0.5 μ M 806R, 2 ng DNA, 0.02 U/ μ L Q5 polymerase) under the following conditions: 98°C for 30 s; followed by 15 cycles at 98°C for 10 s, 52°C for 30 s, and 72°C for 30 s; with a final extension step at 72°C for 2 min for the initial V4 region amplification.

Immediately following the initial amplification, the resulting product was re-amplified using primers (SI Table 3.1) that contained double Hamming barcodes (21). This two-step PCR scheme was used in order to ensure high quality amplicons, as the initial replication occurred before the addition of Illuminia-specific adaptors or sample-specific barcodes. The secondary amplification mix contained 1X Q5 reaction buffer, 200 μ M dNTPS, 0.5 μ M 515F, 0.5 μ M 806R, 2 ng DNA, 0.02 U/ μ L Q5 polymerase. 21 μ L of this mix was added to 9 μ L of the initial reaction product. These reactions were then cycled under the following conditions: 98°C for 30 s; followed by 4 cycles at 98°C for 10 s, 52°C for 10 s, and 72°C for 30 s; followed by 6 cycles at 98°C for 10 s and 72°C for 1 min; concluding with a final extension at 72°C for 2 min.

Two independent PCR reactions with unique barcode combinations were generated for each sample. These technical replicates were pooled and purified using the EZ Cycle Pure kit

(Omega Bio-tek) according to manufacturer's protocols. Samples were eluted in 30 μ L of elution buffer. Purified amplicons were quantified using a NanoDrop Lite spectrophotometer (Thermo Scientific). Amplicons from twelve guts per treatment for the short-term dietary shift, all available guts excluding day 30 for the long-term dietary shift, and all available guts for the wild comparison were normalized and pooled to a concentration of 10 nM based on a predicted total product size of ~400 base pairs. The quality of the prepared library was assessed using the Agilent 2100 Bioanalyzer DNA-HS assay (Agilent Technologies, Santa Clara, CA, USA) before submission to the Georgia Genomics Facility for sequencing (Illumina Miseq 250 x 250 base pairs; Illumina, Inc., San Diego, CA, USA).

American Gut Project (AGP) Data Retrieval

The American Gut Project (AGP) is a collaborative effort to characterize the human gut microbiome through crowd-sourcing fecal samples for 16S rRNA analysis from the public (22, 23). We chose to use data from the AGP as a human comparison for our cockroach data as the AGP uses the same 16S rRNA primers (515F/806R) and sequencing technology (Illumina Miseq) as used in our experiments (22). A file containing all demultiplexed, full length, debloomed sequences from the AGP was downloaded (April 2015 version). From this file, a subset of 157 samples was randomly selected from individuals who provided their sex and were between 20 and 60 years of age. This subset of samples was analyzed using the method described below.

Data Analysis

The Mothur software package was used to analyze the sequences generated from this experiment (24). The MiSeq standard operating protocol was followed (25, 26), with the following modifications: after sequences were assembled, sequences that had any ambiguous

bases or were longer than 275 base pairs were removed; sequences that passed this initial screening process were aligned to the Silva reference database (Release 123) (27-29); aligned sequences were again screened, this time in order to remove sequences that contained homopolymers of 8 or more base pairs; UCHIME was used in order to identify chimeras from the remaining sequences (30); after chimera removal, the Wang method was used for taxonomic classification of samples with the Greengenes reference database (August 2013 Version) (31-33); sequences that were unclassifiable or identified as chloroplasts, mitochondria, Eukaryota, or *Blattabacterium* (cockroach endosymbiont found in fat body cells) were removed. The remaining sequences were clustered into OTUs based on 97% or greater sequence identity.

In order to make an accurate comparison between data generated from this experiment and data provided by the AGP, sequences generated from this experiment were trimmed to match the length of samples provided by the AGP. All sequences were then analyzed using the same pipeline as described above. Figures containing only the unique data generated in this experiment used the original dataset; figures containing comparisons to the AGP data used the trimmed dataset.

Accession Numbers

The sequences generated from this experiment were submitted to the NCBI Sequence Read Archive and are available under the accession numbers SRP075213, SRP075102, and SRP075057.

3.5 Results

Effect of Diet on Hindgut Microbial Community

Laboratory-raised adult cockroaches were maintained for 14 days on a variety of diets including tuna, butter, honey, whole wheat flour, white flour, a mixture of the above, and a

starvation diet. Over the course of the experiment, only the butter and starvation treatments were found to have a significant change in weight (paired t-test, $p < 0.001$ and < 0.05 , respectively). After 14 days on each diet, cockroaches were sacrificed and hindgut lumen contents were used for microbial DNA extraction and 16S rRNA gene amplicon sequencing. A total of 28,742,658 16S rRNA sequences were obtained from 99 unique samples of which 15,754,172 passed quality checks, resulting in an average of 1,294 OTUs per sample.

Bacteroidetes, *Firmicutes*, and *Proteobacteria* were the predominant phyla present in the gut microbiota of all treatments (Figure 3.1, SI Figure 3.1). Within the *Bacteroidetes* phylum, bacteria from the *Porphyromonadaceae*, *Rikenellaceae*, *Bacteroidaceae* families were especially prevalent, accounting for over 40% of the total bacteria in several cockroaches. *Clostridia* represented the majority of *Firmicutes* in the cockroach gut, though there were other classes present, such as *Erysipelotrich* and *Bacilli*. In the *Proteobacteria* phyla, *Desulfobacteraceae* and *Enterobacteriaceae* were the major families represented. The predominant archaeal taxon was *Methanomicrococcus blatticola*, a methanogen most commonly associated with cockroaches (34). These results agree well with previously published studies of cockroaches (17-19). Overall, most identified microbes are typical of an omnivorous diet and are often found in the human gut (13, 35). However, many of the microbes found were unclassifiable past the class or family level, suggesting that they may belong to poorly characterized, insect-specific lineages.

Dietary shifts did not result in large changes in gut microbial community composition. No large differences in the relative abundances of major bacterial phyla or families were observed between dietary treatments (Figure 3.1, SI Figure 3.1). This is in contrast to mammals, where dietary shifts have been shown to change the ratio of *Bacteroidetes*: *Firmicutes* as well as other members of the microbial community (13, 14). This stability in gut microbiome

composition was apparent at all taxonomic resolutions. Ordination analysis did not identify a strong impact of diet on the microbial community composition at the 97% OTU level (SI Figure 3.2). Neither non-metric multidimensional scaling nor principal component based analyses detected clear separation between each diet treatment, suggesting that diet does not have a strong impact on the microbial community composition. PERMANOVA analysis did detect a significant effect for diet on community composition (p -value = 0.001). However, the biological significance of this difference is unclear, as the effect size was small ($R^2 = 0.21$ overall; average R^2 for 100 random permutations of data labels = 0.08). Similarly, pairwise comparisons of individual diets with the dog food control identified small ($R^2 = 0.11$ - 0.23) but significant (p -value = 0.001-0.004) shifts in community composition (SI Table 3.2). In addition, we did not observe large shifts in alpha or beta diversity following treatment (SI Figure 3.3).

The initial short-term dietary perturbation was followed up with an extended time series. This long-term dietary shift included the two additional dietary treatments of bran and filter paper (Table 3.1) as well as more frequent sampling on days 1, 2, 3, 7, 14, 30, 60, and 90. These experiments also showed minimal dietary effects on gut microbiota composition (SI Figures 4-7).

Individual-to-Individual Variation

An initial hypothesis was that diet-driven changes in gut microbiome composition might have been obscured by high individual-to-individual variation. In order to test this, we sought to compare the relative level of individual-to-individual variation observed among *P. americana* to those found in other animals with complex gut communities. For this comparison, we used 157 randomly chosen human fecal samples from the American Gut Project (AGP) (22). This dataset

was chosen as it represents an extensive examination of individual-to-individual variation in gut microbiome composition in an animal that shares many traits (an omnivorous diet and an anoxic, circumneutral hindgut lumen extensively colonized by microbes) with cockroaches. One potential caveat is that the degree to which fecal samples accurately reflect the microbial community composition of the gut lumen is poorly constrained. However, we felt that this comparison served to place our observations of cockroach gut microbial diversity into context. To minimize artifacts resulting from differences in the sequencing technology used, we trimmed our data to similar read lengths and jointly re-processed the combined human and cockroach dataset as described in the methods. After quality control measures a total of 2,768,251 16S rRNA sequences remained from 138 unique human fecal samples, with an average richness of 1075 OTUs per human fecal sample. The reprocessed cockroach data comprised 15,899,340 16S rRNA gene sequences with an average of 1713 OTUs per sample.

Comparison of *P. americana* hindgut community composition with human fecal samples shows that the cockroach gut community consistently exhibits higher alpha diversity at the 97% OTU level than the human gut microbiota (Figure 3.2). In contrast, comparisons of *P. americana* identified much lower beta diversity than that observed between human samples (Figure 3.2). Similar trends were observed in comparisons of our data to datasets from studies of humans and humanized mice (36-38) (data not shown). Pairwise comparisons of individual cockroach gut samples showed significantly lower average Bray-Curtis dissimilarities than a similar comparison of human fecal samples for both abundance-weighted and unweighted measures (Figure 3.2). This suggests that the cockroach population has less individual-to-individual variation than the human population. Moreover, the lower unweighted (presence/absence-based) dissimilarity suggests that the cockroach population has a richer and more extensive core gut

microbiota than the human population (Figure 3.2). A shared core community of 201 OTUs (SI Table 3.3) that were present across all dietary treatment groups was identified, averaging 67% of the sequences recovered from cockroaches within all dietary treatment groups. In contrast, only 5 OTUs were shared among all 138 human samples (SI Table 3.4), accounting for an average of 31% of the sequences recovered from human fecal samples.

Comparison of the Gut Microbiota of Wild-caught and Laboratory-raised Insects

Our dietary perturbations revealed that laboratory-raised *P. americana* host a gut microbiota that has very low individual-to-individual variability relative to human fecal samples. A comparison between laboratory-raised and wild-caught *P. americana* was conducted in order to verify that this low diversity was a common property of this species and not an artifact of laboratory culture conditions. To do so, we examined the gut microbiota of freshly captured *P. americana* individuals both immediately upon capture and following 14 days of culture under laboratory conditions.

At the phylum level, the gut microbiota of wild-caught *P. americana* is similar to that of the laboratory cockroach population. *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* were the predominant phyla present in the gut microbiota of all treatment groups (Figure 3.3A). Wild-caught individuals exhibited a higher abundance of *Proteobacteria* and a relatively lower abundance of *Bacteroidetes* and *Firmicutes*, (t-test, $p < 10^{-5}$ for both time points) which became more similar in abundance following 14 days of cultivation under laboratory conditions.

At the 97% identity OTU level, laboratory-raised and wild-caught populations clustered independently by ordination analysis, with wild-caught cockroaches becoming more similar to the laboratory population following 14 days of housing under laboratory conditions (Figure 3.3B). The wild-caught cockroaches exhibited higher alpha diversity (Figure 3.3C), and showed

increased individual-to-individual variation by unweighted Bray-Curtis dissimilarity metrics (Figure 3.3C). However, the wild-caught and laboratory-raised cockroaches showed similar levels of individual-to-individual variation by abundance-weighted Bray-Curtis metrics (Figure 3.3C), suggesting that much of the difference in beta diversity may be attributed to a greater representation of low-abundance, transiently hosted microbes in the guts of wild-caught cockroaches. This may be a result of environmental exposure to a higher diversity of microbes. Consistent with this hypothesis, alpha diversity in the guts of wild-caught cockroaches decreased following 14 days of cultivation under laboratory conditions.

Direct comparisons of laboratory-raised and wild-caught cockroaches identified significantly greater between-group than within-group dissimilarities (Figure 3.3C). This suggests that there are differences in the specific microbial OTUs hosted by these two populations. However, these between-group dissimilarities are lower than those observed between individual human fecal samples, suggesting that these gut populations still exhibit a large degree of overlap following over a decade of laboratory cultivation. Consistent with this, the three treatment groups, which had an average of 1,575 OTUs per sample, shared 199 microbial OTUs (SI Table 3.5) that made up an average of 47% of the sequences recovered from the initial wild-caught, 55% from the wild-caught after 14 days in laboratory conditions, and 54% from the laboratory-raised cockroach gut communities. Interestingly, while alpha diversity within wild-caught populations does decrease following 14 days in the laboratory, the level of dissimilarity between laboratory and wild-caught cockroach populations does not decrease substantially. This suggests that the core gut microbiome of wild-caught cockroaches was not replaced with laboratory-associated species in that time period.

3.5 Discussion

Diet has a strong role in shaping the structure and function of the mammalian gut microbiome (12-14, 39). Our goal was to determine to what extent the omnivorous insect *P. americana* exhibited similar trends. Our results show that adult *P. americana* has a rich, extensive core gut microbial community with minimal variation between individuals. The cockroach gut core community (SI Table 3.3, 3.4) is primarily composed of bacteria in the *Bacteroidetes* and *Firmicutes* phyla, though members of the *Euryarchaeota*, *Actinobacteria*, *Proteobacteria*, *Synergistetes*, *Tenericutes*, and *Verrucomicrobia* phyla are present as well as multiple unclassified bacteria. This core was found to be present in both laboratory-raised and wild cockroaches. These results contrast strongly with observations of human fecal samples, which exhibit substantial individual-to-individual variation and few, if any, shared microbial OTUs.

P. americana's stable, extensive core community appears to be a unique characteristic of the cockroach and is highly resilient to changes in host diet. Our results are in agreement with a study of the cockroach *S. lateralis*, which found no observable differences among the gut microbiota of cockroaches fed a low-or high fiber diet (11). Similar work in *P. americana* and the related cockroach species *B. germanica* did identify significant changes in their gut communities in response to diet (17, 19). However, both studies used alternate sequencing technologies that resulted in a smaller number of sequences (216 and 48, 527, respectively) and examined fewer treatments (three and two, respectively) (17, 19).

In mammals, different microbial groups are believed to specialize in the utilization of specific dietary substrates, in part because they tend to increase in abundance when these substrates are enriched in the host's diet. For example, *Bacteroidetes* are associated with high-

protein diets while *Firmicutes* are associated with high fiber diets (35). This hypothesis is based on two assumptions: 1) that all gut microbes cannot utilize all substrates equally well and that microbial abundance in the gut is dependent on their ability to obtain substrates for growth and 2) that a change in dietary composition will translate into a change in substrate availability within the gut. The absence of diet-driven changes in cockroach gut microbiome composition suggests that one of these assumptions is not true. One possibility is that cockroach-associated gut microbes are substantially more metabolically versatile than mammalian-associated species, and can therefore survive equally well when presented with a wide range of dietary compositions. Similarly, the ability to utilize the dietary substrates tested may be widely distributed across cockroach gut microbial lineages, such that changes in substrate availability are driving “hidden” changes in microbial representation at a sub-OTU taxonomic resolution. Finally, it may be that cockroach gut microbes are obtaining growth substrates through an alternative pathway, such as metabolic cross-feeding between gut microbes or provision of key substrates by the host. Future investigations of the metabolic capabilities of cockroach gut microbes should provide further insight into these questions.

Cockroaches are among the most diverse and abundant members of the animal kingdom, surviving in a wide variety of habitats from the tropical rainforest and mountainous caves to urban environments (40, 41). The American cockroach, *P. americana*, can be found throughout the world; however it is best known as a common household pest, thriving in warm and moist environments such as steam tunnels or boiler rooms (6, 42). Maturing to adulthood in as few as six months and living for up to two years, adult *P. americana* are opportunistic feeders that can survive on a wide variety of food sources (40, 43) and frequently subsist on no or limited food

for days at a time (5). Thus, a stable resident gut community would provide a remarkable evolutionary advantage.

Insects have evolved diverse mechanisms for the maintenance of stable host-symbiont relationships with their gut microbiota. Heteropteran stinkbugs have developed highly species-specific associations with individual gut symbionts that are either maternally transmitted or acquired in early development (44-46). Other insects have established stable relationships with simple gut communities, including honey and bumble bees (47, 48) as well as ants (49). While the mechanisms by which bees regulate their gut microbiome has not been established, the Sonoran Desert turtle ant, *Cephalotes rohweri*, was recently shown to have a mechanical filter that blocks any bacteria or particles larger than 0.2 μ m from entering into the midgut and hindgut after an initial gut microbiome is established (50). However, stable host/gut symbiont associations have heretofore been primarily found in insects with specialized diets and low-diversity gut microbiota. Thus, it is unlikely that the same mechanisms are at work in *P. americana*, which consumes a wide-ranging, omnivorous diet and hosts a highly-diverse gut microbiome that compositionally resembles that of mammalian omnivores (11).

Termites have long-been known to have a symbiotic relationship with their gut microbial community, which, like the cockroach gut microbiota, is extensive and diverse (1, 51). The termite's more restricted herbivorous diet and social behavior are currently thought to be the key drivers that shape the development of their specialized gut microbiota (51). However, given that molecular analyses suggest that termites fall within the cockroach radiation (52), these results suggest an alternative hypothesis, in which the ability to maintain a stable gut microbiome evolved prior to, and perhaps facilitated, the evolutionary shift to a lignocellulosic diet. Further

work should provide insight into the mechanisms underlying this stability and its role in shaping cockroach (and termite) evolution and ecology.

3.6 Acknowledgements

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3.9 Tables

Table 3.1. Nutrient Information for 100g Serving of Each Diet Treatment

Diet Treatment	Calories	Protein	Carbohydrate	Fat	Fiber
Bran ^L	375	17.5	67.5	5	17.5
Butter ^{S,L}	714	0	0	79	0
Filter Paper ^L	U	U	U	U	U
Honey ^S	286	0	81	0	0
Tuna ^{S,L}	107	27	0	1	0
White Flour ^{S,L}	367	13	73	0	3
Whole Wheat Flour ^S	333	13	67	2	13
Mixed ^S	239	18	27	7	2
Starvation ^{S,L}	N/A	N/A	N/A	N/A	N/A

^S and ^L correspond to treatments used in the short-term and long-term dietary shifts, respectively.

U is unavailable. N/A is not applicable. Nutritional facts as stated by the manufactures of each food product. Note that the mixed diet was based on the general guidelines for a typical human diet. Thus, it was calorically composed of 25% tuna, 25% butter, 16.67% whole wheat flour, 16.67% white flour, and 16.67% honey and the values shown assume a daily diet of 2000 calories.

3.10 Figures

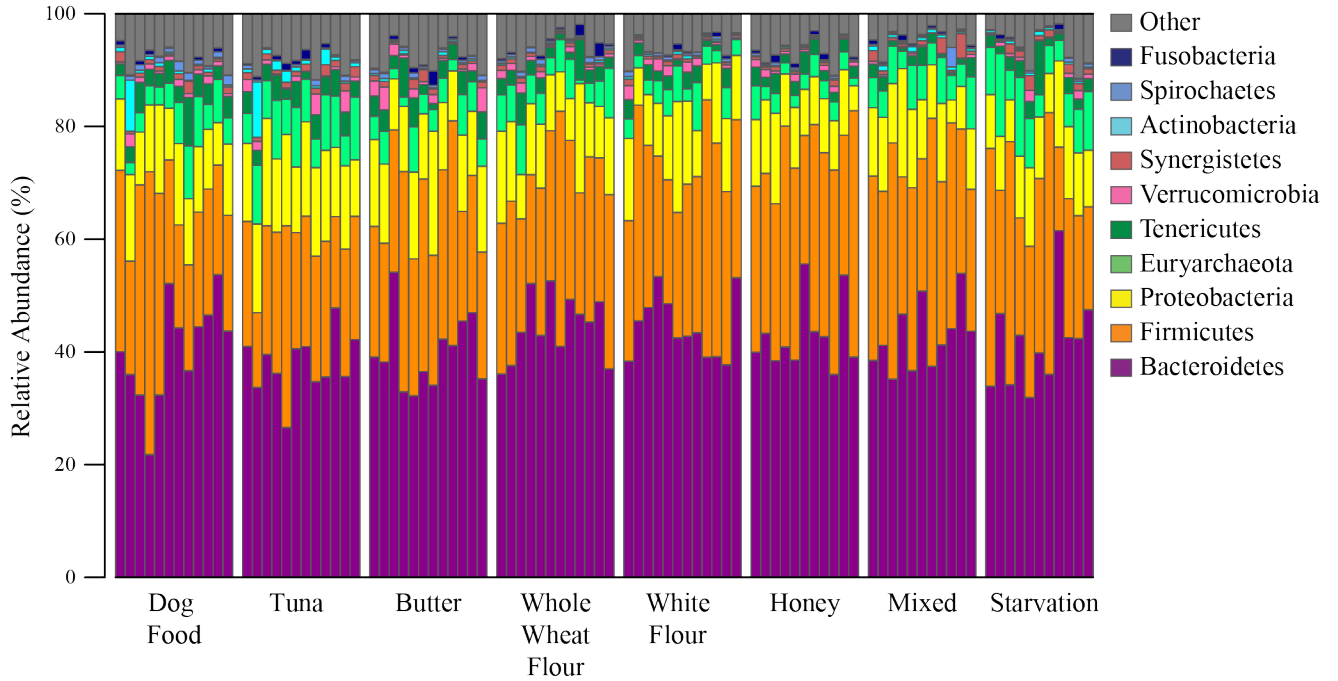


Figure 3.1. Relative abundance of microbial phyla across 14 day diet treatments (Table 3.1).

Each bar represents an individual cockroach gut. The ten most abundant phyla are shown.

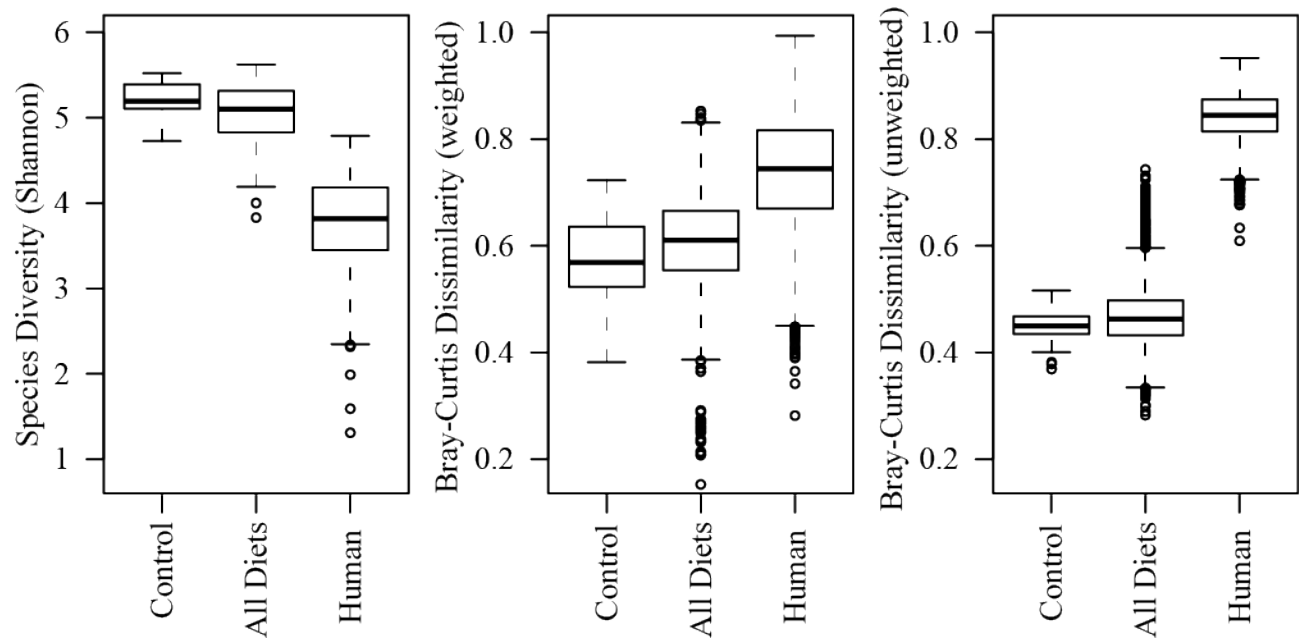


Figure 3.2. Alpha and beta diversity among cockroach gut and human fecal samples. Boxplots show Shannon diversity indices (left) and weighted (middle) and unweighted Bray-Curtis dissimilarity (right) between the laboratory cockroaches raised on a dog food diet, all cockroach treatment groups, and human gut microbial communities at the OTU level (97% sequence identity). Human data was obtained from the American Gut Project (22). Cockroach data was trimmed to the same lengths and alignment positions as the human gut data prior to OTU calling, and all libraries were resampled to a depth of 4000 sequences. For each group, the bar delineates the mean, hinges represent the lower and upper quartile, whiskers extend to the most extreme value which is no more than 1.5 times the interquartile range away from the box, and outliers are plotted if present.

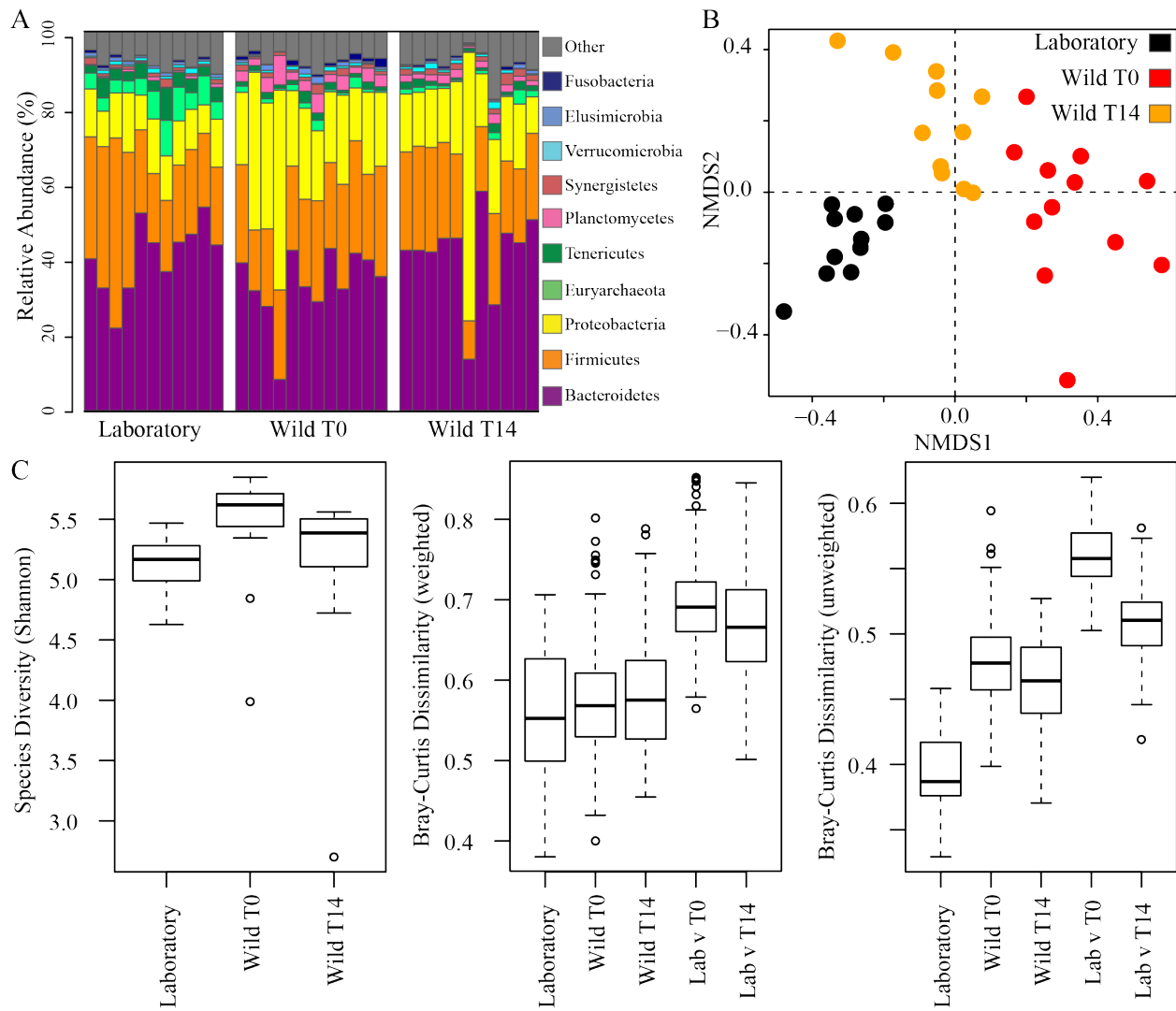


Figure 3.3. Comparison of laboratory-raised and wild-caught cockroach gut microbiota. A) Relative abundances of the 10 most abundant phyla identified among laboratory-raised and wild-caught cockroach gut samples immediately following capture (T0) or following 14 days of culture under laboratory conditions (T14). Each bar represents an individual cockroach gut. B) Non-metric multidimensional scaling (NMDS) plot of laboratory-raised and wild-caught cockroaches. PERMANOVA based on dissimilarities was also conducted (R^2 of 0.242, p -value of 0.001). C) Boxplots comparing Shannon diversity (left) and weighted (middle) and unweighted (right) compositional dissimilarity among and between the three groups at the OTU

level (97%) sequence identity). For analysis in parts B and C, libraries were sampled to a constant depth of 4000. Boxplots were produced as described for Figure 3.2. For each group, the bar delineates the mean, hinges represent the lower and upper quartiles, whiskers extend to the most extreme value which is no more than 1.5 times the interquartile range away from the box, and outliers are plotted if present.

CHAPTER 4

THE PRAYING MANTIS HOSTS A HIGHLY VARIABLE GUT MICROBIOME

DOMINATED BY MANTID-SPECIFIC BACTERIAL LINEAGES¹

¹ Tinker KA and Ottesen EA. To be submitted to PLOS One.

4.1 Abstract

Praying mantids are predators that consume a wide variety of insects, including cockroaches. While closely related to termites and cockroaches, which are known for their diverse gut microbiota, little is known about the mantid gut microbiome or their importance to host health. In order to better understand the mantid gut microbiome and its relationship to host health, we conducted a 16S rRNA gene-based study of gut microbiome composition in adults and late-instar larvae of three mantid species. We found that the praying mantis gut microbiome is highly variable, and often dominated by microbes that are present in low abundance or not found in the guts of their insect prey. Future studies will explore the role of these microbes in the digestion of the dietary substrates and/or the degradation of toxins produced by their insect prey.

4.2 Introduction

Praying mantids are insect predators that consume a carnivorous diet (1-5). The most well studied praying mantis species live in temperate regions such as North America, although the majority are native to tropical or subtropical regions (6). Common species, such as the Chinese mantis (*Tenodera sinensis*), have been used to study and model predator behavior (7-12). These praying mantids are sympatric general predators that typically hunt based on prey size, primarily subsisting on a diet of small insects including fruit flies, crickets, and caterpillars, although adult praying mantids are known to consume small birds or small reptiles (1-5).

Emerging work suggests that praying mantids do not uniformly consume available insect prey (3, 13, 14). Rafter et al. demonstrated that Chinese mantids sometimes gut the body of their insect prey in order to avoid specific nutrients and/or toxins, rather than indiscriminately consuming the entire body of their insect meal (14). These sophisticated behavior patterns suggest that the Chinese mantid has the ability to evaluate the resource quality of available prey.

This behavior may extend beyond predator-prey interactions, as these carnivores are known to ingest a high-protein herbivorous diet of pollen when there is no available prey. Chinese mantids that consume an omnivorous diet are more fit than their strict carnivore counterparts, with larger body mass, higher fecundity, and increased offspring survival (15).

A host's diet and gut microbiota are inherently linked; the gut microbiota assists the host with food digestion and nutrient absorption, provides a layer of protection against opportunistic pathogens, and can metabolize certain toxins and/or environmental pollutants (16-18). Therefore, it is possible that the praying mantis gut microbiome plays a role in shaping the diet and/or predatory behaviors of the host. However, to our knowledge, no studies have been conducted that examine the structure of the praying mantis gut microbiome, likely due to characteristics that make it difficult to study the praying mantis and its gut microbiota. Praying mantids are solitary, cryptic insects that often blend in to the nearby environment (19, 20), which makes capturing a large number of wild insects labor-intensive. Mantids also have a high mortality rate, with only a fraction of the initial cohort surviving to adulthood (21-24). This high mortality rate is partially due to predation, although they have specific temperature, humidity, and dietary requirements that impact their survival and make rearing in a lab environment particularly difficult (3, 6, 15, 19, 25, 26).

We aimed to fill this knowledge gap by completing a survey of the hindgut microbiota from 15 individual praying mantids across three species using 16S rRNA sequencing. Praying mantids were fed a uniform diet of cockroaches, allowing us to 1) characterize the praying mantis gut microbiome and 2) determine if the praying mantis gut microbiome is composed solely of members of the prey microbiome or if it hosts a unique gut microbiota populated by mantid-associated bacterial lineages.

4.3 Methods

Insects and Sample Collection

40 fourth-instar praying mantis nymphs were purchased from Bugs in Cyberspace. Four species, with ten nymphs per species, were represented: *Tenodera sinensis*, *Hierodula venosa*, *Deroplatys lobata*, and *Gongylus Gongylodes*. Nymphs were kept isolated in plastic cups, with a pipe cleaner for perching and a cotton ball damp with water. Nymphs were initially raised on a mixed diet of *Drosophila melanogaster*, *Drosophila hydei*, and *Periplaneta americana* nymphs. After several molts, surviving praying mantids were exclusively fed *P. americana* until their sacrifice. The initial aim of the work was to sacrifice healthy adults at one week past their final molt. Given high rates of insect loss, particularly following the final adult molt, adults and nymphs were sacrificed early when their overall body condition and activity levels suggested that they were unlikely to survive to the target age. *P. americana* were provided by the University of Georgia's entomology department. They are maintained in mixed-age, mixed-sex colonies in aquarium tanks on a diet of dog food (Pet Pride Chunk Style Complete Nutrition Dog Food [Pet Pride], composed of 21% protein, 9% fat, and 4% fiber) *ad libitum*.

Insects were placed in CO₂ chambers or on ice in sterile culture plates until sufficiently torpid (Table 4.1, SI Table 4.1). The insects were then dissected and the whole gut was removed. The whole gut was rinsed with 1XPBS diluted from 20X stock at pH 7.5 (Amresco, Solon, OH), and visible debris was removed with forceps. The whole gut was placed on a sterile surface before separating the hindgut with a scalpel. After separation, the hindgut was placed in 1XTE Buffer and stored -80 °C.

DNA Extraction

A modified version of the EZNA Bacteria Kit (Omega Biotek, Norcross, GA) was used to extract microbial DNA from stored hindgut samples. After thawing on ice, samples were pulverized with a sterile microcentrifuge pestle. Pulverized samples were centrifuged for 10 min at 5,000 g, the supernatant was discarded, and the pellet was resuspended in 100 μ L of 1XTE Buffer. 10 μ L lysozyme (as supplied by kit) was added to the resuspended sample before incubation at 37°C for 30 min. After incubation, approximately 25 mg of glass beads (as supplied by the kit) was added to each sample. Samples were bead beaten for 5 min at 3,000 rpm using a vortex mixer with a horizontal adapter. Next, 100 μ L BTL buffer and 20 μ L proteinase K solution (as supplied by the kit) were added to each sample before incubation at 55°C while shaking at 600 rpm for 1 h. After incubation, the manufacturer's protocol (June 2014 version) was followed beginning at step 11. The final DNA concentrations (ranging from 40 to 800 ng/ μ L) and A260/A280 were measured using a NanoDrop Lite spectrophotometer (Thermo Scientific, Wilmington, DE).

Library Preparation and Sequencing

A previously described two-step PCR method was used to prepare the amplicon library for sequencing (27). During the initial reaction the V4 region of the 16S rRNA gene was amplified with the 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) primers. The initial reaction contained 1X Q5 buffer (NEB), 200 μ M dNTPS, 0.5 μ M 515F, 0.5 μ M 806R, 2 ng template DNA, and 0.02 U/ μ L Q5 Hot Start High-Fidelity DNA polymerase (NEB) for a total reaction volume of 10 μ L. PCR conditions were 98°C for 30 s for denaturation followed by 15 cycles at 98°C for 10 s, 52°C for 30 s, and 72°C for 30 s. The final extension step was 72°C for 2 min.

Immediately after the final extension, 9 μL of the initial reaction product was reamplified using barcoded 515F and 806R primers (27) (SI Table 4.2). The initial reaction product was added to the second reaction, which contained 1X Q5 buffer (NEB), 200 μM dNTPS, 0.5 μM 515F, 0.5 μM 806R, and 0.02 U/ μL Hot Start High-Fidelity DNA polymerase (NEB) at a volume of 21 μL . PCR conditions were for 98°C for 30 s for initial denaturation; 4 cycles at 98°C for 10 s, 52°C for 10 s, and 72°C for 30 s; 6 cycles at 98°C for 10 s and 72°C for 1 min; and the final extension step at 72°C for 2 min.

Amplicons were visualized on a 2% w/v agarose gel. PCR for failed samples was redone with an additional 5 cycles during the initial reaction (SI Table 4.1). Amplicons were purified using the EZ Cycle Pure Kit (Omega Biotek) before quantification with a NanoDrop Lite spectrophotometer (Thermo Scientific). Samples were normalized and pooled to a concentration of 10 nM on the basis of a predicted total product size of ~400bp. The pooled library was submitted to the Georgia Genomics facility for sequencing (Illumina MiSeq 250x250 bp; Illumina, Inc., San Diego, CA).

Data Analysis

The Mothur software was used to analyze 16S rRNA gene sequences generated during this experiment (28). A modified version of the Miseq standard operating protocol (29, 30) was followed. Sequences were assembled and screened to remove any that contained ambiguous bases or were longer than 275 base pairs. Remaining sequences were aligned to the Silva reference database (Release 128) (31-33). After alignment, sequences were screened to remove any that contained homopolymers of 8 or more base pairs. Chimeras were identified via UCHIME and removed (34). Remaining sequences were classified using the DicDB reference database (Version 3.0), a specialized Silva-based reference database that provides high resolution

for gut microbiota associated with cockroaches and termites. Any sequences that were unclassified or identified as chloroplasts, mitochondria, Eukaryota, or *Blattabacterium* (cockroach endosymbiont found in fat body cells) were removed. The remaining sequences were clustered using the OptiClust (35) method into OTUs based on 97% or greater sequence identity.

Accession Numbers

The sequences generated from this experiment were submitted to the NCBI Sequence Read Archive and are available under the accession numbers SRP132948 and SRP132487.

4.4 Results

16S rRNA gene libraries were prepared and sequenced from hindguts of praying mantids fed a diet of laboratory-raised cockroaches and from hindguts of laboratory-raised cockroaches. The laboratory-raised cockroaches were included in our analysis in order to provide context regarding prey gut microbiome composition. This 16S rRNA library resulted in a total of 1,426,892 raw sequences, with 939,355 remaining after quality control filtering (SI Table 4.1).

Praying mantids were found to harbor diverse gut microbiota. As a whole, these insects exhibited lower alpha diversity and higher beta diversity in gut microbiome composition than their cockroach prey. Interestingly, while mean alpha diversity was substantially lower than observed in cockroaches, variance in alpha diversity was substantially higher (Figure 4.2, SI Figure 4.2) such that an individual praying mantis may host a gut microbiota that is equally or more diverse than the typical cockroach gut microbiota. Inter-individual variation among praying mantids was substantially higher than observed in cockroach prey by both weighted and unweighted beta diversity measures (Figure 4.2, SI Figure 4.2). Non-metric multidimensional scaling analyses show a clear separation between praying mantis and cockroach samples (SI Figure 4.1). Similarly, permutational multivariate analysis of variance (PERMANOVA, $p <$

0.001) found significant differences in gut microbiome composition between mantids and their cockroach prey. In contrast, we did not find significant relationships between gut microbiome composition and praying mantis age or body condition (PERMANOVA, $p > 0.05$).

Firmicutes, Proteobacteria, and Bacteroidetes were the predominant phyla present in the hindgut microbiota of all insects. All hindguts also contained a high proportion of microbes from other, less abundant phyla including Actinobacteria, Verrucomicrobia, and Tenericutes. Variability among individual insects was higher in praying mantids, with non-predominant phyla composing 3-40% of the gut microbiota in praying mantids and only 5-13% in cockroaches (SI Figure 4.1).

The Streptococcaceae, Lactobacillaceae, and Enterococcaceae families were prevalent among praying mantids, representing 74.5% of all Firmicutes found in praying mantis hindgut samples (Figure 4.1). In contrast, these families only composed 6.1% of all Firmicutes found in prey hindgut samples. Within the Proteobacteria phylum, Enterobacteriaceae were the most abundant family in the gut microbiota of praying mantids. Enterobacteriaceae represented 41.6% of all Proteobacteria found in praying mantid hindgut samples, but were not present in any of the cockroach samples (Figure 4.1).

While Enterobacteriaceae were found only within praying mantids, other bacterial lineages were common to both praying mantids and their cockroach prey. For example, the majority of Bacteroidetes in both insect orders are members of the Flavobacteriaceae_1, Bacteroidaceae, and Porphyromonadaceae gut group families (Figure 4.1). These families composed a total of 45.6% and 39.2% of all Bacteroidetes in the praying mantid and cockroach hindguts, respectively.

At the 97% OTU level, few lineages were shared between praying mantids and cockroaches. Only 7 OTUs were found in all insect microbiomes in the study, with 52 shared among all praying mantids and 158 shared among all cockroaches (Figure 4.2, SI Tables 4.2-4.7). The most abundant OTUs found in praying mantids are either completely absent from all cockroaches or only found in a few cockroaches at low abundance (Figure 4.3). These highly abundant OTUs are not found at equal depth across all praying mantids (Figure 4.4). A closer examination reveals that the 10 outliers above 20% relative abundance represent 9 unique praying mantis individuals. This suggests that the bulk of an individual praying mantid's gut microbiome is composed of one or several dominant OTUs. These dominant OTUs appear to be mantid-associated bacteria that are blooming, likely in response to conditions within the specific host gut (Figure 4.4).

4.5 Discussion

Published work on the gut microbiome has demonstrated that most insects host simple gut microbial communities that are highly variable among individuals (17). Insects found in the Blattodea order are the exception: cockroaches host a complex gut microbiome composed of hundreds of unique microbial species that is highly stable between individuals (27, 36-40). One of the goals of this work was to determine the extent to which the gut microbiome of praying mantids resemble those of other members of superorder Dictyoptera, such as cockroaches and termites. Our results demonstrate that, unlike cockroaches and termites, the praying mantis hosts a gut microbial community that is highly variable between individuals, with lower average alpha diversity and higher beta diversity than observed in cockroaches.

Emerging work on the caterpillar gut microbiome demonstrates that not all insects have a symbiotic relationship with their gut microbiota; rather some insects have no resident microbiota

(41). Our work demonstrates praying mantids host a unique, host-associated gut microbiota, exhibiting a distinct phylogenetic profile from the gut microbiota of the prey and dominated by lineages that are either not found or not abundant in cockroaches.. This suggests that the praying mantis has a resident gut microbiota that is distinct from the microbes ingested with prey, although at present it is impossible to determine the strength of this relationship or its role in host health.

The fact that many of the specific bacterial lineages that dominate the praying mantis gut are not found within their cockroach prey implies that some of members of the praying mantis gut microbiota are not acquired directly from their prey. Potential sources of these microbes include their environment and prey insects from early in life. While all of the study insects were fed cockroaches in their final weeks of the study, they were fed a combination of *Drosophila melanogaster* and *Drosophila hydei* until large enough to safely consume cockroach nymphs. It is also possible that unique microbes were acquired from nymphal cockroach prey, as only adult cockroaches were included in the comparison dataset. However, work by Carrasco et al. suggests that cockroach nymphs share highly similar microbiota to their adult relatives (42).

Many of these predominant bacterial lineages showed substantial variance in overall abundance across individuals. Due to the destructive sampling techniques used, it is impossible to say whether this variability is the result of inter-individual variability that is paired with intra-individual temporal stability or whether the observed variability is the result of frequent microbial blooms and temporal variability among individual mantids. Further, due to the variable health of some insects were cannot rule out that some of these taxa may be opportunistic pathogens responding to dysbiotic events in the gut. Only 5% of Chinese praying mantids survive to adulthood in the environment [35], which implies that most praying mantids present

within an ecosystem are likely to be in poor health and/or under stress. However, while our sample size is limited, no obvious differences were identified between healthy adult mantids and individuals sacrificed early due to poor body condition, suggesting that observed microbiome composition may be typical of these mantid species.

In conclusion, this work demonstrates that mantids host a diverse and highly variable gut community. Overall, this gut microbiome is more typical of that observed in other insects than the highly diverse and stable microbiota found in other members of the superorder Dictyoptera. This suggests that the strong symbiotic relationships between cockroaches (and termites) and their gut microbiota evolved following the evolutionary split between the Mantodea and the Blattodea. However, praying mantids do host a gut microbiota that is unique in composition from that of their prey, and the role of this microbiota in host health and nutrition is ripe for investigation.

4.6 Acknowledgements

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4.8 Tables

Table 4.1. Metadata for Praying Mantids

Sample			Age	Body Condition
ID	Order	Species	(Adult or Nymph)	(Excellent/Poor/Very Poor)
W1	Mantodae	<i>Tenodera sinensis</i>	Adult	Excellent
W2	Mantodae	<i>Tenodera sinensis</i>	Adult	Excellent
W3	Mantodae	<i>Tenodera sinensis</i>	Nymph	Poor
W4	Mantodae	<i>Tenodera sinensis</i>	Nymph	Poor
W5	Mantodae	<i>Tenodera sinensis</i>	Nymph	Poor
X1	Mantodae	<i>Hierodula venosa</i>	Adult	Excellent
X2	Mantodae	<i>Hierodula venosa</i>	Nymph	Poor
X3	Mantodae	<i>Hierodula venosa</i>	Nymph	Poor
X4	Mantodae	<i>Hierodula venosa</i>	Nymph	Poor
X5	Mantodae	<i>Hierodula venosa</i>	Nymph	Poor
X6	Mantodae	<i>Hierodula venosa</i>	Nymph	Poor
X7	Mantodae	<i>Hierodula venosa</i>	Nymph	Very Poor
X8	Mantodae	<i>Hierodula venosa</i>	Nymph	Very Poor
Y1	Mantodae	<i>Deroplatys lobata</i>	Nymph	Poor
Y2	Mantodae	<i>Deroplatys lobata</i>	Nymph	Poor

4.9 Figures

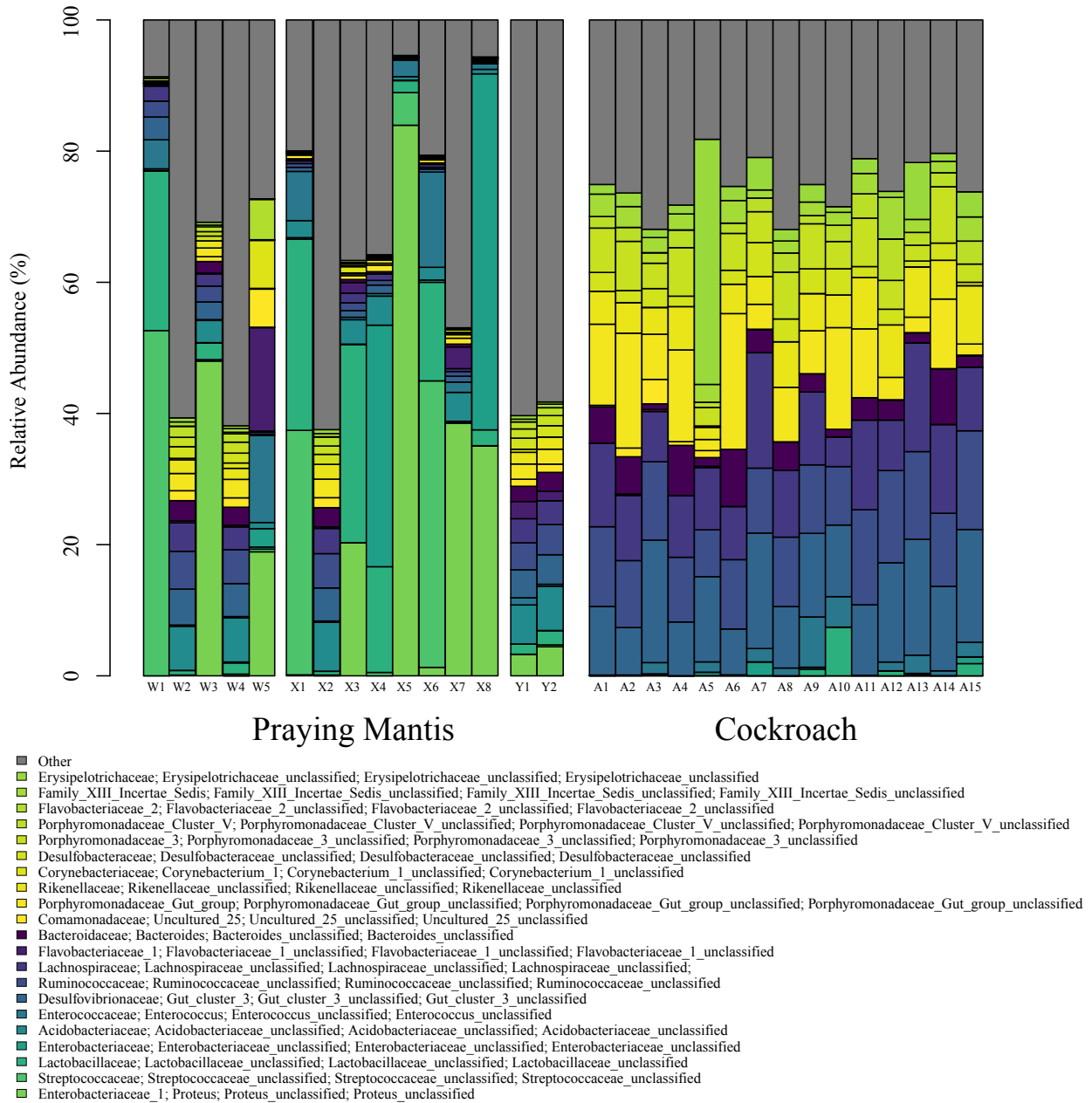


Figure 4.1. Relative abundances of microbial families in praying mantids and cockroaches. Each bar represents an individual insect gut. All families that represent $\geq 5\%$ from any one sample are displayed, less abundant families are listed as Other.

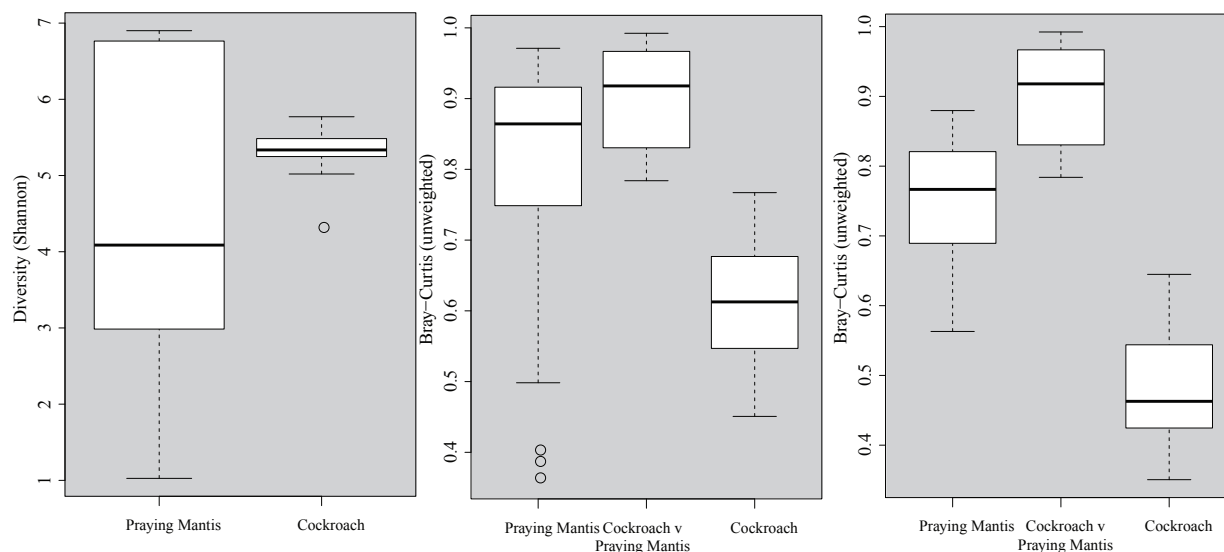


Figure 4.2. Alpha and beta diversities among praying mantids and cockroaches. Boxplots show Shannon diversity indices (left), and weighted (middle) and unweighted (left) Bray-Curtis dissimilarities among praying mantid and cockroach gut microbial communities at 97% sequence identity. Libraries were resampled to a depth of the sample with the fewest sequences (3901). For each group, the bars delineate the median, the hinges represent the lower and upper quartiles, the whiskers extend to the most extreme values (which are no more than 1.5 times the interquartile range from the box), and outliers are plotted, if present.

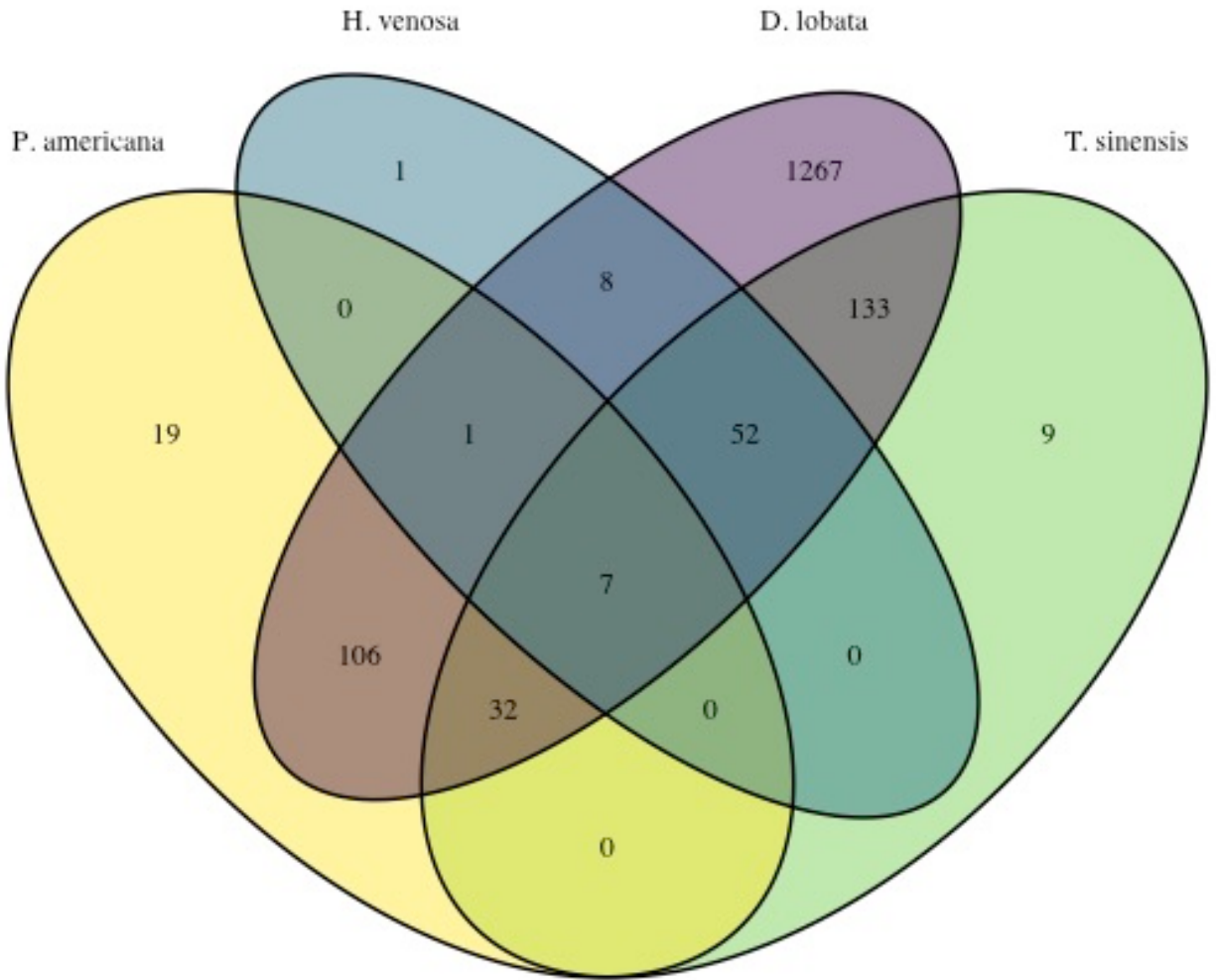


Figure 4.3. Venn diagram of number of core OTUs shared among all praying mantis and cockroach species. 7 OTUs were shared among all insect species, an unclassified: *Enterococcus*; *Ruminococcaceae* associated with the *Blattodea* order; *Comamonadaceae*; *Rhodoferrax*; *Desulfobacteraceae*; *Bacteroides*; and *Porphyromonadaceae* associated with termites (SI Table 4.3). A list of OTUs shared among all praying mantids is shown in SI Table 4.3, all cockroaches in SI Table 4.4, and all insects in SI Table 4.5. OTUs shared among each species of praying mantid are found in SI Tables 4.6-4.8.

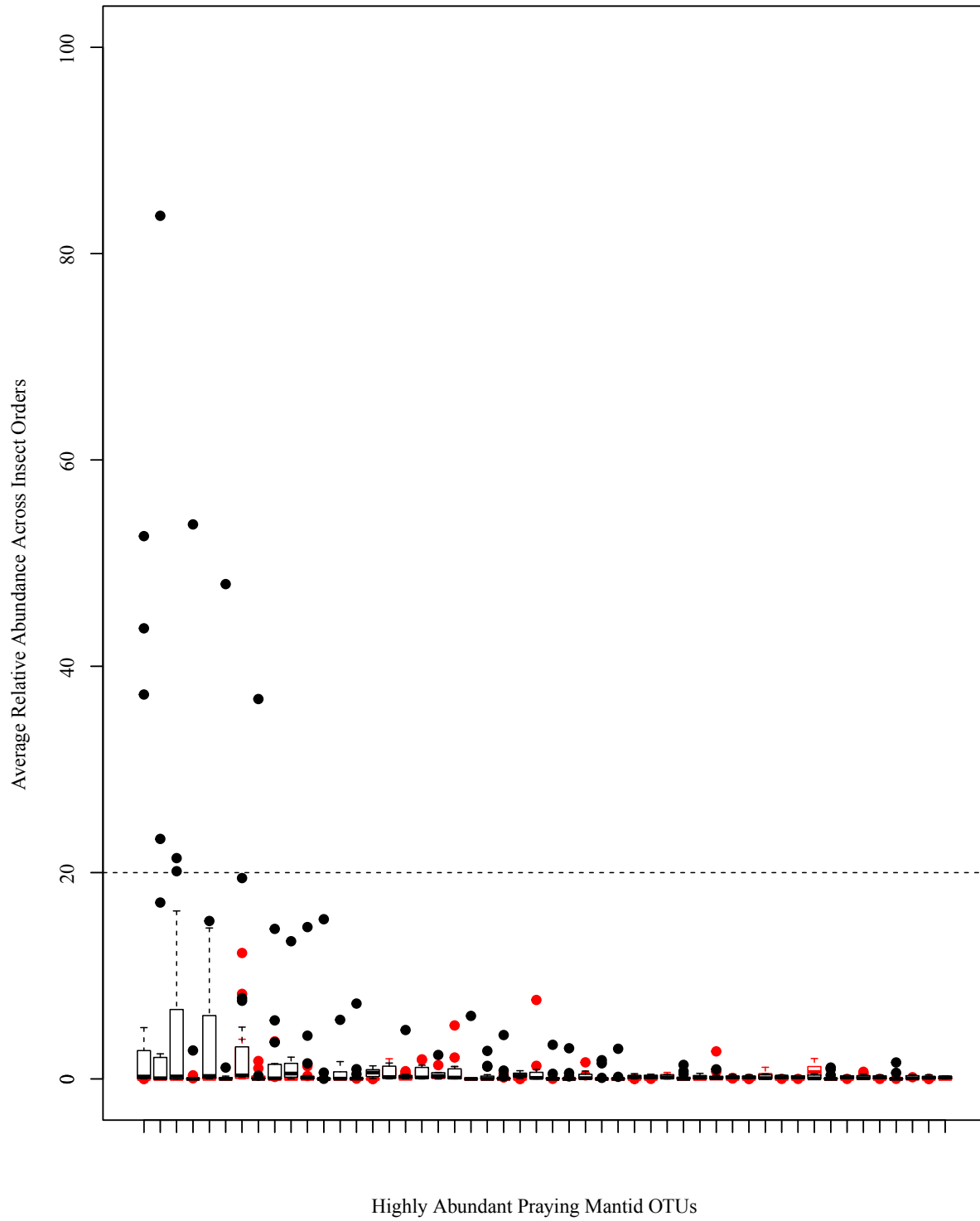


Figure 4.4. Relative abundances in praying mantids and cockroaches of the 50 most abundant praying mantis OTUs. Two boxplots are shown at each OTU; they depict the relative abundance of that OTU among all praying mantids (black) and all cockroaches (red). For each boxplot, the bars delineate the median, the hinges represent the lower and upper quartiles, the whiskers extend to the most extreme values (which are no more than 1.5 times the interquartile range from the box), and outliers are plotted, if present. There are 10 outliers above 20% relative abundance, which represent 9 unique praying mantis individuals.

CHAPTER 5

CONCLUSIONS

Cockroaches are versatile insects that can be found in a wide variety of habitats from residential homes and commercial warehouses to tropical rainforests and mountainous caves. This versatility is likely in part due to their gut microbiota (1-8). The gut microbiome plays an important role in the health and fitness of the host: it protects against colonization by opportunistic pathogens; breaks down dietary substrates and toxins; provides the host with bioavailable nutrients for absorption; and facilitates communication through the production of pheromones and/or kairomones (9-16). My dissertation is an exploration of the complex cockroach gut microbial community. The work presented seeks to identify how host and environmental factors shape the structure and activity of the gut microbiome as well as provide a framework for examining host and/or ecological impacts of the gut microbiota.

The exact structure and activity of the gut microbiome are shaped by a wide-range of host-mediated and environmental factors including host genotype, age, sex, housing, and antibiotic usage (17-27). Previous work suggests that changing host dietary needs was the primary driver in the specialization of the termite gut microbiome (28-30). However, my comprehensive survey of the cockroach gut microbiota in chapter two reveals that host phylogeny and the gut microbiota are tightly linked in cockroaches. This suggests that specialization of the cockroach gut microbiome began before the emergence of termites and the shift to a lignocellulose-based diet.

Although host phylogeny drives the initial composition of the cockroach gut microbiome, environmental factors could have a significant effect on the structure and function of the cockroach gut microbiome. Through a series of dietary manipulations presented in my third chapter, I found that dietary shifts have minimal impact on the cockroach gut microbiota (31). This is highly surprising, as diet has been shown to be the environmental factor that has the strongest impact on the gut microbiome of humans and other mammals. This suggests that there may be a host-mediated mechanism that assists with maintaining the cockroach gut microbiota. If such a mechanism exists, it may represent an intermediate step in the evolution of the obligate host-microbe symbiosis observed in termites.

Cockroaches have the ability to survive on a significantly wider range of dietary substrates than most mammals. This stable core gut microbiota may contribute to their ability to consume a wide, omnivorous diet and survive harsh living conditions. Climate change and anthropomorphic stressors have resulted in rising temperatures, longer periods of drought, and an increased rise in antibiotic resistance in bacterial populations. These factors have been shown to alter environmentally- and host-associated microbial populations (10, 26, 32-34). Cockroaches are particularly important to the ecosystem, acting both as an important food source for other animals and as detritivores in their natural habitats. Their ability to fulfill these roles could diminish if their stable gut microbiota is consistently disrupted. Thus, future work should be conducted in order to explore the effects of other environmental factors, such as those listed above, on the insect gut microbiome.

The work presented in chapters 2 and 3 demonstrate that the cockroach hosts a highly stable, curated gut microbial community. This community may be maintained by a host-mediated mechanism. However, another possibility is uncharacterized, insect-associated strains are highly

adaptable and/or possess novel properties that allow them to maintain homeostasis despite a constant flux in nutrient availability. One way to explore this possibility is through an extensive metatranscriptomic analysis on the cockroach gut microbiota activity in response to dietary perturbations. I present the foundational work for such an analysis in Appendix A. Recent work suggests the cockroach gut microbiome may play a role in cockroach aggregation (13).

Therefore, another possibility is that the core cockroach gut microbial community is maintained through trophallaxis, coprophagy, and/or social contact that occurs in response to aggregation events.

A stable gut microbiome that is uniform across a cockroach population would act as a natural reservoir for highly conserved, insect-associated microbial strains. One way to access this reservoir is through inoculation by predation. The praying mantis is a close relative and predator of cockroaches (35-40). Recent studies suggest that praying mantids do not uniformly consume available insect prey (41-43). Rather, they possess some mechanism by which to evaluate their prey. In my final chapter, I explore this possibility of inoculation by predation by examining the praying mantis gut microbiome. Interestingly, there was little overlap between the predominant praying mantis- and cockroach-associated gut microbiota. Instead, the praying mantis gut microbiome was dominated by highly variable, mantid-specific lineages. The source of these mantid-specific lineages is unknown, however they may contribute to their ability to succeed as predators.

Superorder Dictyoptera contains praying mantids, cockroaches, and termites. These insects represent a wide range of dietary needs: carnivorous, omnivorous, and herbivorous. The events preceding the emergence of these insect lineages are unknown. However, it seems likely

that gut microbiota hosted by these insects facilitated this dietary specialization and represent a rich, untapped source of microbial diversity ripe for future study.

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APPENDIX A

METHODS FOR EXAMINING THE RESPONSE OF THE
AMERICAN COCKROACH GUT MICROBIOME TO DIETARY SHIFTS

Abstract

The American cockroach hosts a complex gut microbial community that has minimal response to dietary shifts. This is highly unusual, as dietary shifts have been linked to changes in the gut microbiome across the animal kingdom. We aim to explore how metabolic patterns in the gut shift in response to dietary change by using a metatranscriptomic approach. However, there are many challenges associated with preparing metatranscriptomic libraries from environmental samples. Here we report our method for RNA sequence library preparation, which relies on using a minimum starting amount of hindgut material in order to avoid RNA degradation during the extraction process.

Introduction

Diet has been shown to be the environmental factor that has the strongest impact on the mammalian gut microbiome, with different microbial groups increasing in abundance when specific substrates are enriched in the host's diet (1). For example, *Bacteroidetes* are associated with high-protein diets, while *Firmicutes* are associated with high-fiber diets (2). In contrast the American cockroach, *Periplaneta americana*, hosts a highly stable core gut microbiota that has minimal community change in response to dietary shifts (3). This stability has not been reported in any other organism that hosts such an extensive, diverse gut microbial community.

The composition of the cockroach gut microbial community does not change in response to dietary shifts; however this does not preclude a change in the transcriptional and metabolic responses of the gut microbiota to dietary shifts. Previous work demonstrates that dietary shifts can alter hindgut metabolic activity in the cockroach (4, 5). This suggests dietary shifts induce a shift in the use of metabolic networks, perhaps facilitated by host-microbe or microbe-microbe cross feeding.

We aim to explore how metabolic patterns in the gut shift in response to dietary change by using community RNA sequencing to identify patterns of microbial gene expression under four dietary treatments. Our initial attempts at extracting RNA from the entire preserved hindgut sample failed due to high levels of RNA degradation from host inhibitors. Here we report our successful method of RNA sequence library preparation and provide a cursory analysis of associated metatranscriptomic sequences.

Methods

Cockroach Hindgut Sample Collection

Laboratory cultures of *P. americana* are maintained in mixed age, mixed sex colonies in aquarium tanks at room temperature on a diet of dog food *ad libitum*. Each culture tank is provided with corn cob bedding, cardboard tubes for nesting, and a cellulose sponge saturated with water. 25 adult cockroaches per treatment group were selected from the culture tanks, weighed, and marked for later identification. Each dietary treatment group was housed in a single plastic tank that contained pebbles for bedding, a large weigh boat for shelter, and food and water in weigh boats. Food, water, and weigh boats were changed as needed, and any ootheca or deceased cockroaches were removed daily. Treatments included a control diet of dog food (Kroger Nutritionally Complete Bite Size Adult Dog Food; composed of 21% protein, 8% fat, and 6% fiber), bran (Bob's Red Mill Organic High Fiber Oat Bran Hot Cereal), butter (Kroger Unsalted Butter Sticks), tuna (StarKist Selects Low Sodium Chunk Light Tuna in Water), and starvation (Table 4.1).

On day 14 of the dietary-shift, all cockroaches were sacrificed and their hindgut microbiota was preserved for DNA (9 hindguts) or RNA extraction (16 hindguts). Individual cockroaches were removed from tanks, weighed, and placed on ice in sterile culture plates. Once

sufficiently torpid, cockroaches were dissected and the entire gut was removed. Any visible debris, including fat bodies or exoskeleton, was removed with forceps. The hindgut was then separated from the rest of the gut using a scalpel and submerged in either 100 μ L of 1XTE buffer or RNALater (Ambion, Austin, TX, USA). Hindgut samples placed in 1XTE buffer were immediately stored at -80°C . Hindgut samples placed in RNALater were crushed with a sterile glass stirring rod. The suspended gut lumen was then removed and stored at -80°C .

DNA Extraction

Microbial DNA was extracted from hindgut samples using a modified version of the EZNA Bacteria Kit (Omega Biotek, Norcross, GA). Hindgut samples were thawed, pulverized with a sterile microcentrifuge pestle, and centrifuged for 10 min at 5,000 g. After discarding the supernatant, the remaining pellet was resuspended in 100 μ L of 1XTE buffer before adding 10 μ L lysozyme (as supplied by kit) and incubating at 37°C for 30 min. Next, approximately 25 mg of glass beads (as supplied by the kit) was added to each sample before bead beating for 5 min at 3,000 rpm. 100 μ L BTL buffer and 20 μ L proteinase K solution (as supplied by the kit) was added to each sample before incubating at 55°C while shaking at 600 rpm for 1 h. The manufacturer's protocol (June 2014 version) was followed beginning at step 11. Final DNA concentrations (ranging from 30 to 1800 ng/ μ L) and A260/A280 were measured using a NanoDrop Lite spectrophotometer (Thermo Scientific, Wilmington, DE).

Total RNA Extraction

Initial attempts at extracting RNA from the entire preserved hindgut sample failed due to high levels of RNA degradation from host inhibitors (Figure A.1). Thus, microbial RNA was extracted from one half of the total volume (50 μ L) of sample using a modified version of the HP Total RNA Kit (Omega Biotek, Norcross, GA). DNases were removed using Invitrogen's Turbo

DNA-free Kit (Thermo Scientific, Wilmington, DE) before cleaning and concentrating the extracted RNA using the EZNA MicroElute RNA Clean-Up Kit (Omega Biotek, Norcross, GA).

Preserved hindgut samples were thawed on ice and a 50 μ l sample was aliquoted for RNA extraction. 200 μ l PBS was added to each sample before centrifuging at 5,000 x rcf for 5 min. The supernatant was removed, samples were suspended in 50 μ l lysozyme (30 mg lysozyme per mL TE buffer), and 4 μ l Superase-In RNase Inhibitor (Thermo Scientific, Wilmington, DE) was added to each sample before vortexing for 30 s. Samples were incubated at 30°C for 10 minutes at 300rpm. 700 μ l GTC Lysis buffer (as supplied by kit) was added before transferring the sample to a new tube containing 25-40mg glass beads. Samples were vortexed for 30 s and placed in ice for 30 s. This was repeated three times, for a total of 2 min bead beating. From this point forward, the manufacturer's protocol (HP Total RNA Kit, December 2010 version) was followed beginning at step 5. In brief, lysate was transferred to a DNA clearance column. After clearance, the column was discarded and an equal volume (700 μ l) of 70% ethanol was added to the lysate. The sample was applied to a HiBind RNA column, washed once with 500 μ l RNA Wash Buffer I (as supplied by kit), and washed twice with 500 μ l RNA Wash Buffer II (as supplied by kit). 50 μ l of DEPC water was added to the column before incubation at room temperature for 5 min followed by elution.

0.1 volume 10X Turbo DNase Buffer (5 μ l) and 1 μ l TURBO DNase (as supplied by the Turbo DNA-free kit) were gently mixed into the eluted RNA before incubation at 37°C for 25 min. After DNase removal, the manufacturer's protocol for the EZNA MicroElute RNA Clean Up Kit (July 2014 version) was followed. Quality of total RNA was confirmed on the Bioanalyzer using the RNA 6000 Pico Total RNA Kit. The top four total RNA samples per

dietary treatment were identified and used for rRNA depletion and library preparation (Agilent, Santa Clara, CA).

Preparation of RNA depletion probes

Custom biotin-labeled antisense RNA probes were made using pooled extracted microbial DNA (SI Methods) from the hindguts of one representative cockroach per dietary treatment. First, extracted DNA was amplified using T7-appended primers that target universal archaeal, bacterial, and eukaryotic ribosomal gene regions and cockroach host tissue (Table 4.2). PCR reactions had a total volume of 50 μ L and were composed of 5X Q5 buffer (NEB, Ipswich, MA), 200 μ M dNTPS, 0.5 F primer, 0.5 μ M R primer, 100 ng template DNA, and 0.02 U/ μ L Q5 Hot Start High-Fidelity DNA polymerase (NEB). PCR conditions were 98°C for 2 min; 35 cycles at 98°C for 20 s, variable for 20 s, and 72°C for 2 min; and 72°C for 3 min.

Next, the Ampliscribe T-7 Flash Biotin-RNA Transcription Kit (Lucigen Corp., Middleton, WI) was used to complete in vitro transcription of biotin-labeled antisense RNA probes. Transcription reactions had a total volume of 10 μ L and were composed of 1 μ L AmpliScribe T-7 Flash 10X buffer, 4 μ L NTP/Biotin-UTP premix, 100mM DDT, 0.25 μ L Riboguard RNase, 1 μ L AmpliScribe T-7 Flash Enzyme Solution, and 500ng of template DNA from the previous reaction. Transcription reactions were incubated at 37°C for 4 hrs. After transcription, the reactions were purified using the MEGAclear Kit Purification for Large Scale Transcription Reactions (Life Technologies, Carlsbad, CA). In brief, the reaction volume was brought to 100 μ L and 350 μ L of Binding Solution Concentrate (as supplied by kit) and 250 μ L of 100% ethanol was added to the sample. The mixture was applied to a Filter Cartridge (as supplied by kit) before two washes with 500 μ L Wash Solution (as supplied by kit). RNA was eluted into 50 μ L Elution Solution (as supplied by kit) after incubation at 65°C for 5 min.

rRNA Depletion and Library Preparation

rRNA was depleted from total RNA through hybridization to custom RNA probes before removal with magnetic beads. Hybridization reactions had a total volume of 50 μL and were composed of 1 μL Superase-In RNase Inhibitor (Thermo Scientific), 2.5 μL 20X Sodium chloride-citrate (SSC) buffer, 10 μL 100% Formamide, antisense RNA probes (Table 4.3), and extracted total RNA. Reactions were incubated at 70°C for 5 min followed by a rampdown to 25°C using 5°C increments for 1 min each before incubating at room temperature for 2-5 min.

100 μL streptavidin-coated magnetic beads (NEB) per sample were prepared during the hybridization reaction. Beads were applied to a magnetic separation rack and the supernatant was removed. Beads were washed three times with an equal volume of 0.1N NaOH (first wash) or 1X SSC buffer (second and third wash), aliquoted into 100 μL volumes, and kept on ice until the hybridization reaction was complete. After completion, supernatant was removed from pre-aliquoted beads and 40 μL of 1X SSC and 10 μL 20% formamide was added to the completed hybridization reaction before applying to the mixture to the dried beads.

The mixture was incubated at room temperature for 10 min, with occasional flicking to mix. Beads were captured on a magnetic rack and supernatant containing the depleted RNA was collected in a new tube. Remaining beads were resuspended with 100 μL 1X SSC and captured on the magnetic rack. Supernatant was transferred to the new tube, for a total volume of 200 μL depleted RNA. Depleted RNA was cleaned using the RNeasy MinElute kit (Omega) before quality confirmation, as described above.

Depleted RNA was prepared using the NEBNext Ultra Directional RNA Library Prep Kit for Illumina (NEB) following the product guidelines with an RNA fragmentation time of 10 min. The prepared library was submitted to the Georgia Genomics facility for normalization, pooling,

and sequencing (Illumina HiSeq; Illumina, Inc., San Diego, CA). Returned sequences were deposited into MG-RAST and run through the default pipeline with no removal of artificial replicate sequences.

Results

After 14 days on each diet, cockroaches were sacrificed and hindgut lumen contents were used for microbial RNA extraction and metatranscriptomic sequencing. A total of 94,225,324 sequences were obtained from 20 unique samples, with each sample having an average depth of 4,711,266 (Table 4.4).

Discussion

The cockroach gut microbiome hosts a complex microbial community that exhibits minimal structural changes in response to dietary shifts. However, previously published work indicates that dietary shifts cause a significant change in the metabolic activity of the cockroach gut microbiota (4, 5) An examination of how metabolic patterns in the cockroach gut microbiome shift in response to changing nutrient inputs may provide insight into why changes in microbial activity do not translate into alterations in microbial community composition.

The stability of the cockroach gut microbial community may be in part due to cross-feeding in the form of metabolic pathways that remain active in the absence of a dietary source of the target substrate. In order to explore the extent of host-microbe and/or microbe-microbe cross-feeding events we used a metatranscriptomic approach to identify patterns of microbial gene expression under four dietary treatments. Metatranscriptomic is often used for examining patterns of gene expression in complex microbial communities. However, preparing metatranscriptomic libraries from environmental samples can often be challenging due to factors such as contamination by host-associated compounds, adsorption to nearby particles, or low

yield. Here, we provide our method for RNA sequence library preparation from insect hindgut microbiota while laying the foundation for future work examining the effect of dietary shifts on the cockroach gut microbiome. Our proposed work will provide insight into the metabolic capabilities of cockroach gut microbes and the distribution of these capabilities between microbial species. This is especially relevant as the bulk of the cockroach gut is composed of poorly studied, insect-associated bacterial lineages (3, 6).

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Tables

Table A.1. Nutrient Information for 100g Serving of Each Diet Treatment

Diet Treatment	Calories	Protein	Carbohydrate	Fat	Fiber
Bran	375	17.5	67.5	5	17.5
Butter	714	0	0	79	0
Tuna	107	27	0	1	0
Starvation	N/A	N/A	N/A	N/A	N/A

All cockroaches were raised on our laboratory control diet of dog food, composed of 21% protein, 8% fat, and 6% fiber.

Table A.2. Primers used for custom rRNA probes

	Target Region	Forward Sequence	Reverse Sequence	Annealing Temp (°C)	Reference(s)
Archaea	16S	TCCGGTTGATCCY GCCGG	GCCAGTGAATTGTAATACGACTCACTATAG GGGGYYACCTTGTTACGACTT	70	(7-9)
	23S	ASAGGGTGAHAR YCCCGTA	GCCAGTGAATTGTAATACGACTCACTATAG GGCTGTCTCRCGACGGTCTRAACCCA		
Bacteria	16S	AGAGTTTGATCCT GGCTCAG	GCCAGTGAATTGTAATACGACTCACTATAG GGACGGCTACCTTGTTACGACTT	39	(8-10)
	23S	GAASTGAAACAT CTHAGTA	GCCAGTGAATTGTAATACGACTCACTATAG GGCGACATCGAGGTGCCAAAC		
Eukaryote	18S	ACCTGGTTGATCC TGCCAG	AATTATAATACGACTCACTATAGATTCTYGC AGGTTCACCTAC	55	(8, 9)
	28S	ACCCGCYGAAYT TAAGCATA	AATTATAATACGACTCACTATAGATTCTGR YTTAGAGGCGTTCAG		
Cockroach Specific	CITS	CCTGCGGAAGGA TCATTAAC	GCCAGTGAATTGTAATACGACTCACTATAG GGCTTAAATTCAGCGGGTAGTCTC	60	This study

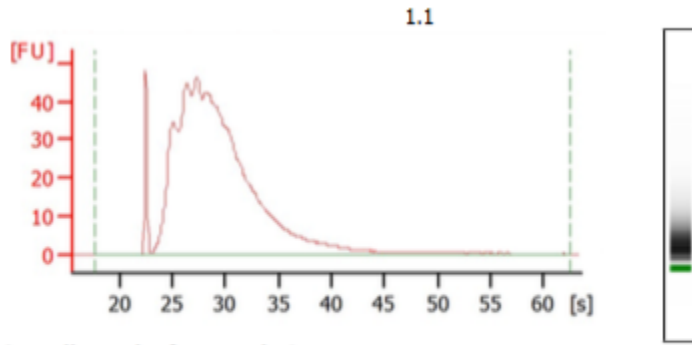
A.3. Ratio of antisense RNA probes used for rRNA depletion

RNA probe target	ng of probe per 250ng total RNA
Archaea 16S	200
Archaea 23S	200
Bacteria 16S	500
Bacteria 23S	500
Eukaryote 18S	250
Eukaryote 28S	250
Cockroach CITS	250

A.4 Number of reads per sample

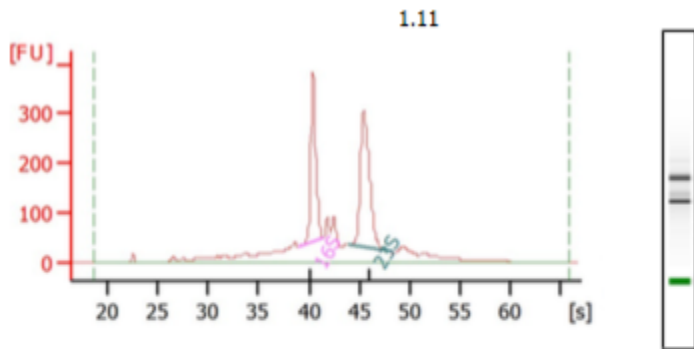
Diet Treatment	Sample Name	No. Raw Sequences
Dog Food (Control)	1.7	3,486,172
	1.10	4,446,812
	1.11	1,606,242
	1.12	6,256,092
Butter	2.15	17,591,556
	2.9	4,503,789
	2.12	3,681,767
	2.14	5,476,953
Tuna	3.10	4,859,621
	3.13	2,118,730
	3.14	4,450,622
	3.15	2,143,103
Bran	4.11	5,229,592
	4.14	4,569,328
	4.15	3,056,378
	4.16	2,905,847
Starvation	5.6	5,515,452
	5.10	4,249,210
	5.11	1,612,214
	5.14	6,465,844

Figures



Overall Results for sample 3 : 1.1

RNA Area: 1,178.7
 RNA Concentration: 2,925 pg/ μ l
 rRNA Ratio [23s / 16s]: 0.0
 RNA Integrity Number (RIN): 2.3 (B.02.08)



Overall Results for sample 1 : 1.11

RNA Area: 2,823.6
 RNA Concentration: 8,340 pg/ μ l
 rRNA Ratio [23s / 16s]: 1.3
 RNA Integrity Number (RIN): 8.4 (B.02.08)

Fragment table for sample 1 : 1.11

Name	Start Time [s]	End Time [s]	Area	% of total Area
16S	38.98	41.42	514.3	18.2
23S	43.85	48.15	662.0	23.4

Figure A.1. Sample quality information for total RNA extracted from an entire cockroach hindgut sample (top) or half of a cockroach hindgut sample (bottom). Sample quality information was obtained by using a Bioanalyzer with the RNA 6000 Pico Total RNA Kit.

APPENDIX B
CHAPTER 2 SUPPLEMENTAL INFORMATION¹

¹ Tinker KA and Ottesen EA. To be submitted to AEM.

Supplemental Table 2.1. Sample metadata

Species Id.	Internal Sample Id.	Species	Family	Origin	Sex	Dissection Notes	raw sequences	make contigs	screen seqs (maxambig = 0, maxlength = 275)	screen seqs (maxhomop = 8)	chimeral removal	remove lineage
Bb-A-1	K1	<i>Lucihormetica verrucosa</i>	Blaberidae	Roachcrossing	N/A	N/A	67264	66497	50416	50332	50153	48048
Bb-A-2R	K2	<i>Lucihormetica verrucosa</i>	Blaberidae	Roachcrossing	N/A	N/A	126633	126633	105977	105874	104812	85105
Bb-A-3	K3	<i>Lucihormetica verrucosa</i>	Blaberidae	Roachcrossing	N/A	N/A	108189	108189	88422	88288	87894	79265
Bb-A-4	K4	<i>Lucihormetica verrucosa</i>	Blaberidae	Roachcrossing	N/A	N/A	85774	85774	70252	70180	69823	62869
Bb-A-5	K5	<i>Lucihormetica verrucosa</i>	Blaberidae	Roachcrossing	N/A	N/A	83892	83892	66367	66215	65998	62726
Bb-A-6	K6	<i>Lucihormetica verrucosa</i>	Blaberidae	Roachcrossing	N/A	N/A	95285	95285	76459	76375	76179	76060
Bb-A-7	K7	<i>Lucihormetica verrucosa</i>	Blaberidae	Roachcrossing	N/A	N/A	97396	97396	79679	79581	79041	72885
Bb-A-8	K8	<i>Lucihormetica verrucosa</i>	Blaberidae	Roachcrossing	N/A	N/A	32039	32039	24342	24306	24290	18910
Bb-A-9	K9	<i>Lucihormetica verrucosa</i>	Blaberidae	Roachcrossing	N/A	N/A	77479	77479	62584	62479	61968	57351
Bb-A-10	K10	<i>Lucihormetica verrucosa</i>	Blaberidae	Roachcrossing	N/A	N/A	113328	67264	54695	54646	54505	51133
Bb-A-11	K11	<i>Lucihormetica verrucosa</i>	Blaberidae	Roachcrossing	N/A	tore hindgut	105422	113328	93059	92977	91844	84470
Bb-A-12	K12	<i>Lucihormetica verrucosa</i>	Blaberidae	Roachcrossing	N/A	N/A	66497	105422	85510	85433	84649	76151
Bb-B-1	R1	<i>Blaberus craniifer</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	156347	156347	122548	121901	121325	119287
Bb-B-2	R2	<i>Blaberus craniifer</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	93005	93005	71350	71108	70548	68907
Bb-B-3	R3	<i>Blaberus craniifer</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	124978	124978	98400	98259	96414	93613
Bb-B-4	R4	<i>Blaberus craniifer</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	180918	180918	142518	142257	141296	135145
Bb-B-5 ^R	R5	<i>Blaberus craniifer</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	147488	147488	112686	112473	110535	105098
Bb-B-6	R6	<i>Blaberus craniifer</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	143789	143789	116204	116098	115026	112274
Bb-B-7	R7	<i>Blaberus craniifer</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	144441	144441	116749	116527	115788	111809
Bb-B-8	R8	<i>Blaberus craniifer</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	151393	151393	119389	119136	118851	117280

Bb-B-10	R10	<i>Blaberus craniifer</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	132383	132383	104077	103677	102862	100437
Bb-B-11	R11	<i>Blaberus craniifer</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	110941	110941	87887	87799	87584	85940
Bb-C-1	C1	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	F	N/A	75980	75980	33393	33254	25614	25520
Bb-C-2	C2	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	F	pregnant	13414	13414	9917	9893	8166	8123
Bb-C-3	C3	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	M	N/A	91866	91866	46661	46297	31481	30480
Bb-C-4 ^R	C4	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	F	N/A	15039	15039	10531	10510	8617	8482
Bb-C-5	C5	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	F	N/A	12120	12120	8688	8676	7673	7602
Bb-C-6	C6	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	F	N/A	98001	98001	68991	68856	65909	65309
Bb-C-7	C7	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	F	N/A	38915	38915	24966	24916	22667	22478
Bb-C-8	C8	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	F	N/A	101947	101947	71861	71745	67444	66969
Bb-C-9	C9	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	F	N/A	43607	43607	19697	19517	18179	18149
Bb-C-10	C10	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	M	N/A	88640	88640	37905	37818	35396	35287
Bb-C-11	C11	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	M	N/A	91938	91938	51663	51431	48894	48885
Bb-C-12	C12	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	M	N/A	154693	154693	106436	106290	103748	103165
Bb-C-13	C13	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	M	N/A	150313	150313	105652	103219	98991	98923
Bb-C-14	C14	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	M	N/A	171299	171299	125533	125402	121797	119219
Bb-C-15	C15	<i>Diploptera punctata</i>	Blaberidae	UGA Entomology	F	N/A	145406	145406	102050	101926	99656	98294
Bb-D-1 ^R	Q1	<i>Gromphadorhina portentosa</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	37329	37329	27857	27784	27655	26485
Bb-D-2	Q2	<i>Gromphadorhina portentosa</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	71427	71427	54955	54682	54251	52837
Bb-D-3	Q3	<i>Gromphadorhina portentosa</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	26918	26918	19622	19570	19312	17704
Bb-D-4	Q4	<i>Gromphadorhina portentosa</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	13667	13667	10441	10393	10360	10069
Bb-D-5	Q5	<i>Gromphadorhina portentosa</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	59485	59485	46249	46029	45647	43713
Bb-D-6	Q6	<i>Gromphadorhina portentosa</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	17736	17736	12876	12847	12786	12450
Bb-D-7	Q7	<i>Gromphadorhina portentosa</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	75478	75478	59406	59293	58900	55095

Bb-D-8	Q8	<i>Gromphadorhina portentosa</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	37549	37549	29408	29338	29328	29299
Bb-D-9	Q9	<i>Gromphadorhina portentosa</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	124544	124544	97881	97644	96501	94085
Bb-D-10	Q10	<i>Gromphadorhina portentosa</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	110238	110238	84866	84561	84156	78252
Bb-D-11	Q11	<i>Gromphadorhina portentosa</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	111689	111689	87806	87510	87027	78959
Bb-D-12	Q12	<i>Gromphadorhina portentosa</i>	Blaberidae	UGA Entomology	N/A	dissected under CO ₂	110809	110809	84572	84164	83864	80547
Bb-E-1	B1	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	M	tore hindgut at midgut connection	53377	53377	35194	35152	30666	30212
Bb-E-2	B2	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	M	N/A	67880	67880	43710	43622	40419	39345
Bb-E-3	B3	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	F	tore hindgut at midgut connection	45909	45909	28649	28618	25703	25217
Bb-E-4 ^R	B4	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	M	N/A	123896	123896	84286	84174	75649	73118
Bb-E-5	B5	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	M	tore hindgut at midgut connection	1932	1932	443	442	425	411
Bb-E-6	B6	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	F	pregnant	146054	146054	110033	109885	101167	98685
Bb-E-7	B7	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	F	pregnant	121526	121526	88687	88516	85083	83245
Bb-E-8	B8	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	M	N/A	79620	79620	56141	56021	52919	52401
Bb-E-9	B9	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	M	N/A	33709	33709	22108	22053	19529	19224
Bb-E-10	B10	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	M	N/A	165277	165277	119258	119033	109233	108060
Bb-E-11	B11	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	M	N/A	56026	56026	37511	37479	36162	35872
Bb-E-12	B12	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	M	N/A	120827	120827	84766	84422	80611	79815
Bb-E-13	B13	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	F	N/A	156703	156703	115799	115646	107887	107244
Bb-E-14	B14	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	F	N/A	102567	102567	75388	75303	70018	69560
Bb-E-15	B15	<i>Nauphoeta cinerea</i>	Blattidae	UGA Entomology	M	N/A	103680	103680	75665	75554	68428	68148
Bb-F-1 ^{AR}	G1	<i>Oxyhaloa deusta</i>	Blaberidae	Roachcrossing	N/A	tore hindgut	31049	31049	24838	24777	22556	21621
Bb-F-2	G2	<i>Oxyhaloa deusta</i>	Blaberidae	Roachcrossing	N/A	tore hindgut	204766	204766	141175	141071	140113	137559
Bb-F-3	G3	<i>Oxyhaloa deusta</i>	Blaberidae	Roachcrossing	N/A	N/A	174197	174197	112472	112368	109508	108380
Bb-F-4	G4	<i>Oxyhaloa deusta</i>	Blaberidae	Roachcrossing	N/A	N/A	111095	111095	70860	70809	70352	69996
Bb-F-5	G5	<i>Oxyhaloa deusta</i>	Blaberidae	Roachcrossing	N/A	pregnant	222529	222529	86978	86910	85350	84769
Bb-F-6	G6	<i>Oxyhaloa deusta</i>	Blaberidae	Roachcrossing	N/A	N/A	59319	59319	19402	19384	18895	18257

Bb-F-9	G9	<i>Oxyhaloa deusta</i>	Blaberidae	Roachcrossing	N/A	N/A	14118	14118	10368	10359	10347	10316
Bb-F-10	G10	<i>Oxyhaloa deusta</i>	Blaberidae	Roachcrossing	N/A	N/A	30181	30181	21988	21967	21945	21781
Bb-F-11	G11	<i>Oxyhaloa deusta</i>	Blaberidae	Roachcrossing	N/A	N/A	13261	13261	9651	9640	9614	9571
Bb-F-12	G12	<i>Oxyhaloa deusta</i>	Blaberidae	Roachcrossing	N/A	N/A	12365	12365	9021	9011	9000	8988
Bb-F-13	G13	<i>Oxyhaloa deusta</i>	Blaberidae	Roachcrossing	N/A	N/A	27593	27593	21285	21260	21095	20943
Bb-F-14	G14	<i>Oxyhaloa deusta</i>	Blaberidae	Roachcrossing	N/A	N/A	16894	16894	12955	12941	12823	12467
Bb-G-1	N1	<i>Panchlora nivea</i>	Blaberidae	Roachcrossing	N/A	N/A	12194	12194	8774	8764	8751	8695
Bb-G-2 ^A	N2	<i>Panchlora nivea</i>	Blaberidae	Roachcrossing	N/A	N/A	85418	85418	66139	66010	61537	61100
Bb-G-3 ^{AR}	N3	<i>Panchlora nivea</i>	Blaberidae	Roachcrossing	N/A	N/A	90466	90466	68801	68603	66190	64349
Bb-G-4 ^A	N4	<i>Panchlora nivea</i>	Blaberidae	Roachcrossing	N/A	N/A	83020	83020	65718	65635	62461	61735
Bb-G-5 ^A	N5	<i>Panchlora nivea</i>	Blaberidae	Roachcrossing	N/A	N/A	86688	86688	68159	68083	63109	62690
Bb-G-6 ^A	N6	<i>Panchlora nivea</i>	Blaberidae	Roachcrossing	N/A	N/A	88228	88228	70243	70082	66446	63888
Bb-G-7 ^A	N7	<i>Panchlora nivea</i>	Blaberidae	Roachcrossing	N/A	N/A	70323	70323	55752	55620	52609	50240
Bb-G-8 ^A	N8	<i>Panchlora nivea</i>	Blaberidae	Roachcrossing	N/A	N/A	85831	85831	68681	68472	66240	64360
Bb-G-9 ^A	N9	<i>Panchlora nivea</i>	Blaberidae	Roachcrossing	N/A	N/A	69940	69940	55354	55253	52336	49741
Bb-G-10 ^A	N10	<i>Panchlora nivea</i>	Blaberidae	Roachcrossing	N/A	N/A	31142	31142	24553	24511	24470	24412
Bb-G-11 ^A	N11	<i>Panchlora nivea</i>	Blaberidae	Roachcrossing	N/A	N/A	79407	79407	61545	61480	54561	54396
Bb-G-12 ^A	N12	<i>Panchlora nivea</i>	Blaberidae	Roachcrossing	N/A	N/A	85016	85016	66948	66858	62254	61923
Bb-H-1	M1	<i>Paraplecta sp.</i> "Kenya"	Blaberidae	Roachcrossing	N/A	N/A	80048	296238	233649	233429	232898	231618
Bb-H-2	M2	<i>Paraplecta sp.</i> "Kenya"	Blaberidae	Roachcrossing	N/A	N/A	98705	98705	77618	77520	77359	75542
Bb-H-3 ^R	M3	<i>Paraplecta sp.</i> "Kenya"	Blaberidae	Roachcrossing	N/A	N/A	32560	32560	25154	25104	24769	24630
Bb-H-4 ^A	M4	<i>Paraplecta sp.</i> "Kenya"	Blaberidae	Roachcrossing	N/A	N/A	82105	82105	63864	63749	61763	60143
Bb-H-5 ^A	M5	<i>Paraplecta sp.</i> "Kenya"	Blaberidae	Roachcrossing	N/A	N/A	94158	94158	73670	73543	71267	69174
Bb-H-6 ^A	M6	<i>Paraplecta sp.</i> "Kenya"	Blaberidae	Roachcrossing	N/A	N/A	64753	64753	51325	51222	48537	45401
Bb-H-7 ^A	M7	<i>Paraplecta sp.</i> "Kenya"	Blaberidae	Roachcrossing	N/A	N/A	87201	87201	67236	67137	65109	63068
Bb-H-8 ^A	M8	<i>Paraplecta sp.</i> "Kenya"	Blaberidae	Roachcrossing	N/A	N/A	74202	74202	57745	57591	56146	53830
Bb-H-9	M9	<i>Paraplecta sp.</i> "Kenya"	Blaberidae	Roachcrossing	N/A	N/A	23497	23497	18361	18330	18321	18241
Bb-H-10	M10	<i>Paraplecta sp.</i> "Kenya"	Blaberidae	Roachcrossing	N/A	N/A	17419	80048	60975	60911	60672	59858

Bb-H-11	M11	<i>Paraplecta sp.</i> "Kenya"	Blaberidae	Roachcrossing	N/A	N/A	74098	17419	12319	12296	12264	12107
Bb-H-12	M12	<i>Paraplecta sp.</i> "Kenya"	Blaberidae	Roachcrossing	N/A	N/A	296238	74098	58984	58917	58728	58257
Bb-I-1	P1	<i>Pycnoscelus</i> <i>surinamensis</i>	Blaberidae	Roachcrossing	N/A	N/A	44867	44867	35656	35594	35467	35149
Bb-I-2	P2	<i>Pycnoscelus</i> <i>surinamensis</i>	Blaberidae	Roachcrossing	N/A	N/A	73996	73996	58438	58364	57686	56155
Bb-I-3 ^R	P3	<i>Pycnoscelus</i> <i>surinamensis</i>	Blaberidae	Roachcrossing	N/A	N/A	54580	54580	43352	43302	42987	41826
Bb-I-4	P4	<i>Pycnoscelus</i> <i>surinamensis</i>	Blaberidae	Roachcrossing	N/A	N/A	50092	50092	39966	39908	39801	39196
Bb-I-5	P5	<i>Pycnoscelus</i> <i>surinamensis</i>	Blaberidae	Roachcrossing	N/A	N/A	92782	92782	73753	73633	73305	72300
Bb-I-6 ^A	P6	<i>Pycnoscelus</i> <i>surinamensis</i>	Blaberidae	Roachcrossing	N/A	N/A	47197	47197	37650	37592	36125	34845
Bb-I-7	P7	<i>Pycnoscelus</i> <i>surinamensis</i>	Blaberidae	Roachcrossing	N/A	N/A	58068	58068	46611	46551	46274	45590
Bb-I-8	P8	<i>Pycnoscelus</i> <i>surinamensis</i>	Blaberidae	Roachcrossing	N/A	N/A	56612	56612	45135	45058	44899	44471
Bb-I-9	P9	<i>Pycnoscelus</i> <i>surinamensis</i>	Blaberidae	Roachcrossing	N/A	N/A	27041	27041	21553	21518	21108	20534
Bb-I-10	P10	<i>Pycnoscelus</i> <i>surinamensis</i>	Blaberidae	Roachcrossing	N/A	N/A	54720	54720	42599	42520	41863	41197
Bb-I-11	P11	<i>Pycnoscelus</i> <i>surinamensis</i>	Blaberidae	Roachcrossing	N/A	N/A	54319	54319	41841	41760	41601	41111
Bb-I-12	P12	<i>Pycnoscelus</i> <i>surinamensis</i>	Blaberidae	Roachcrossing	N/A	N/A	50393	50393	39055	38995	38540	38305
Bb-J-1	E1	<i>Schultesia</i> <i>lampyridiformis</i>	Blaberidae	UGA Entomology	M	N/A	77763	77763	37761	37662	24920	24722
Bb-J-2	E2	<i>Schultesia</i> <i>lampyridiformis</i>	Blaberidae	UGA Entomology	M	N/A	52407	52407	25045	24993	17711	17629
Bb-J-3	E3	<i>Schultesia</i> <i>lampyridiformis</i>	Blaberidae	UGA Entomology	F	N/A	70140	70140	33531	33309	27411	27305
Bb-J-4	E4	<i>Schultesia</i> <i>lampyridiformis</i>	Blaberidae	UGA Entomology	F	N/A	76699	76699	36504	36392	30079	29826
Bb-J-5	E5	<i>Schultesia</i> <i>lampyridiformis</i>	Blaberidae	UGA Entomology	M	N/A	24235	24235	17315	17269	16056	16006
Bb-J-6	E6	<i>Schultesia</i> <i>lampyridiformis</i>	Blaberidae	UGA Entomology	M	N/A	85920	85920	58767	58681	56354	56175
Bb-J-7	E7	<i>Schultesia</i> <i>lampyridiformis</i>	Blaberidae	UGA Entomology	M	N/A	44045	44045	30177	30151	29080	28984
Bb-J-8	E8	<i>Schultesia</i> <i>lampyridiformis</i>	Blaberidae	UGA Entomology	M	N/A	122242	122242	88361	88215	77768	76755
Bb-J-9	E9	<i>Schultesia</i> <i>lampyridiformis</i>	Blaberidae	UGA Entomology	F	tore hindgut	84222	84222	59044	58975	54377	53662
Bb-J-10 ^R	E10	<i>Schultesia</i> <i>lampyridiformis</i>	Blaberidae	UGA Entomology	F	pregnant	49333	49333	33968	33930	32329	32189

Bb-J-11	E11	<i>Schultesia lampyridiformis</i>	Blaberidae	UGA Entomology	M	N/A	130355	130355	89929	89780	87422	86891
Bb-J-12	E12	<i>Schultesia lampyridiformis</i>	Blaberidae	UGA Entomology	M	N/A	17848	17848	6881	6863	6770	6688
Bb-J-13	E13	<i>Schultesia lampyridiformis</i>	Blaberidae	UGA Entomology	M	N/A	115015	115015	76804	76707	76339	76057
Bb-J-14	E14	<i>Schultesia lampyridiformis</i>	Blaberidae	UGA Entomology	F	N/A	63441	63441	30794	30664	21883	21726
Bb-J-15	E15	<i>Schultesia lampyridiformis</i>	Blaberidae	UGA Entomology	M	N/A	87657	87657	65358	65250	59642	59442
Bt-A-1	A1	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	M	N/A	108720	108720	75784	75729	67337	65598
Bt-A-2	A2	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	M	N/A	26531	26531	15024	15005	13798	13583
Bt-A-3	A3	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	M	tore hindgut	16180	16180	5323	5307	4382	4282
Bt-A-4 ^R	A4	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	M	N/A	53389	53389	33946	33908	29386	28918
Bt-A-5	A5	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	F	N/A	29338	29338	16773	16696	16067	15984
Bt-A-6	A6	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	M	N/A	99741	99741	74495	74388	71011	69373
Bt-A-7	A7	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	M	N/A	38892	38892	27845	27786	27361	27108
Bt-A-8	A8	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	M	N/A	66640	66640	48155	48065	45909	45351
Bt-A-9	A9	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	M	N/A	56766	56766	41540	41467	40342	39888
Bt-A-10	A10	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	M	N/A	119319	119319	84401	84212	82309	81013
Bt-A-11	A11	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	M	N/A	98573	98573	69828	69740	61621	60656
Bt-A-12	A12	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	F	N/A	43348	43348	28162	28104	24430	23987
Bt-A-13	A13	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	M	N/A	65516	65516	45350	45276	43851	43597
Bt-A-14	A14	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	M	N/A	80745	80745	57288	57224	54169	53708
Bt-A-15	A15	<i>Periplaneta americana</i>	Blattidae	UGA Entomology	F	N/A	54729	54729	36512	36443	34017	33517
Bt-B-1	TWSBG1 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	F	N/A	115129	115129	89481	89389	88235	87216
Bt-B-2	TWSBG2 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	F	N/A	53394	53394	40620	40558	39033	38652
Bt-B-3	TWSBG3 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	F	N/A	53205	53205	41720	41659	41439	41108
Bt-B-4	TWSBG4 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	F	N/A	48727	48727	39223	39166	36224	35237

Bt-B-5	TWSBG5 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	F	tore hindgut at midgut connection	89503	89503	71262	71070	67143	64628
Bt-B-6 ^R	TWSBG6 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	M	N/A	96052	96052	77047	76925	75375	75019
Bt-B-7	TWSBG7 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	F	N/A	101870	101870	79988	79888	77982	75633
Bt-B-8	TWSBG8 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	F	N/A	95502	95502	75775	75622	73316	72731
Bt-B-9	TWSBG9 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	F	N/A	87475	87475	66496	66409	64015	63535
Bt-B-10	TWSBG10 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	M	N/A	75845	75845	59275	59183	54319	53466
Bt-B-11	TWSBG11 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	F	N/A	80176	80176	61821	61683	57816	57139
Bt-B-12	TWSBG12 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	F	N/A	81795	81795	64207	64080	61054	60647
Bt-B-13	TWSBG13 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	M	N/A	75273	75273	58502	58361	56361	55768
Bt-B-14	TWSBG14 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	F	N/A	125766	125766	100023	99862	95359	94373
Bt-B-15	TWSBG15 T14	<i>Periplaneta fuliginosa</i>	Blattidae	Field-collected	M	N/A	151706	151706	121416	121218	117683	115604
C-A-1	H1	<i>Eragula pilosa</i>	Corydiidae	Roachcrossing	N/A	N/A	40158	40158	28647	28556	26777	25360
C-A-2	H2	<i>Eragula pilosa</i>	Corydiidae	Roachcrossing	N/A	N/A	92048	92048	73939	73827	72995	66088
C-A-3	H3	<i>Eragula pilosa</i>	Corydiidae	Roachcrossing	N/A	N/A	11802	11802	8013	7993	7938	7306
C-A-4	H4	<i>Eragula pilosa</i>	Corydiidae	Roachcrossing	N/A	N/A	86035	86035	59998	59829	57912	53515
C-A-5	H5	<i>Eragula pilosa</i>	Corydiidae	Roachcrossing	N/A	N/A	50807	50807	33125	32980	32200	30840
C-A-6 ^R	H6	<i>Eragula pilosa</i>	Corydiidae	Roachcrossing	N/A	N/A	158345	158345	114086	113744	111315	110357
C-A-7	H7	<i>Eragula pilosa</i>	Corydiidae	Roachcrossing	N/A	N/A	193054	193054	139174	138787	133745	124805
C-A-8	H8	<i>Eragula pilosa</i>	Corydiidae	Roachcrossing	N/A	N/A	30294	30294	21561	21482	19110	18106
C-B-1	J1	<i>Ergaula capucina</i>	Corydiidae	Roachcrossing	M	N/A	111715	111715	79890	79661	78188	76883
C-B-2	J2	<i>Ergaula capucina</i>	Corydiidae	Roachcrossing	M	N/A	104496	104496	71983	71760	70624	69140
C-B-3	J3	<i>Ergaula capucina</i>	Corydiidae	Roachcrossing	M	N/A	171659	171659	115685	115370	112777	110374
C-B-4	J4	<i>Ergaula capucina</i>	Corydiidae	Roachcrossing	F	N/A	163917	163917	113616	113357	110959	107893
C-B-5	J5	<i>Ergaula capucina</i>	Corydiidae	Roachcrossing	M	N/A	215657	215657	146950	146636	145837	144941
C-B-6	J6	<i>Ergaula capucina</i>	Corydiidae	Roachcrossing	F	N/A	229612	229612	155662	155164	149599	145274
C-B-7	J7	<i>Ergaula capucina</i>	Corydiidae	Roachcrossing	M	N/A	29887	29887	23869	23811	23115	22506
C-B-8	J8	<i>Ergaula capucina</i>	Corydiidae	Roachcrossing	M	N/A	37309	37309	29880	29813	29060	27153
C-B-9	J9	<i>Ergaula capucina</i>	Corydiidae	Roachcrossing	F	pregnant	68240	68240	53783	53701	53317	52386

C-B-10 ^R	J10	<i>Ergaula capucina</i>	Corydiidae	Roachcrossing	M	N/A	56861	56861	45632	45584	45427	43660
C-B-11	J11	<i>Ergaula capucina</i>	Corydiidae	Roachcrossing	F	N/A	121293	121293	96915	96814	96044	93107
C-B-12	J12	<i>Ergaula capucina</i>	Corydiidae	Roachcrossing	M	N/A	108240	108240	84739	84625	83732	82568
C-C-1 ^R	O1	<i>Polyphaga aegyptiaca</i>	Corydiidae	Roachcrossing	M	N/A	179910	179910	123713	123385	120372	118657
C-C-2	O2	<i>Polyphaga aegyptiaca</i>	Corydiidae	Roachcrossing	F	N/A	65653	65653	45255	45107	44814	43967
C-C-3	O3	<i>Polyphaga aegyptiaca</i>	Corydiidae	Roachcrossing	M	N/A	187540	187540	127574	127280	125350	123957
C-C-4	O4	<i>Polyphaga aegyptiaca</i>	Corydiidae	Roachcrossing	F	N/A	66147	66147	46358	46225	45936	44725
C-C-5	O5	<i>Polyphaga aegyptiaca</i>	Corydiidae	Roachcrossing	F	N/A	103019	103019	72759	72518	72028	68734
C-C-6	O6	<i>Polyphaga aegyptiaca</i>	Corydiidae	Roachcrossing	F	N/A	178304	178304	122562	122169	121446	114765
C-C-7 ^A	O7	<i>Polyphaga aegyptiaca</i>	Corydiidae	Roachcrossing	F	N/A	35946	35946	27578	27524	25750	24148
C-C-8 ^A	O8	<i>Polyphaga aegyptiaca</i>	Corydiidae	Roachcrossing	F	N/A	36921	36921	28974	28932	26918	24728
C-C-9	O9	<i>Polyphaga aegyptiaca</i>	Corydiidae	Roachcrossing	M	N/A	84858	84858	66940	66877	66481	65406
C-C-11	O11	<i>Polyphaga aegyptiaca</i>	Corydiidae	Roachcrossing	F	N/A	71070	71070	54886	54795	54522	52804
C-C-12	O12	<i>Polyphaga aegyptiaca</i>	Corydiidae	Roachcrossing	F	N/A	29738	29738	23746	23725	23590	22046
C-D-1	L1	<i>Therea olegrandjeani</i>	Corydiidae	Roachcrossing	N/A	N/A	19150	194395	128837	128373	124863	118431
C-D-2	L2	<i>Therea olegrandjeani</i>	Corydiidae	Roachcrossing	N/A	N/A	150404	150404	104917	104668	100492	98962
C-D-3	L3	<i>Therea olegrandjeani</i>	Corydiidae	Roachcrossing	N/A	N/A	143258	143258	100839	100548	96262	94337
C-D-4	L4	<i>Therea olegrandjeani</i>	Corydiidae	Roachcrossing	N/A	N/A	332835	332835	232936	232450	224097	217177
C-D-5	L5	<i>Therea olegrandjeani</i>	Corydiidae	Roachcrossing	N/A	N/A	157519	157519	105505	105169	96185	94690
C-D-6	L6	<i>Therea olegrandjeani</i>	Corydiidae	Roachcrossing	N/A	N/A	110432	110432	75983	75763	75020	74454
C-D-7	L7	<i>Therea olegrandjeani</i>	Corydiidae	Roachcrossing	N/A	N/A	16863	16863	13349	13322	13215	12809
C-D-8	L8	<i>Therea olegrandjeani</i>	Corydiidae	Roachcrossing	N/A	N/A	26942	26942	21451	21403	20940	20651
C-D-9	L9	<i>Therea olegrandjeani</i>	Corydiidae	Roachcrossing	N/A	N/A	119883	119883	92515	92391	92161	91179
C-D-10 ^R	L10	<i>Therea olegrandjeani</i>	Corydiidae	Roachcrossing	N/A	N/A	136823	19150	13500	13482	13455	13170
C-D-11	L11	<i>Therea olegrandjeani</i>	Corydiidae	Roachcrossing	N/A	N/A	104151	136823	104567	104440	103727	100026

C-D-12	L12	<i>Therea olegrandjeani</i>	Corydiidae	Roachcrossing	N/A	N/A	194395	104151	83270	83130	82795	79678
E-A-1	D1	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	F	pregnant	64335	64335	32390	32283	29897	29781
E-A-2	D2	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	M	N/A	91447	91447	46116	45904	40396	40167
E-A-3	D3	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	F	N/A	83773	83773	41439	41286	34224	33923
E-A-4	D4	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	M	tore gut	86783	86783	43020	42785	39656	39452
E-A-5	D5	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	F	pregnant	82033	82033	40293	40212	32007	31810
E-A-6	D6	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	M	N/A	55294	55294	36694	36601	33279	33187
E-A-7	D7	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	F	N/A	16826	16826	10707	10687	10394	10169
E-A-8	D8	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	F	N/A	97076	97076	71391	71301	69065	68784
E-A-9	D9	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	F	N/A	122901	122901	88485	88372	86224	86117
E-A-10	D10	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	M	N/A	41587	41587	30195	30154	28680	28645
E-A-11 ^R	D11	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	M	tore hindgut	145848	145848	104090	103966	100416	100061
E-A-12	D12	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	M	N/A	123399	123399	84692	84619	83304	82551
E-A-13	D13	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	M	N/A	51513	51513	31904	31861	31572	31449
E-A-14	D14	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	M	N/A	90545	90545	62837	62801	62177	62057
E-A-15	D15	<i>Blattella germanica</i>	Ectobiidae	UGA Entomology	M	N/A	143949	143949	101314	101199	99601	98707
E-B-1 ^R	F1	<i>Parcoblatta fulvescens</i>	Ectobiidae	Roachcrossing	F	pregnant	102262	102262	68217	68051	67780	67553
E-B-2	F2	<i>Parcoblatta fulvescens</i>	Ectobiidae	Roachcrossing	F	N/A	141190	141190	90971	90706	89737	88796
E-B-3	F3	<i>Parcoblatta fulvescens</i>	Ectobiidae	Roachcrossing	F	N/A	135615	135615	89591	89356	88079	87407
E-B-4	F4	<i>Parcoblatta fulvescens</i>	Ectobiidae	Roachcrossing	F	N/A	200452	200452	133901	133534	130490	128329
E-B-5	F5	<i>Parcoblatta fulvescens</i>	Ectobiidae	Roachcrossing	F	N/A	177590	177590	118359	117999	116346	116013
E-B-6	F6	<i>Parcoblatta fulvescens</i>	Ectobiidae	Roachcrossing	F	N/A	173297	173297	111748	111389	109115	108393
E-B-7	F7	<i>Parcoblatta fulvescens</i>	Ectobiidae	Roachcrossing	F	N/A	32970	32970	26555	26517	26317	26214
E-B-8	F8	<i>Parcoblatta fulvescens</i>	Ectobiidae	Roachcrossing	F	N/A	26185	26185	20607	20582	20454	20297

E-B-9	F9	<i>Parcoblatta fulvescens</i>	Ectobiidae	Roachcrossing	F	N/A	184913	184913	128739	128644	125664	125227
E-B-10	F10	<i>Parcoblatta fulvescens</i>	Ectobiidae	Roachcrossing	F	N/A	160946	160946	112692	112608	109169	108667
E-B-11	F11	<i>Parcoblatta fulvescens</i>	Ectobiidae	Roachcrossing	M	N/A	123681	123681	40541	40490	37502	36874
E-C-1	I1	<i>Symploce pallens</i>	Ectobiidae	Roachcrossing	F	N/A	79536	79536	54304	54155	53724	53510
E-C-2	I2	<i>Symploce pallens</i>	Ectobiidae	Roachcrossing	F	N/A	152248	152248	107412	107133	104986	103327
E-C-3	I3	<i>Symploce pallens</i>	Ectobiidae	Roachcrossing	F	N/A	133232	133232	94095	93928	92709	92140
E-C-4 ^R	I4	<i>Symploce pallens</i>	Ectobiidae	Roachcrossing	F	N/A	155016	155016	105725	105556	104326	103957
E-C-6	I6	<i>Symploce pallens</i>	Ectobiidae	Roachcrossing	F	N/A	161019	161019	114682	114497	113716	113325
E-C-7	I7	<i>Symploce pallens</i>	Ectobiidae	Roachcrossing	F	N/A	104770	104770	79033	78941	78820	78664
E-C-8	I8	<i>Symploce pallens</i>	Ectobiidae	Roachcrossing	F	N/A	110572	110572	85119	85051	84762	83645
E-C-9	I9	<i>Symploce pallens</i>	Ectobiidae	Roachcrossing	F	N/A	49151	49151	36356	36259	36113	35743
E-C-10	I10	<i>Symploce pallens</i>	Ectobiidae	Roachcrossing	F	N/A	21525	21525	16774	16756	16676	16539
E-C-11	I11	<i>Symploce pallens</i>	Ectobiidae	Roachcrossing	F	N/A	89047	89047	69703	69533	69385	68737
E-C-12	I12	<i>Symploce pallens</i>	Ectobiidae	Roachcrossing	M	N/A	30918	30918	23741	23716	23658	23518

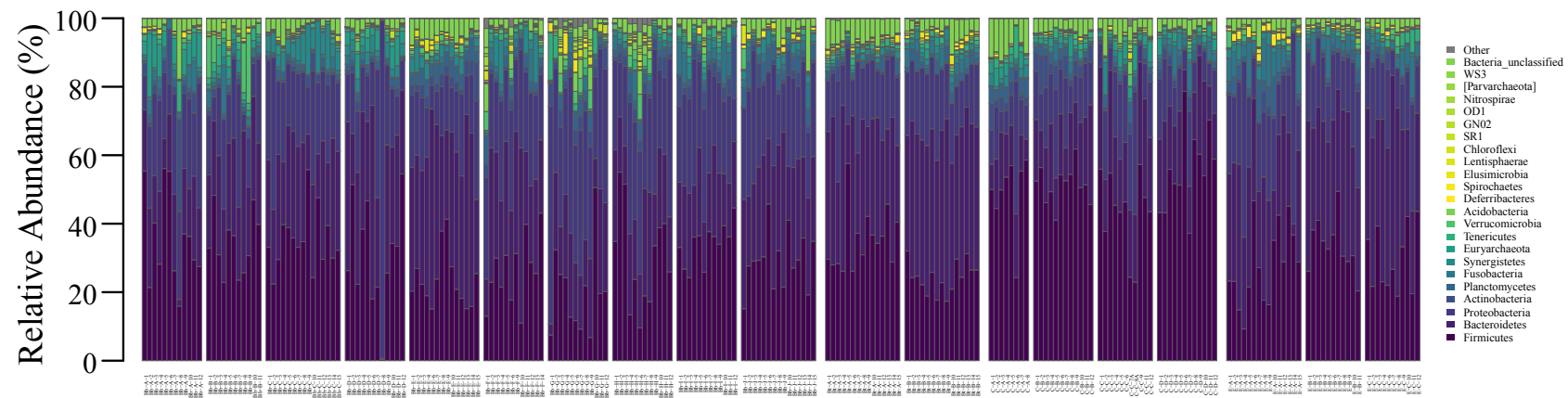
^A Additional 5 cycles during amplification

^R Representative insect used for COII Sequencing

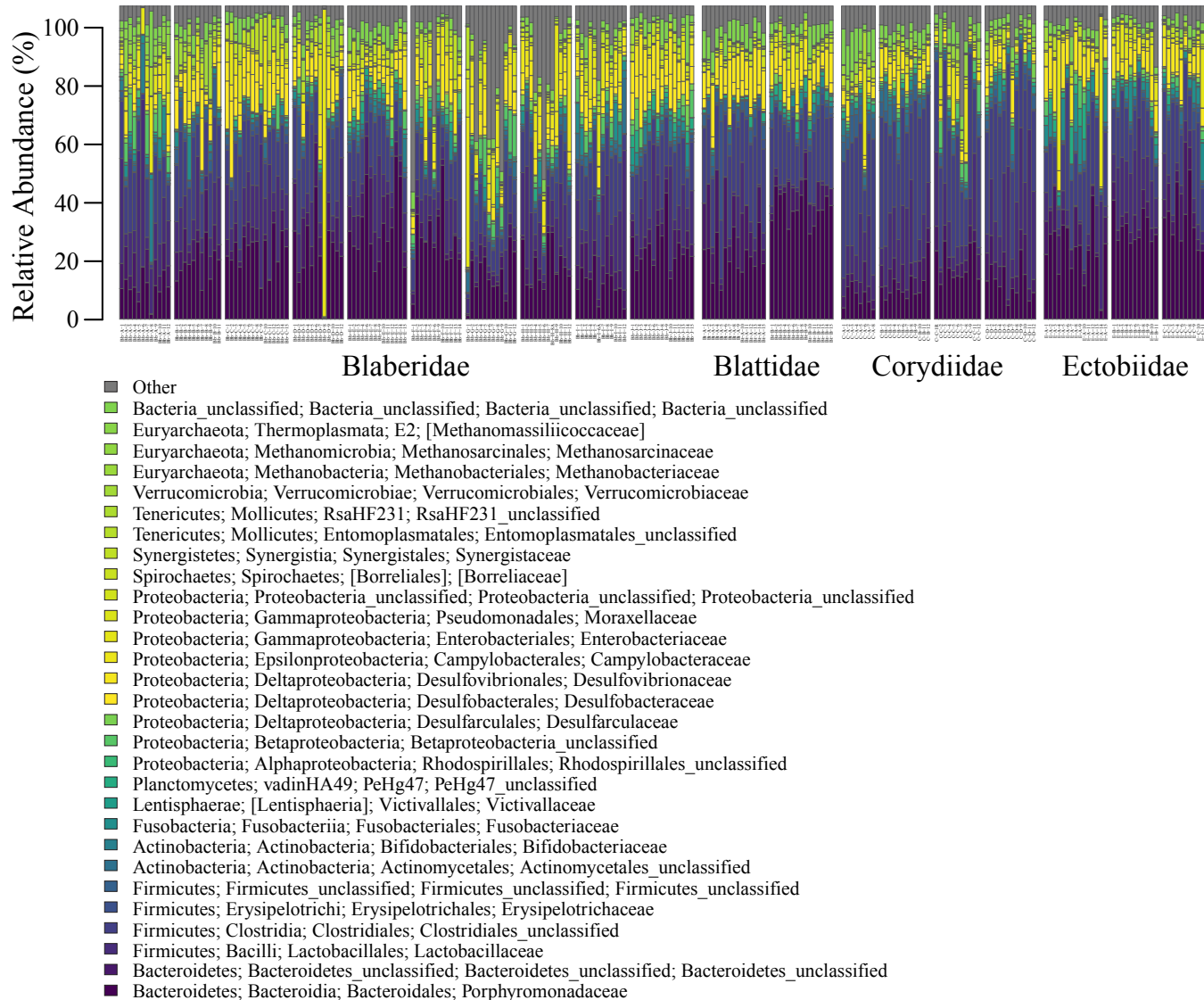
Supplemental Table 2.2. Barcodes used in Caporaso Primers

<u>Forward Barcode</u>	<u>Reverse Barcode</u>
1. AACCAACC	1. GTGTGTGT
2. CCAACCAA	2. AACGAACG
3. GGTTGGTT	3. TGTCTCAC
4. TTGGTTGG	4. CCAACGTA
5. AGTCGACT	5. CGTAGCAT
6. CCATCCTA	6. TTCGTTTCG
7. GTCAAGAG	7. ACACAGTC
8. TAGGTTGC	8. GAGTCAGA
9. AAGCAAGC	9. CGATGGTT
10. CGTTCGTT	10. ATCGTTGG
11. GCAAGCAA	11. TAGCAACC
12. TTCGTTTCG	12. GCTACCAA
13. AGGTGAAC	13. CACTGAGT
14. CTACAGCA	14. AGTGTCTG
15. GACACTGT	15. TCACAGAC
16. TCTGTGTC	16. GTGACTCA

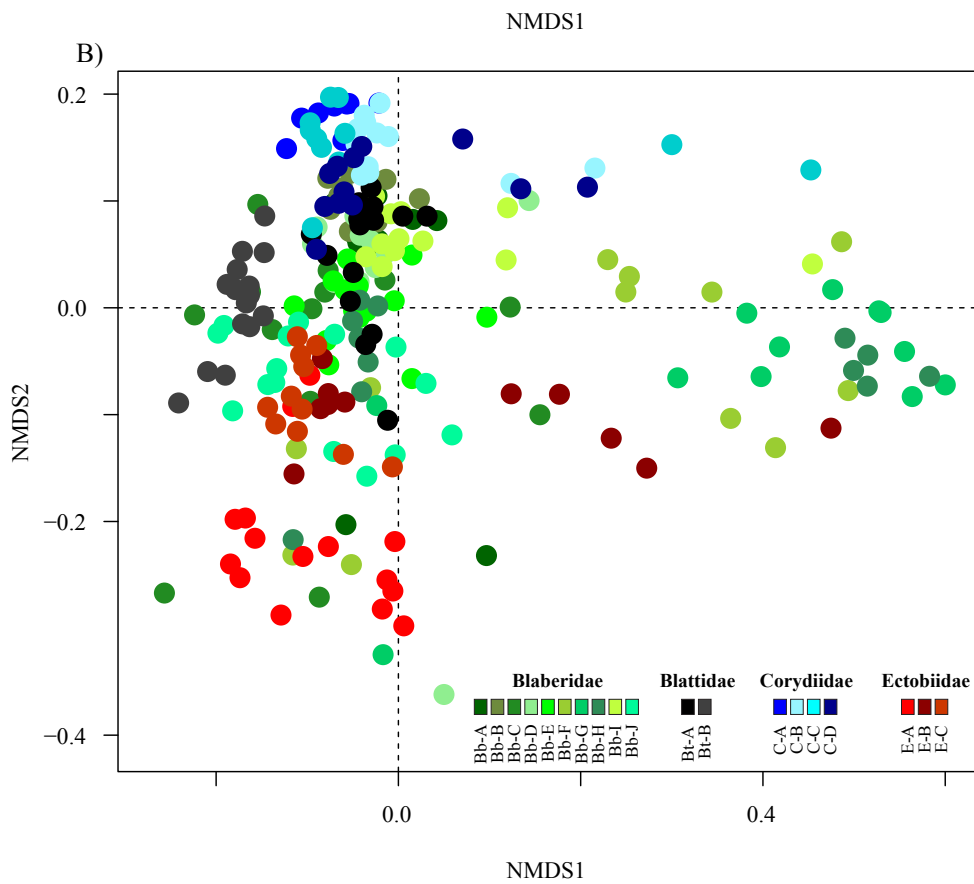
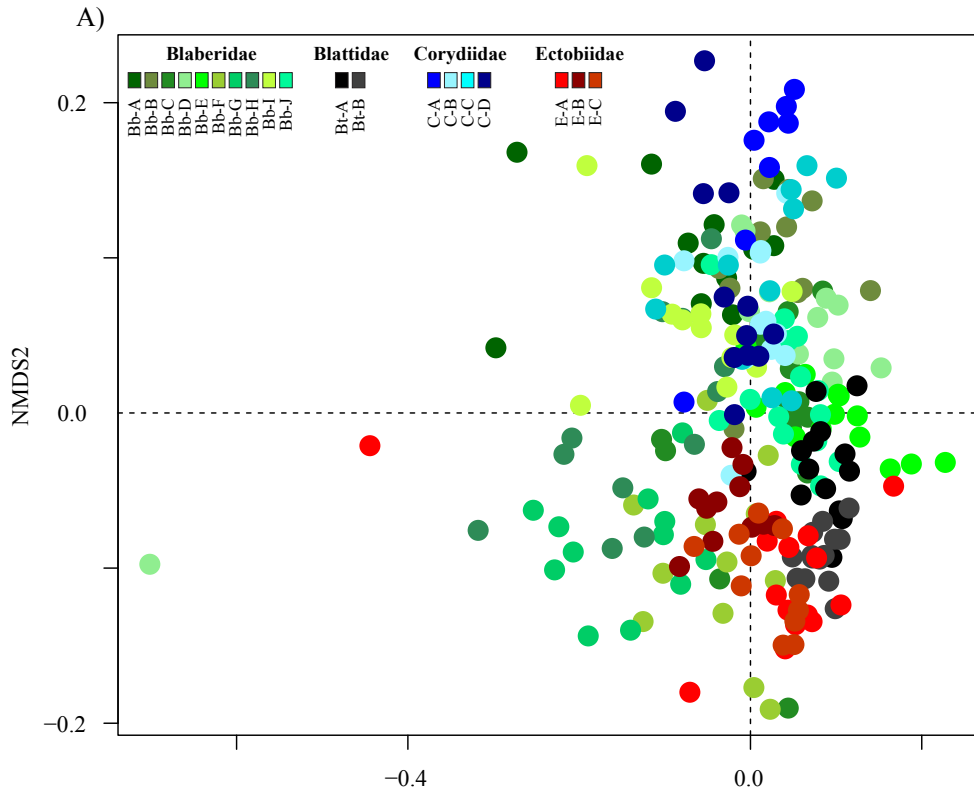
515F Primer: AATGATACGGCGACCACCGAGA TCTACAC XXXXXXXX
TATGGTAATT CA GTGCCAGCMGCCGCGGTAA; 806R Primer:
CAAGCAGAAGACGGCATAACGAGAT XXXXXXXX AGCAGCTCCAG AC
GGACTACHVGGGTWTCTAA



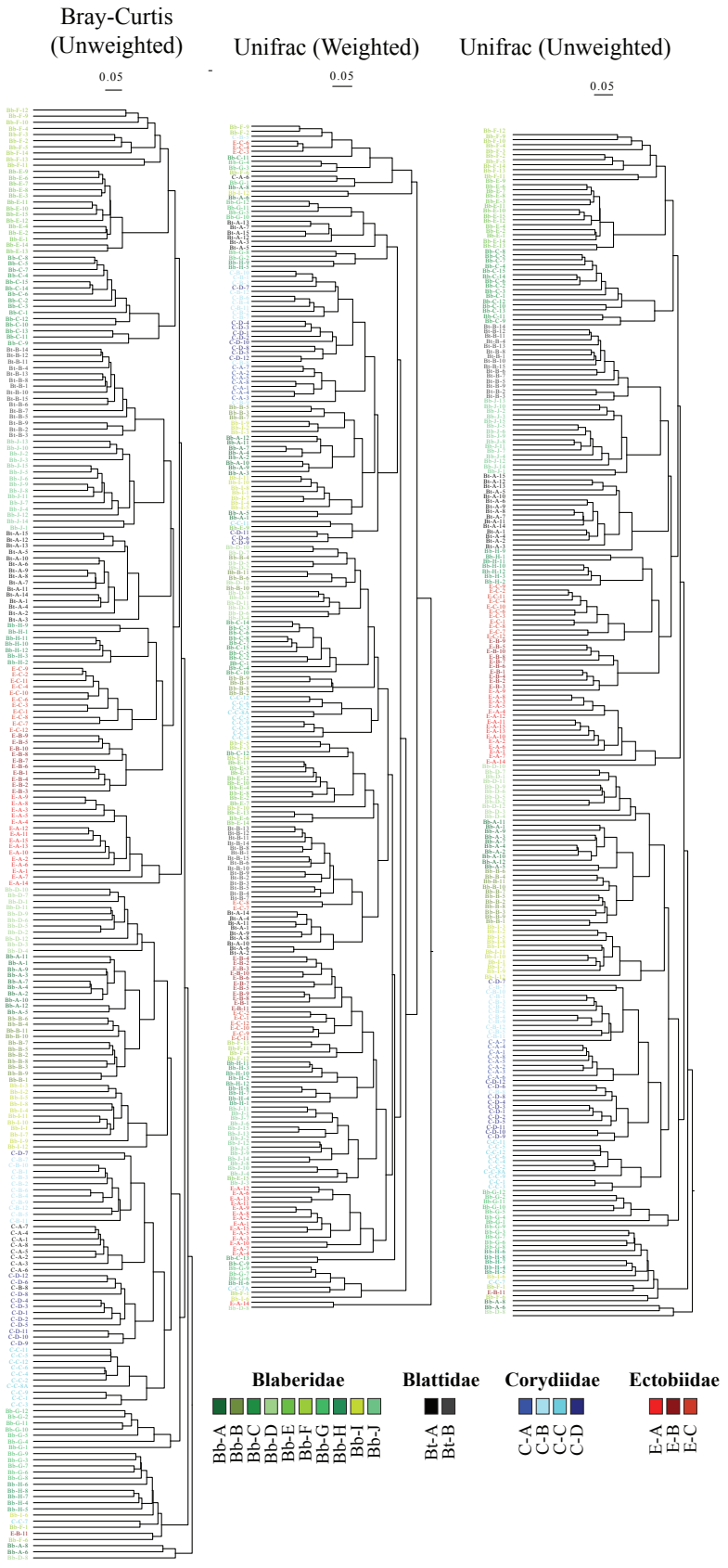
Supplemental Figure 2.1. Relative abundance of microbial phyla from individual insect guts. All families that represent $\geq 1\%$ from any one insect host are shown.



Supplemental Figure 2.2. Relative abundance of microbial families from individual insect guts. All families that represent $\geq 5\%$ from any one insect host are shown.



Supplemental Figure 2.3. Nonmetric multidimensional scaling (NMDS) plots of Blattodea. Plot was constructed with Unifrac (A) weighted and (B) unweighted metrics based on the distribution of OTUs (97% sequence identity). Libraries were resampled to a depth of the sample with the fewest sequences (4365). Clustering by host family (Weighted: $R=0.19$, $P<0.001$; Unweighted: $R=0.18$, $P<0.001$) and species (Weighted: $R=0.73$, $P<0.001$; Unweighted: $R=0.74$, $P<0.001$) was supported by ANOSIM.

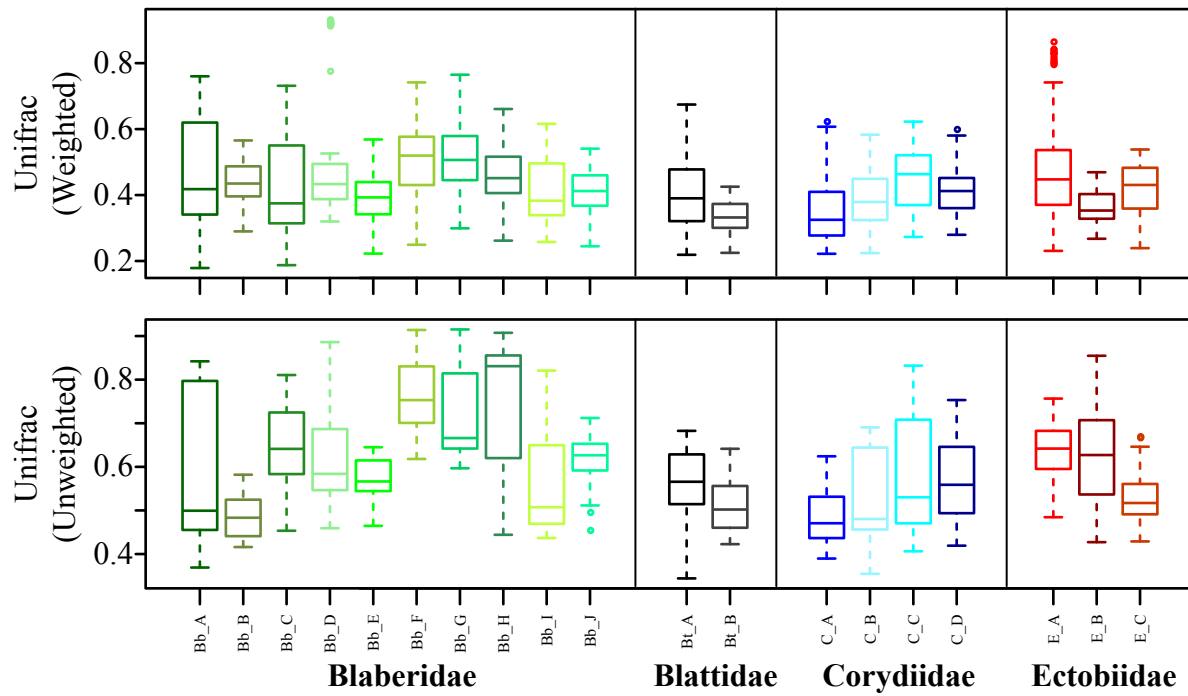


Supplemental Figure 2.4. Dendrogram of hierarchical cluster analysis using A) Bray-Curtis dissimilarity (unweighted) and Unifrac B) (weighted) and C) (unweighted) measurements calculated from OTU (97% sequence identity) measurements.

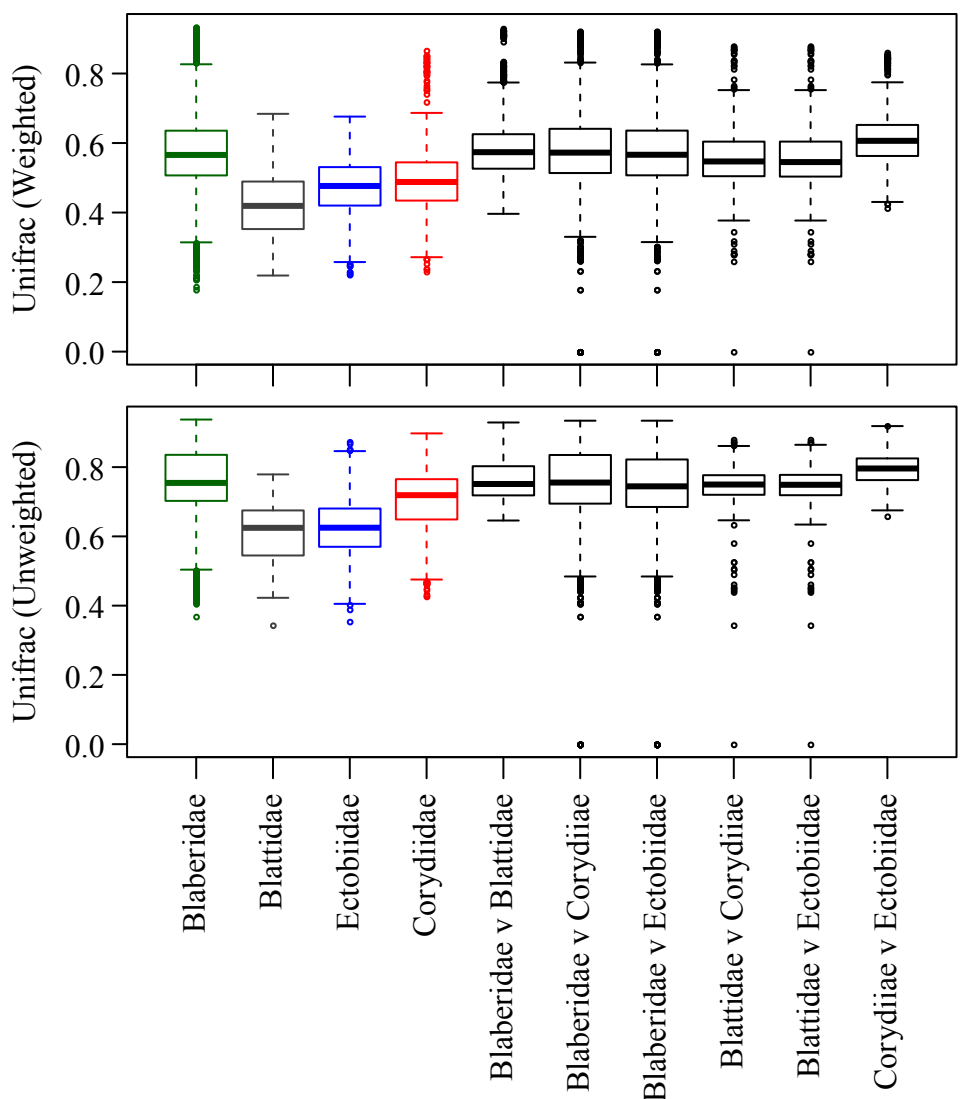
Supplemental Table 2.3 ANOSIM R Values

	Braycurtis (Weighted)	Bray-Curtis (Unweighted)	Unifrac (Weighted)	Unifrac (Unweighted)
All Families*	0.3005	0.3392	0.1912	0.182
All Species*	0.8469	0.851	0.732	0.741
Blaberidae (Bb)*	0.7936	0.6194	0.4185	0.5686
Blattidae (Bt)*	0.8291	0.9021	0.6131	0.7624
Corydiidae (C)*	0.5867	0.6865	0.3714	0.3399
Ectobiidae (E)*	0.7951	0.8173	0.4465	0.5808

*P-Value < 0.001 for all metrics



Supplemental Figure 2.5. Box-plots showing Unifrac measurements within species. For each box, the bars delineate the median, the hinges represent the lower and upper quartiles, the whiskers extend to the most extreme values (which are no more than 1.5 times the interquartile range from the box), and outliers are plotted, if present.



Supplemental Figure 2.6. Box-plots showing Unifrac measurements within and between families. For each box, the bars delineate the median, the hinges represent the lower and upper quartiles, the whiskers extend to the most extreme values (which are no more than 1.5 times the interquartile range from the box), and outliers are plotted, if present.

APPENDIX C

CHAPTER 3 SUPPLEMENTAL INFORMATION¹

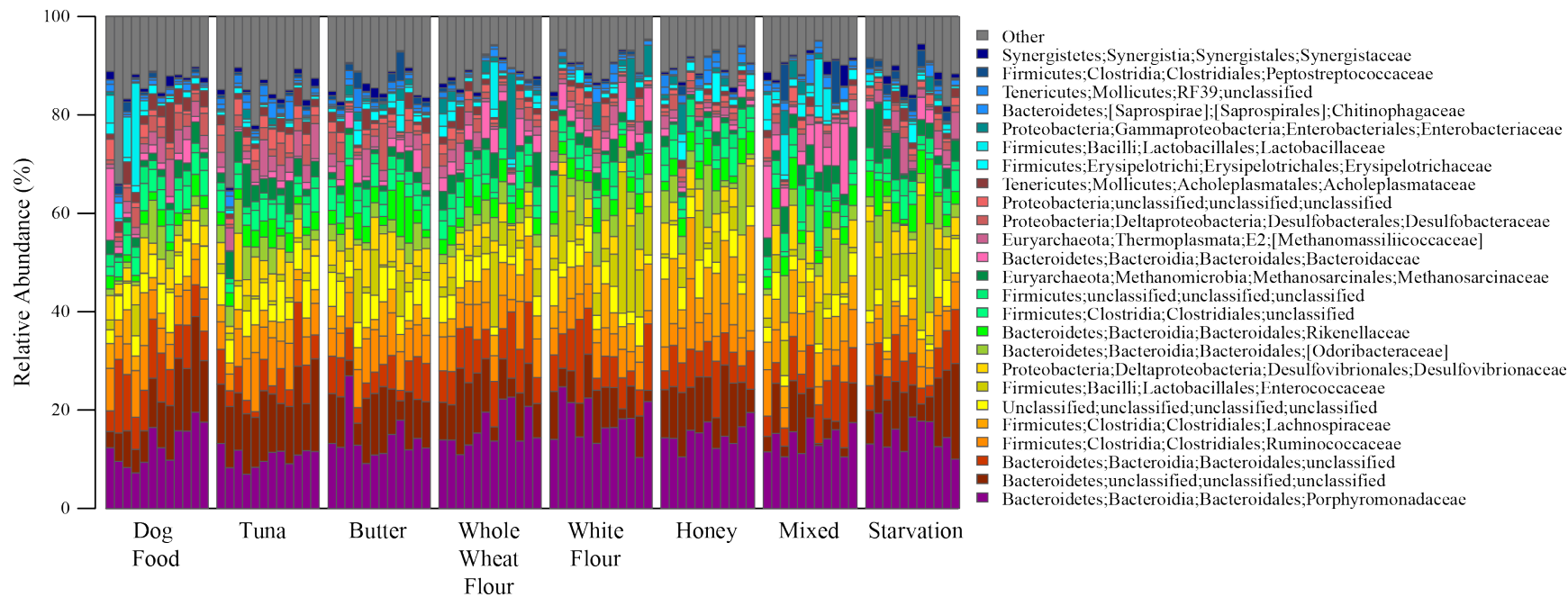
¹ Tinker KA and Ottesen EA. 2016. *Appl Environ Microbiol* 82:6603-6610.

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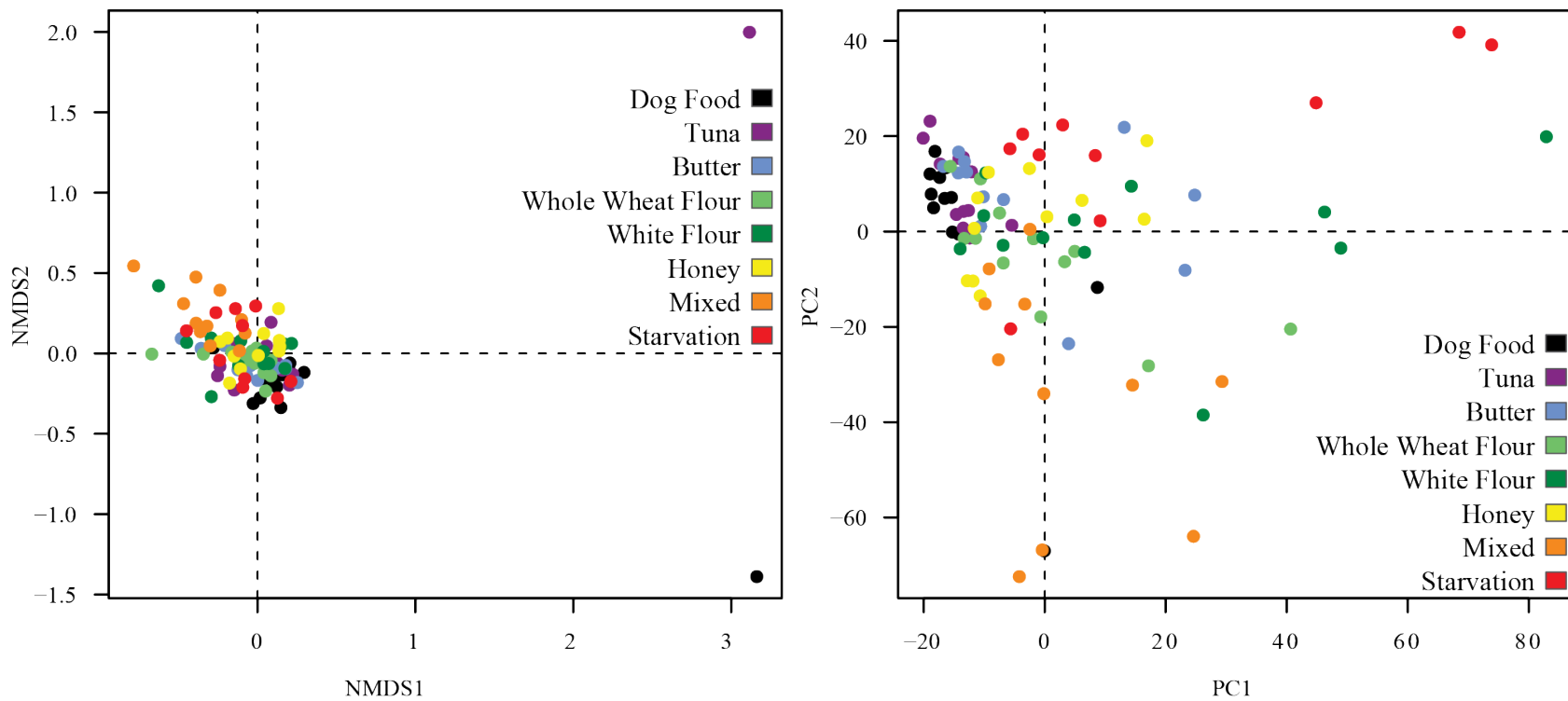
Supplemental Table 3.1. Barcodes used in Caporaso Primers

Forward Barcode	Reverse Barcode
1. AACCAACC	1. GTGTGTGT
2. CCAACCAA	2. AACGAACG
3. GGTTGGTT	3. TGTCTCAC
4. TTGGTTGG	4. CCAACGTA
5. AGTCGACT	5. CGTAGCAT
6. CCATCCTA	6. TTCGTTTCG
7. GTCAAGAG	7. ACACAGTC
8. TAGGTTGC	8. GAGTCAGA
9. AAGCAAGC	9. CGATGGTT
10. CGTTCGTT	10. ATCGTTGG
11. GCAAGCAA	11. TAGCAACC
12. TTCGTTTCG	12. GCTACCAA
13. AGGTGAAC	13. CACTGAGT
14. CTACAGCA	14. AGTGTCTG
15. GACACTGT	15. TCACAGAC
16. TCTGTGTC	16. GTGACTCA

515F Primer: AATGATACGGCGACCACCGAGA TCTACAC XXXXXXXX
TATGGTAATT CA GTGCCAGCMGCCGCGGTAA; 806R Primer:
CAAGCAGAAGACGGCATAACGAGAT XXXXXXXX AGCAGCTCCAG AC
GGACTACHVGGGTWTCTAA



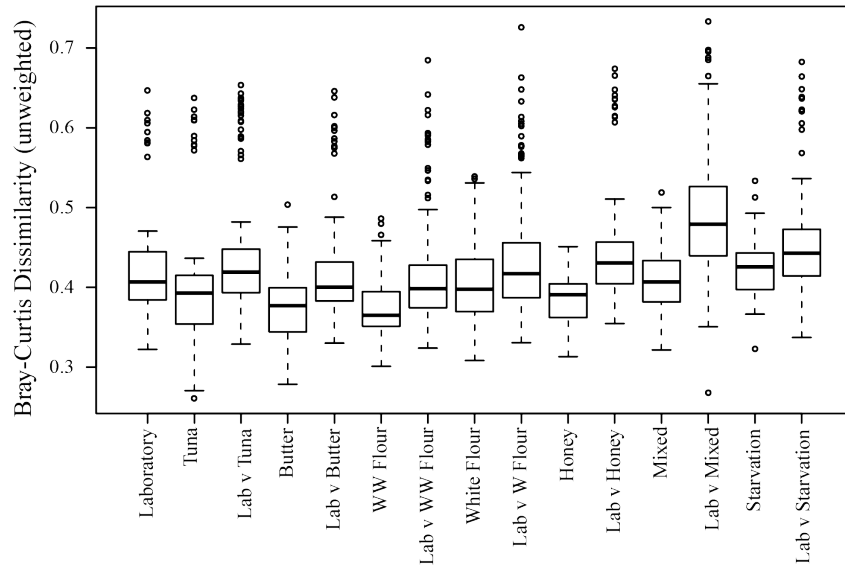
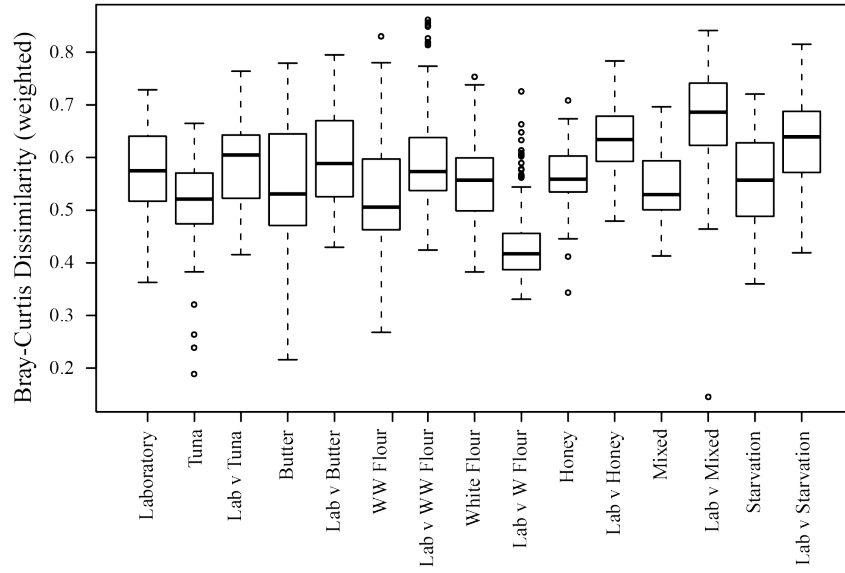
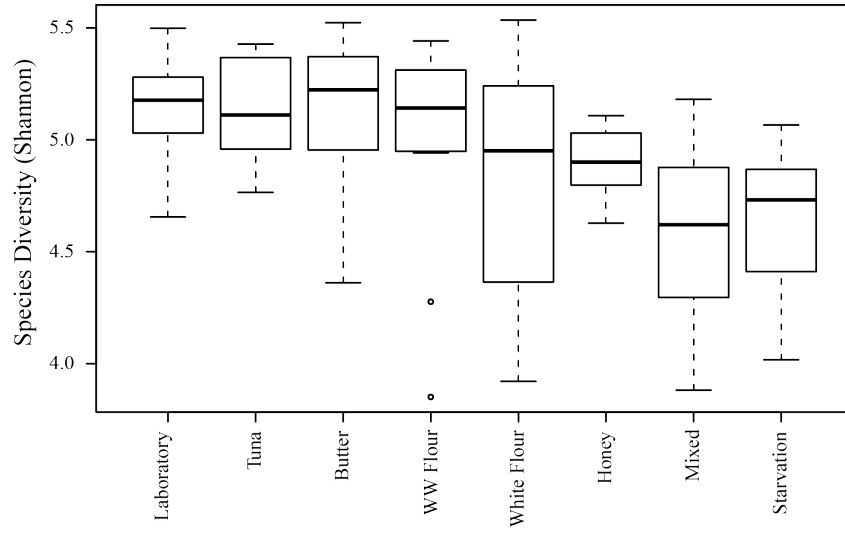
Supplemental Figure 3.1. Relative abundance of microbial families across 14 day diet treatments (Table 1). Each bar represents an individual cockroach gut. The 25 most abundant families are shown.



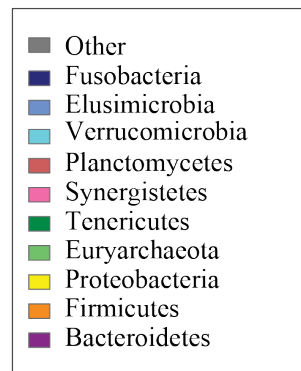
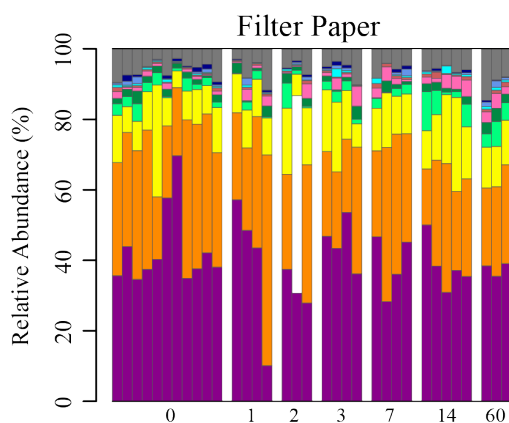
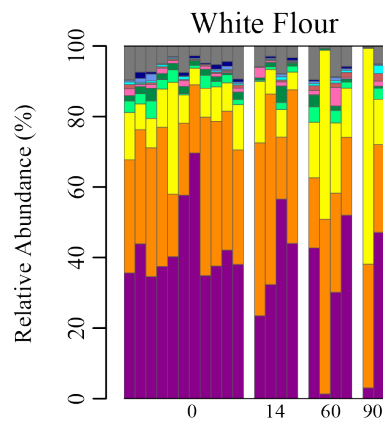
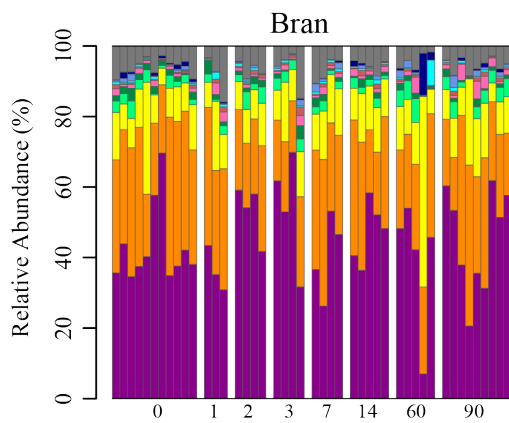
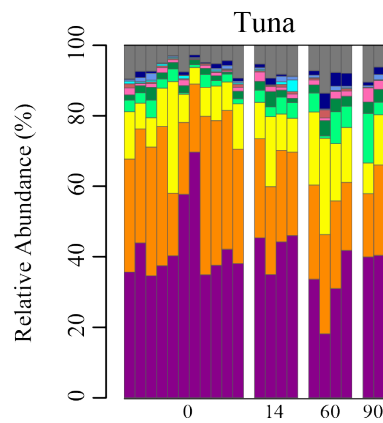
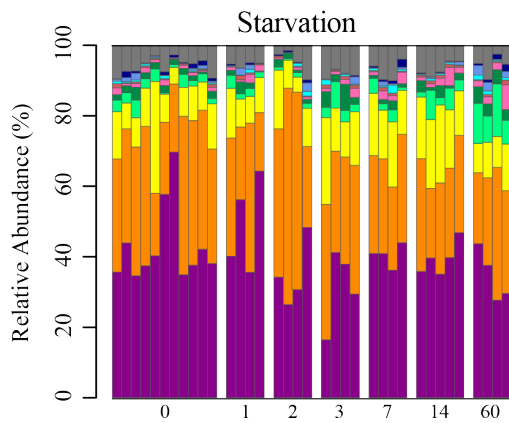
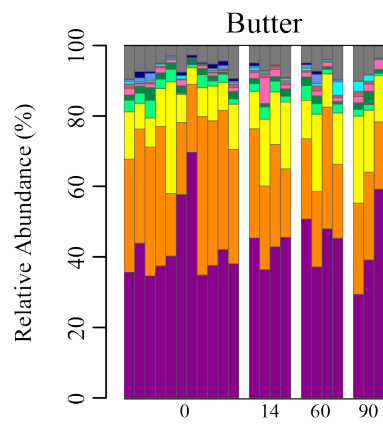
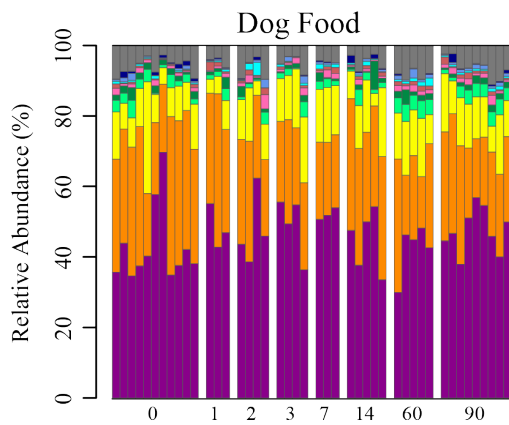
Supplemental Figure 3.2. Ordination analysis of 14-day diet treatments. Non-metric multidimensional scaling (left) and principal components (right) analyses of short-term dietary exposure treatments. All libraries were resampled to a constant depth of 4000 sequences. PERMANOVA based on dissimilarities was also conducted (R^2 of 0.217, p-value of 0.001).

Supplemental Table 3.2. PERMANOVA based on Bray-Curtis dissimilarity measurements between the laboratory dog food diet and other diet treatments

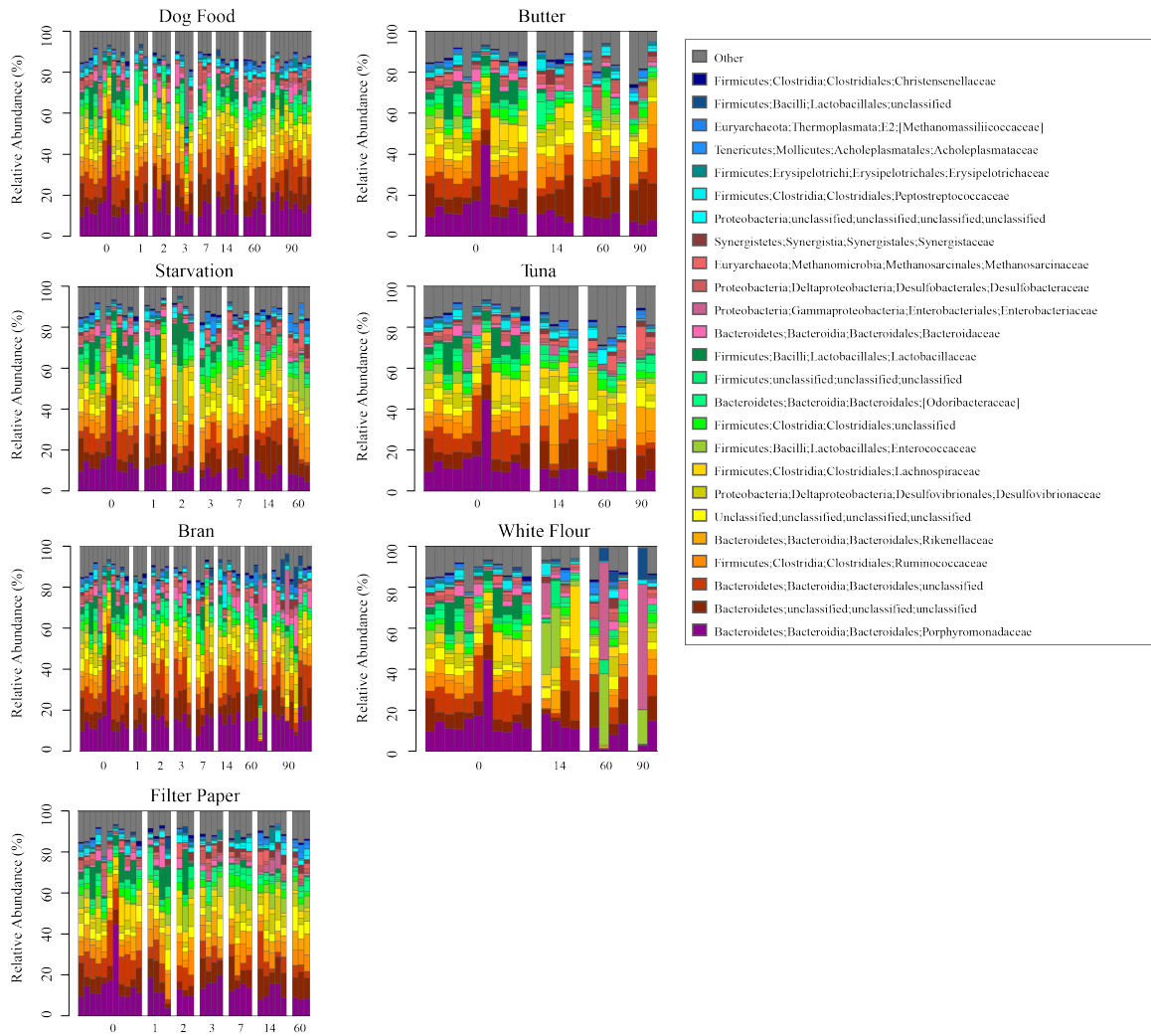
	P-value	R ²
Lab v Tuna	0.001	0.118
Lab v Butter	0.004	0.106
Lab v Whole Wheat Flour	0.002	0.117
Lab v White Flour	0.001	0.144
Lab v Honey	0.001	0.161
Lab v Mixed	0.001	0.232
Lab v Starvation	0.001	0.186



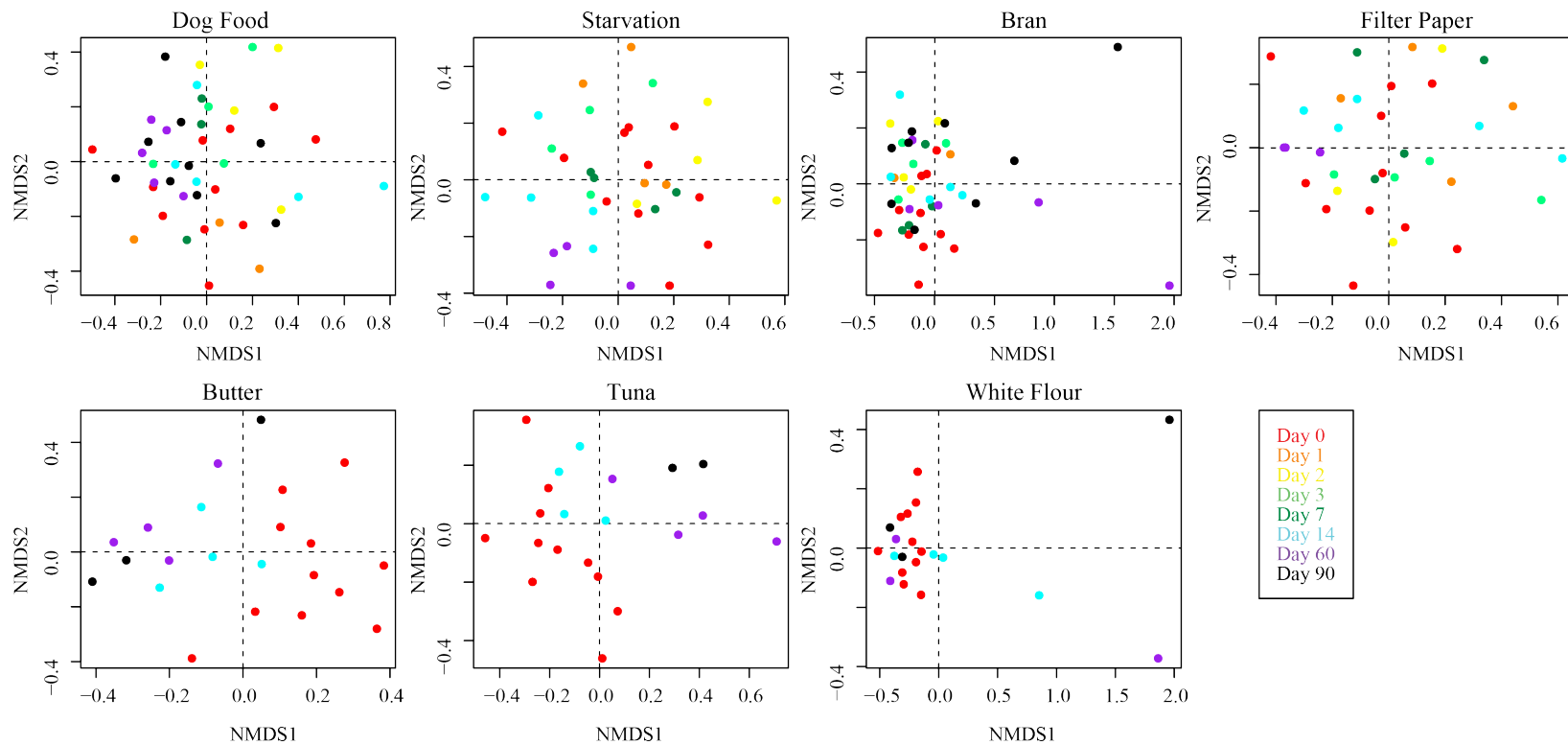
Supplemental Figure 3.3. Diversity analyses of short-term dietary treatments. Boxplots show Shannon diversity (top), weighted (middle), and unweighted (bottom) Bray-Curtis dissimilarities for each diet treatment. Dissimilarity analyses show within-treatment pairwise dissimilarities for each treatment as well as pairwise comparisons against dog food-fed control (laboratory) samples. All libraries were resampled to a constant depth of 4000 sequences. For each group, the bar delineates the mean, hinges represent the lower and upper quartiles, whiskers extend to the most extreme value which is no more than 1.5 times the interquartile range away from the box, and outliers are plotted if present.



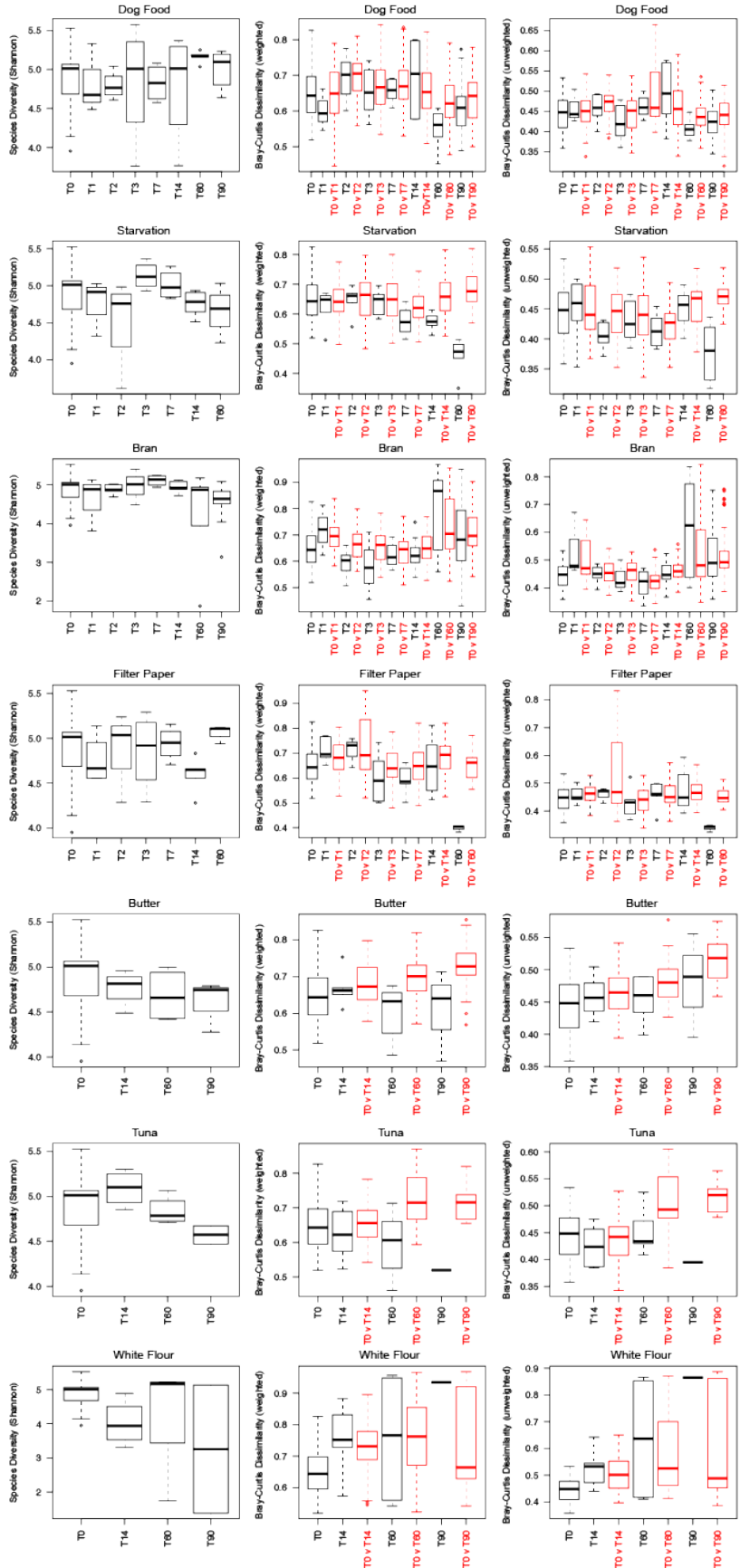
Supplemental Figure 3.4. Relative abundances of microbial phyla across long-term dietary treatments. The ten most abundant phyla are shown. Each bar represents an individual cockroach gut sample. Day 0 control samples are included in all graphs for ease of comparison.



Supplemental Figure 3.5. Relative abundances of microbial families across long-term dietary treatments. The 25 most abundant families are shown. Each bar represents an individual cockroach gut sample. Day 0 control samples are included in all graphs for ease of comparison.



Supplemental Figure 3.6. Non-metric multidimensional scaling of long-term diet treatments. Each point represents an individual cockroach sample. Points are colored according to position in the time series. Time zero for all graphs are the same laboratory raised, dog food-fed cockroach samples. All libraries were resampled to 4000 sequences. PERMANOVA based on dissimilarities was also conducted: dog food (R^2 of 0.192, p-value of 0.011), starvation (R^2 of 0.261, p-value of 0.001), bran (R^2 of 0.201, p-value of 0.007), filter paper (R^2 of 0.231, p-value of 0.002), butter (R^2 of 0.229, p-value of 0.001), tuna (R^2 of



Supplemental Figure 7. Diversity analyses of long-term dietary treatments. Boxplots show Shannon diversity (left), weighted (middle), and unweighted (right) Bray-Curtis dissimilarities for each diet treatment. Dissimilarity analyses show within-time point pairwise dissimilarities for each treatment as well as pairwise comparisons against time zero samples. All libraries were resampled to a constant depth of 4000 sequences. For each group, the bar delineates the mean, hinges represent the lower and upper quartiles, whiskers extend to the most extreme value which is no more than 1.5 times the interquartile range away from the box, and outliers are plotted if present.

Supplemental Table 3. Core gut microbiota found in cockroaches across all dietary treatment groups in the 14-day dietary shift.

k__Archaea(100);p__Euryarchaeota(100);c__Methanomicrobia(100);o__Methanosarcinales(100);f__Methanosarcinaceae(100);g__Methanimicrococcus(100);s__blatticola(100);

k__Archaea(100);p__Euryarchaeota(100);c__Thermoplasmata(100);o__E2(100);f__[Methanomassiliicoccaceae](100);g__vadinCA11(100);unclassified(100);

k__Archaea(100);p__Euryarchaeota(100);c__Thermoplasmata(100);o__E2(100);f__[Methanomassiliicoccaceae](100);g__vadinCA11(100);unclassified(100);.1

k__Bacteria(100);p__Actinobacteria(100);c__Coriobacteriia(100);o__Coriobacteriales(100);f__Coriobacteriaceae(100);g__Enterococcus(100);s__casseliflavus(100);

k__Bacteria(100);p__Actinobacteria(100);c__Coriobacteriia(100);o__Coriobacteriales(100);f__Coriobacteriaceae(100);g__Enterococcus(100);s__casseliflavus(100);.1

k__Bacteria(100);p__Actinobacteria(100);c__Coriobacteriia(100);o__Coriobacteriales(100);f__Coriobacteriaceae(100);g__Enterococcus(100);s__casseliflavus(100);.2

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__[Odoribacteraceae](100);g__Odoribacter(100);unclassified(100);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__[Odoribacteraceae](100);g__Odoribacter(100);unclassified(100);.2

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Bacteroidaceae(100);g__Bacteroides(100);unclassified(100);.5

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Candidatus_Azobacteroides(100);unclassified(100);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Candidatus_Azobacteroides(100);unclassified(100);.1

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Candidatus_Azobacteroides(92);unclassified(92);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Dysgonomonas(100);unclassified(100);.1

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Paludibacter(100);unclassified(100);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Parabacteroides(68);s__gordonii(67);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Parabacteroides(95);s__gordonii(90);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Tannerella(100);unclassified(100);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Tannerella(100);unclassified(100);.1

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Tannerella(62);unclassified(62);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Tannerella(93);unclassified(93);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);unclassified(100);unclassified(100);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);unclassified(100);unclassified(100);.10

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);unclassified(100);unclassified(100);.14

100);.4

k__Bacteria(100);p__Proteobacteria(100);c__Deltaproteobacteria(100);o__Desulfovibrionales(100);f__Desulfovibrionaceae(93);g__Desulfovibrio(59);unclassified(59);

k__Bacteria(100);p__Proteobacteria(100);c__Deltaproteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);

k__Bacteria(100);p__Proteobacteria(100);c__Gammaproteobacteria(100);o__Enterobacteriales(100);f__Enterobacteriaceae(100);unclassified(100);unclassified(100);

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.1

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.13

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.16

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.172

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.2

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.20

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.23

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.25

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.30

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.36

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.38

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.4

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.5

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.8

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.84

k__Bacteria(100);p__Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.9

k__Bacteria(100);p__Spirochaetes(100);c__Spirochaetes(100);o__Spirochaetales(100);f__Spirochaetaceae(100);unclassified(93);unclassified(93);

k__Bacteria(100);p__SR1(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);

k__Bacteria(100);p__SR1(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.1

k__Bacteria(100);p__Tenericutes(100);c__Mollicutes(100);o__Acholeplasmatales(100);f__Acholeplasmataceae(100);g__Acholeplasma(100);unclassified(100);.1

k__Bacteria(100);p__Tenericutes(100);c__Mollicutes(100);o__Acholeplasmatales(100);f__Acholeplasmataceae(100);g__Acholeplasma(91);unclassified(91);

k__Bacteria(100);p__Tenericutes(100);c__Mollicutes(100);o__RsaHF231(100);unclassified(100);unclassified(100);unclassified(100);

k__Bacteria(100);p__Verrucomicrobia(100);c__Opitutae(100);o__Opitutaes(100);f__Opitutaceae(100);g__Opitutus(100);unclassified(100);.1

k__Bacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);

Supplemental Table 4. Core gut microbiota found in all human samples.

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Bacteroidaceae(100);g__Bacteroides(100);s__ovatus(77);
k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Bacteroidaceae(100);g__Bacteroides(100);s__uniformis(95);
k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Bacteroidaceae(100);g__Bacteroides(100);unclassified(100);
k__Bacteria(100);p__Firmicutes(100);c__Clostridia(100);o__Clostridiales(100);f__Lachnospiraceae(100);g__Roseburia(99);s__faecis(89);
k__Bacteria(100);p__Firmicutes(100);c__Clostridia(100);o__Clostridiales(100);f__Ruminococcaceae(100);g__Faecalibacterium(100);s__prausnitzii(100);

Supplemental Table 5. Core gut microbiota found in laboratory-raised cockroaches, initial wild-caught cockroaches, and wild-caught cockroaches after 14 days in laboratory conditions.

k__Archaea(100);p__Euryarchaeota(100);c__Methanomicrobia(100);o__Methanosarcinales(100);f__Methanosarcinaceae(100);g__Methanimicrococcus(100);s__blatticola(100);*

k__Archaea(100);p__Euryarchaeota(100);c__Thermoplasmata(100);o__E2(100);f__[Methanomassiliicoccaceae](100);g__vadinCA11(100);unclassified(100);.1*

k__Bacteria(100);p__Actinobacteria(100);c__Coriobacteriia(100);o__Coriobacteriales(100);f__Coriobacteriaceae(100);g__Enterococcus(100);s__caseliflavus(100);.1*

k__Bacteria(100);p__Actinobacteria(100);c__Coriobacteriia(100);o__Coriobacteriales(100);f__Coriobacteriaceae(100);g__Enterococcus(100);s__caseliflavus(100);*

k__Bacteria(100);p__Actinobacteria(100);c__Coriobacteriia(100);o__Coriobacteriales(100);f__Coriobacteriaceae(100);unclassified(100);unclassified(100);

k__Bacteria(100);p__Bacteroidetes(100);c__[Saprosirae](100);o__[Saprosirales](100);f__Chitinophagaceae(100);unclassified(100);unclassified(100);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__[Odoribacteraceae](100);g__Odoribacter(100);unclassified(100);.1

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__[Odoribacteraceae](100);g__Odoribacter(100);unclassified(100);*

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__[Odoribacteraceae](100);unclassified(100);unclassified(100);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Bacteroidaceae(99);g__Bacteroides(99);unclassified(99);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Candidatus_Azobacteroides(100);unclassified(100);.1*

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Candidatus_Azobacteroides(100);unclassified(100);.2

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Candidatus_Azobacteroides(100);unclassified(100);.3

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Candidatus_Azobacteroides(100);unclassified(100);.4

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Candidatus_Azobacteroides(100);unclassified(100);.7

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Candidatus_Azobacteroides(100);unclassified(100);*

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Dysgonomonas(100);unclassified(100);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Paludibacter(100);unclassified(100);.3

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Parabacteroides(55);unclassified(55);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Parabacteroides(89);unclassified(89);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Tannerella(100);unclassified(100);.2

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);g__Tannerella(100);unclassified(100);*

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);unclassified(100);unclassified(100);.1

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);unclassified(100);unclassified(100);.2*

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);unclassified(100);unclassified(100);*

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);unclassified(51);unclassified(51);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(100);unclassified(65);unclassified(65);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Porphyromonadaceae(97);g__Candidatus_Azobacteroides(90);unclassified(90);

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Rikenellaceae(100);unclassified(100);unclassified(100);.1*

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Rikenellaceae(100);unclassified(100);unclassified(100);.15

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Rikenellaceae(100);unclassified(100);unclassified(100);.2

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Rikenellaceae(100);unclassified(100);unclassified(100);.4

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Rikenellaceae(100);unclassified(100);unclassified(100);.5

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Rikenellaceae(100);unclassified(100);unclassified(100);.6

k__Bacteria(100);p__Bacteroidetes(100);c__Bacteroidia(100);o__Bacteroidales(100);f__Rikenellaceae(97);unclassified(97);unclassified(97);

k__Bacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.2*
k__Bacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.3*
k__Bacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.39*
k__Bacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.40
k__Bacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.5*
k__Bacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.7*
k__Bacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.87
k__Bacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);.*

*Also found in all cockroaches in the 14-day dietary shift, listed in SI Table 2.

APPENDIX D
CHAPTER 4 SUPPLEMENTAL INFORMATION¹

¹ Tinker KA and Ottesen EA. To be submitted to PLOS One.

Supplemental Information Table 4.3. Sample metadata for all insects

ID	Species	Date Dissected	Sex	Notes	raw seqs	contigs	screen seqs	screen seqs	chimeral removal	remove lineage
W1	<i>Tenodera sinensis</i>	14-Jul-2017	N/A	Final/adult molt occurred on 10-Jul-2017.	47025	47025	35772	35719	34540	34123
W2 ^A	<i>Tenodera sinensis</i>	21-Jul-2017	N/A	Final/adult molted occurred on 17-Jul-2017. Wings never extended/uncrumpled.	39736	39736	31872	31813	29176	26059
W3 ^A	<i>Tenodera sinensis</i>	21-Jul-2017	N/A	Dying after failed final/adult molt; dissected within 24 hours of molt.	51889	51889	41188	41089	38820	37581
W4 ^A	<i>Tenodera sinensis</i>	17-Jul-2017	N/A	Dying after failed final/adult molt; dissected within 24 hours of molt. May have torn foregut.	51522	51522	41055	40983	37217	34910
W5	<i>Tenodera sinensis</i>	26-Jul-2017	N/A	Dying. Last molt date unrecorded; one molt from adulthood.	25132	25132	20354	20331	20155	19998
X1	<i>Hierodula venosa</i>	29-Aug-2017	N/A	Final/adult molt occurred on 24-Aug-2017.	10705	10705	8134	8126	8001	7841
X2 ^A	<i>Hierodula venosa</i>	7-Sep-2017	N/A	Dying. Last molt occurred 14-Aug-2017; 1-2 molts from adulthood.	47133	47133	36683	36375	32943	30833
X3	<i>Hierodula venosa</i>	8-Aug-2017	N/A	Dying. Last molt occurred 22-Jul-2017; 1-2 molts from adulthood.	9887	9887	7511	7485	7285	7008
X4	<i>Hierodula venosa</i>	7-Sep-2017	N/A	Dying. Last molt occurred 3-Aug-2017; 1-2 molts from adulthood.	7144	7144	5380	5355	5224	5050
	<i>Hierodula</i>	6-Aug-		Dying. Last molt date unrecorded; 1-2 molts from						

X6	<i>Hierodula venosa</i>	29-Aug-2017	N/A	Dying. Last molt occurred 9-Aug-2017; 1-2 molts from adulthood.	9674	9674	7584	7572	7433	7314
X7	<i>Hierodula venosa</i>	16-Aug-2017	N/A	Dying. Last molt date unrecorded; 1-2 molts from adulthood. Insect was full of worms.	5167	5167	4107	4101	4014	3911
X8	<i>Hierodula venosa</i>	8-Aug-2017	N/A	Dying. Last molt occurred 23-Jul-2017; 1-2 molts from adulthood. Outside of insect was covered with worms.	37590	37590	30728	30672	30336	30088
Y1 ^A	<i>Deroplatys lobata</i>	8-Aug-2017	N/A	Dying after failed molt; dissected within 24 hours of molt. Last molt date unrecorded; unknown number of molts from adulthood.	46486	46486	35413	35234	32247	30394
Y2 ^A	<i>Deroplatys lobata</i>	14-Aug-2017	N/A	Dying after failed molt; dissected within 24 hours of molt. Last molt date unrecorded; unknown number of molts from adulthood.	46903	46903	36720	36445	33383	31297
A1	<i>Periplaneta americana</i>	26-Jul-2016	M	N/A	108720	108720	75784	75729	67337	65598
A2	<i>Periplaneta americana</i>	26-Jul-2016	M	N/A	26531	26531	15024	15005	13798	13583
A3	<i>Periplaneta americana</i>	26-Jul-2016	M	Tore hindgut.	16180	16180	5323	5307	4382	4282
A4	<i>Periplaneta americana</i>	26-Jul-2016	M	N/A	53389	53389	33946	33908	29386	28918
A5	<i>Periplaneta americana</i>	26-Jul-2016	F	N/A	29338	29338	16773	16696	16067	15984

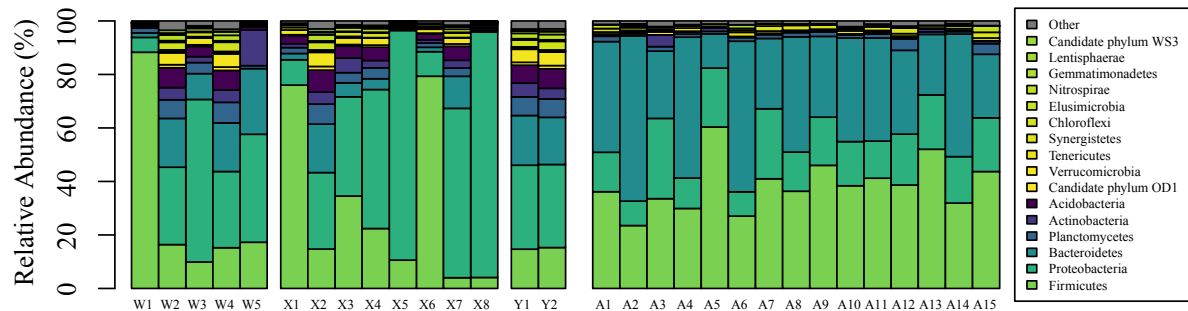
A6	<i>Periplaneta americana</i>	26-Jul-2016	M	N/A	99741	99741	74495	74388	71011	69373
A7	<i>Periplaneta americana</i>	1-Sep-2016	M	N/A	38892	38892	27845	27786	27361	27108
A8	<i>Periplaneta americana</i>	1-Sep-2016	M	N/A	66640	66640	48155	48065	45909	45351
A9	<i>Periplaneta americana</i>	1-Sep-2016	M	N/A	56766	56766	41540	41467	40342	39888
A10	<i>Periplaneta americana</i>	26-Jul-2016	M	N/A	119319	119319	84401	84212	82309	81013
A11	<i>Periplaneta americana</i>	26-Jul-2016	M	N/A	98573	98573	69828	69740	61621	60656
A12	<i>Periplaneta americana</i>	26-Jul-2016	F	N/A	43348	43348	28162	28104	24430	23987
A13	<i>Periplaneta americana</i>	1-Sep-2016	M	N/A	65516	65516	45350	45276	43851	43597
A14	<i>Periplaneta americana</i>	1-Sep-2016	M	N/A	80745	80745	57288	57224	54169	53708
A15	<i>Periplaneta americana</i>	1-Sep-2016	F	N/A	54729	54729	36512	36443	34017	33517

^A Additional amplification cycles

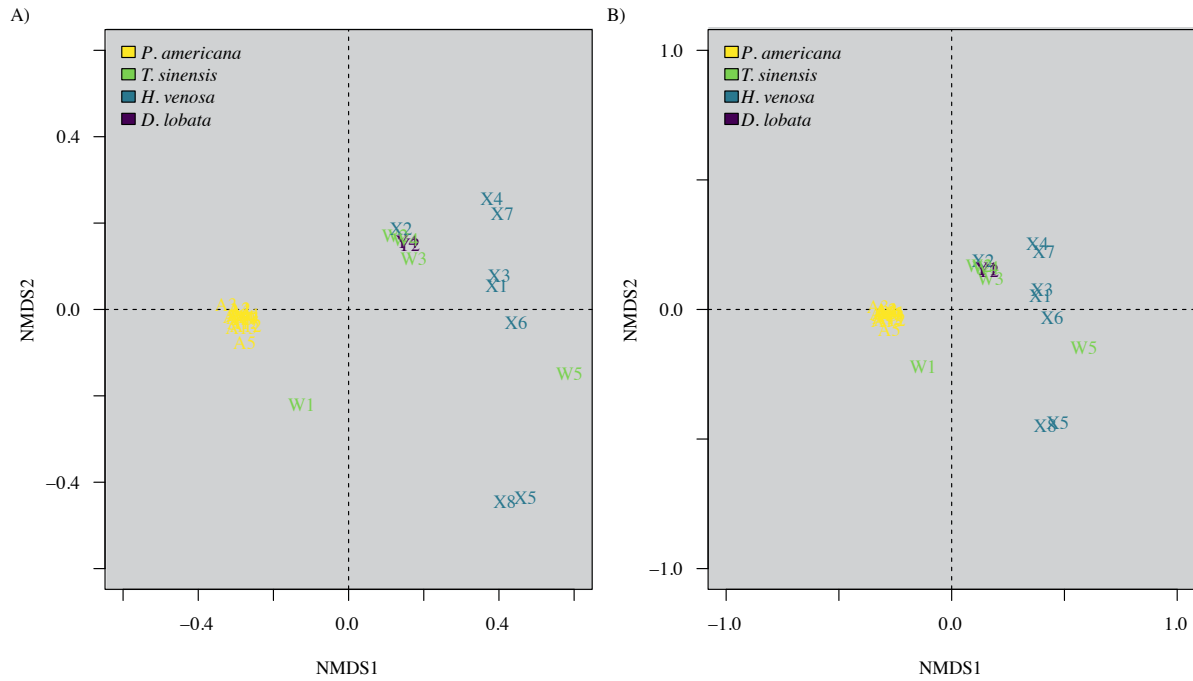
Supplemental Table 4.2. Barcodes used in primers

<u>Forward Barcode</u>	<u>Reverse Barcode</u>
1. AACCAACC	1. GTGTGTGT
2. CCAACCAA	2. AACGAACG
3. GGTTGGTT	3. TGTCTCAC
4. TTGGTTGG	4. CCAACGTA
5. AGTCGACT	5. CGTAGCAT
6. CCATCCTA	6. TTCGTTCG
7. GTCAAGAG	7. ACACAGTC
8. TAGGTTGC	8. GAGTCAGA
9. AAGCAAGC	9. CGATGGTT
10. CGTTCGTT	10. ATCGTTGG
11. GCAAGCAA	11. TAGCAACC
12. TTCGTTCG	12. GCTACCAA
13. AGGTGAAC	13. CACTGAGT
14. CTACAGCA	14. AGTGTCTG
15. GACACTGT	15. TCACAGAC
16. TCTGTGTC	16. GTGACTCA

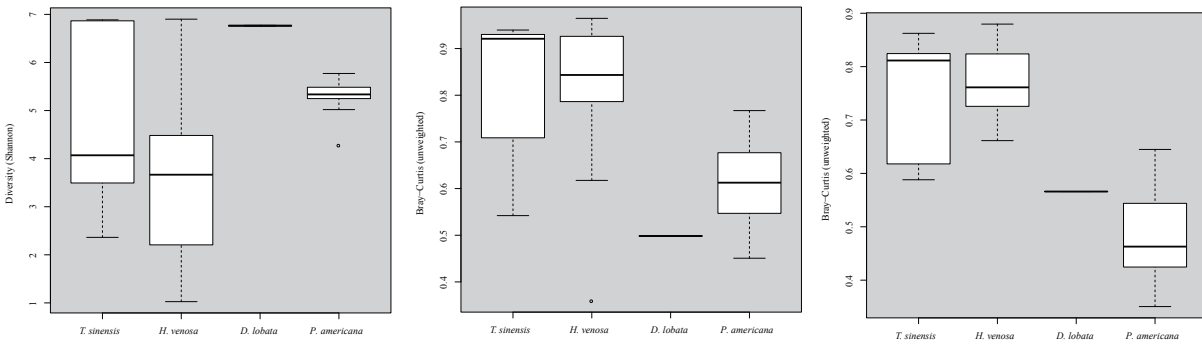
515F Primer: AATGATACGGCGACCACCGAGA TCTACAC XXXXXXXX
TATGGTAATT CA GTGCCAGCMGCCGCGGTAA; 806R Primer:
CAAGCAGAAGACGGCATAACGAGAT XXXXXXXX AGCAGCTCCAG A
GGACTACHVGGGTWTCTAA



Supplemental Figure 4.1. Relative abundances of microbial phyla in praying mantids and cockroaches. Each bar represents an individual insect gut. All phyla that represent $\geq 1\%$ from any one sample is shown.



Supplemental Figure 4.2. Nonmetric multidimensional scaling (NMDS) plots of insects. Plots were constructed with Bray-Curtis weighted (A) and unweighted (B) metrics. Libraries were resampled to a depth of the sample with the fewest sequences (3901). PERMANOVA based on weighted dissimilarities was also conducted: insect family (R^2 of 0.211, p -value <0.001), insect species (R^2 of 0.302, p -value <0.001); praying mantis body condition (R^2 of 0.165, p -value >0.05), praying mantis age (R^2 of 0.071, p -value >0.05).



Supplemental Figure 4.3. Alpha and beta diversities among praying mantids and cockroach species. Boxplots show Shannon diversity indices (left), and weighted (middle) and unweighted (left) dissimilarities among praying mantid and cockroach gut microbial communities at 97% sequence identity. Libraries were resampled to a depth of the sample with the fewest sequences (3901). For each group, the bars delineate the means, the hinges represent the lower and upper quartiles, the whiskers extend to the most extreme values (which are no more than 1.5 times the interquartile range from the box), and outliers are plotted, if present.

Supplemental Table 4.3. Core OTUs found in the gut microbiota of all insects

Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;Bacteroides_unclassified;Bacteroides_unclassified;.14
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Gut_group;Termite_cluster_III;Termite_cluster_III_unclassified;Termite_cluster_III_unclassified;

Firmicutes;Bacilli;Lactobacillales;Enterococcaceae;Enterococcus;Enterococcus_unclassified;Enterococcus_unclassified;
Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Termite_cockroach_cluster;Termite_cockroach_cluster_unclassified;Termite_cockroach_cluster_unclassified;

Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Comamonadaceae_unclassified;Comamonadaceae_unclassified;Comamonadaceae_unclassified;

Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Rhodoferrax;Rhodoferrax_unclassified;Rhodoferrax_unclassified;
Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobacteraceae;Desulfobacteraceae_unclassified;Desulfobacteraceae_unclassified;Desulfobacteraceae_unclassified;.1

Supplemental Table 4.4. Core OTUs found in the gut microbiota of all praying mantids

Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_18;Uncultured_18_unclassified;Uncultured_18_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_9;Uncultured_9_unclassified;Uncultured_9_unclassified;
Acidobacteria;RB25;RB25_unclassified;RB25_unclassified;RB25_unclassified;RB25_unclassified;RB25_unclassified;
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;.1
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;.2
Actinobacteria;Actinobacteria;Corynebacteriales;Mycobacteriaceae;Mycobacterium;Mycobacterium_unclassified;Mycobacterium_unclassified;
Actinobacteria;Actinobacteria;Frankiales;Nakamurellaceae;Nakamurella;Nakamurella_unclassified;Nakamurella_unclassified;
Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;Bacteroides_unclassified;Bacteroides_unclassified;.14*
Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;Bacteroides_unclassified;Bacteroides_unclassified;.5
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Gut_group;Termite_cluster_III;Termite_cluster_III_unclassified;Termite_cluster_III_unclassified
;*
Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae_1;Flavobacterium_1;Flavobacterium_1_unclassified;Flavobacterium_1_unclassified;
Chloroflexi;Caldilineae;Caldilineales;Caldilineaceae;Uncultured_5;Uncultured_5_unclassified;Uncultured_5_unclassified;
Firmicutes;Bacilli;Lactobacillales;Enterococcaceae;Enterococcus;Enterococcus_unclassified;Enterococcus_unclassified;*
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillaceae_unclassified;Lactobacillaceae_unclassified;Lactobacillaceae_unclassified;
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_1;Lactobacillus_1_unclassified;Lactobacillus_1_unclassified;
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_2;Lactobacillus_2_unclassified;Lactobacillus_2_unclassified;
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_sp_a;Lactobacillus_sp_a_unclassified;Lactobacillus_sp_a_unclassified;
Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Streptococcaceae_unclassified;Streptococcaceae_unclassified;Streptococcaceae_unclassified;
Firmicutes;Clostridia_1;Clostridiales;Peptostreptococcaceae;Peptostreptococcaceae_unclassified;Peptostreptococcaceae_unclassified;Peptostreptococcaceae_unclassified;.1
Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Termite_cockroach_cluster;Termite_cockroach_cluster_unclassified;Termite_cockroach_cluster_unclassified;*
Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;.1
Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;
Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;.1
Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;.2

Nitrospirae;Nitrospira;Nitrospirales;Nitrospiraceae;Nitrospira;Nitrospira_unclassified;Nitrospira_unclassified;

Planctomycetes;Pla4_lineage;Pla4_lineage_unclassified;Pla4_lineage_unclassified;Pla4_lineage_unclassified;Pla4_lineage_unclassified;Pla4_lineage_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;

Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;.1

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Bradyrhizobiaceae;Bradyrhizobium_12;Bradyrhizobium_12_unclassified;Bradyrhizobium_12_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Xanthobacteraceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Xanthobacteraceae;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Xanthobacteraceae;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales;Methylocystaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.2

Proteobacteria;Betaproteobacteria;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;.2

Proteobacteria;Betaproteobacteria;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;.7

Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Comamonadaceae_unclassified;Comamonadaceae_unclassified;Comamonadaceae_unclassified;.1

Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Comamonadaceae_unclassified;Comamonadaceae_unclassified;Comamonadaceae_unclassified;.1

Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Comamonadaceae_unclassified;Comamonadaceae_unclassified;Comamonadaceae_unclassified;Comamonadaceae_unclassified;*

Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Rhodofera;Rhodofera_unclassified;Rhodofera_unclassified;*

Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;

Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;

Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;

Proteobacteria;Betaproteobacteria;SC-I-84;SC-I-84_unclassified;SC-I-84_unclassified;SC-I-84_unclassified;SC-I-84_unclassified;

Proteobacteria;Betaproteobacteria;TRA3-20;TRA3-20_unclassified;TRA3-20_unclassified;TRA3-20_unclassified;TRA3-20_unclassified;

Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobacteraceae;Desulfobacteraceae_unclassified;Desulfobacteraceae_unclassified;Desulfobacteraceae_unclassified;.1*

Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.10

Proteobacteria;Deltaproteobacteria;GR-WP33-30;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;Uncultured_1_unclassified;
Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;
Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_8;Uncultured_8_unclassified;Uncultured_8_unclassified;
Verrucomicrobia;OPB35_soil_group;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;.1
Verrucomicrobia;OPB35_soil_group;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;.3
Verrucomicrobia;S-BQ2-57_soil_group;S-BQ2-57_soil_group_unclassified;S-BQ2-57_soil_group_unclassified;S-BQ2-57_soil_group_unclassified;S-BQ2-57_soil_group_unclassified;S-BQ2-57_soil_group_unclassified;
Verrucomicrobia;Spartobacteria;Chthoniobacterales;Xiphinematobacteraceae;Candidatus_Xiphinematobacter;Candidatus_Xiphinematobacter_unclassified;Candidatus_Xiphinematobacter_unclassified;
Verrucomicrobia;Spartobacteria;DA101_soil_group;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;
Verrucomicrobia;Spartobacteria;DA101_soil_group;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;.1

*OTUS found in all insect gut microbiota

Supplemental Table 4.5. Core OTUs found in the gut microbiota of all cockroaches

Actinobacteria;Actinobacteria;Acidimicrobiales;marine_group_2;marine_group_2_unclassified;marine_group_2_unclassified;marine_group_2_unclassified;
Actinobacteria;Actinobacteria;Coriobacteriales;Coriobacteriaceae;Uncultured_10;Uncultured_10_unclassified;Uncultured_10_unclassified;
Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;Bacteroides_unclassified;Bacteroides_unclassified;.1
Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;Bacteroides_unclassified;Bacteroides_unclassified;.12
Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;Bacteroides_unclassified;Bacteroides_unclassified;.13
Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;Bacteroides_unclassified;Bacteroides_unclassified;.14*
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_2;Porphyromonadaceae_2_unclassified;Porphyromonadaceae_2_unclassified;Porphyromonadaceae_2_unclassified;
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Cluster_IV;Cluster_IV_unclassified;Cluster_IV_unclassified;.4
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Cluster_IV;Cluster_IV_unclassified;Cluster_IV_unclassified;.5
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Cluster_IV;Cluster_IV_unclassified;Cluster_IV_unclassified;.8
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Cluster_IV;Cluster_IV_unclassified;Cluster_IV_unclassified;
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;.1
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;.10
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;.2
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;.3
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;.6
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;.1
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Tannerella;Tannerella_unclassified;Tannerella_unclassified;.3
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Tannerella;Tannerella_unclassified;Tannerella_unclassified;.4

Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_6;Odoribacter;Odoribacter_unclassified;Odoribacter_unclassified;.1
 Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Cluster_V;Porphyromonadaceae_Cluster_V_unclassified;Porphyromonadaceae_Cluster_V_uncla
 ssified;Porphyromonadaceae_Cluster_V_unclassified;.11
 Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Cluster_V;Termite_Cockroach_cluster;Termite_Cockroach_cluster_unclassified;Termite_Cockro
 ach_cluster_unclassified;
 Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Gut_group;Butyricimonas;Butyricimonas_unclassified;Butyricimonas_unclassified;
 Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Gut_group;Butyricimonas;Butyricimonas_unclassified;Butyricimonas_unclassified;.2
 Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Gut_group;Mixed_gut_cluster;Mixed_gut_cluster_unclassified;Mixed_gut_cluster_unclassified;
 Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Gut_group;Mixed_gut_cluster;Mixed_gut_cluster_unclassified;Mixed_gut_cluster_unclassified;.1
 1
 Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Gut_group;Mixed_gut_cluster;Mixed_gut_cluster_unclassified;Mixed_gut_cluster_unclassified;.2
 2
 Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Gut_group;Termite_cluster_I;Termite_cluster_I_unclassified;Termite_cluster_I_unclassified;
 Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Gut_group;Termite_cluster_I;Termite_cluster_I_unclassified;Termite_cluster_I_unclassified;.1
 Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Gut_group;Termite_cluster_III;Termite_cluster_III_unclassified;Termite_cluster_III_unclassified
 ;*
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Alisitpes_IV;Alisitpes_IV_unclassified;Alisitpes_IV_unclassified;.13
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Alisitpes_IV;Alisitpes_IV_unclassified;Alisitpes_IV_unclassified;.16
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Alisitpes_IV;Alisitpes_IV_unclassified;Alisitpes_IV_unclassified;
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Alistipes_III;Alistipes_III_unclassified;Alistipes_III_unclassified;
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;gut_cluster_c;gut_cluster_c_unclassified;gut_cluster_c_unclassified;
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;RC9_gut_group;RC9_gut_group_unclassified;RC9_gut_group_unclassified;.3
 Deferribacteres;Deferribacteres;Deferribacterales;Deferribacteraceae;Deferribacteraceae_unclassified;Deferribacteraceae_unclassified;Deferribacteraceae_unclas
 sified;
 Deferribacteres;Deferribacteres;Deferribacterales;Deferribacteraceae;Mucispirillum;Mucispirillum_unclassified;Mucispirillum_unclassified;.2
 Deferribacteres;Deferribacteres;Deferribacterales;Deferribacteraceae;Mucispirillum;Mucispirillum_unclassified;Mucispirillum_unclassified;.5
 Firmicutes;Bacilli;Lactobacillales;Enterococcaceae;Enterococcus;Enterococcus_unclassified;Enterococcus_unclassified;.*
 Firmicutes;Clostridia_1;Clostridiales;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;
 Firmicutes;Clostridia_1;Clostridiales;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;.1
 Firmicutes;Clostridia_1;Clostridiales;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;.2
 Firmicutes;Clostridia_1;Clostridiales;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;.3
 Firmicutes;Clostridia_1;Clostridiales;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;.7
 Firmicutes;Clostridia_1;Clostridiales;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;.8
 Firmicutes;Clostridia_1;Clostridiales;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;
 Firmicutes;Clostridia_1;Clostridiales;Eubacteriaceae_1;Anaerofustis;Anaerofustis_unclassified;Anaerofustis_unclassified;
 Firmicutes;Clostridia_1;Clostridiales;Family_XIII_Incertae_Sedis;Family_XIII_Incertae_Sedis_unclassified;Family_XIII_Incertae_Sedis_unclassified;Family_
 XIII_Incertae_Sedis_unclassified;
 Firmicutes;Clostridia_1;Clostridiales;Family_XIII_Incertae_Sedis;Family_XIII_Incertae_Sedis_unclassified;Family_XIII_Incertae_Sedis_unclassified;Family_
 XIII_Incertae_Sedis_unclassified;.2

Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.7
Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.70
Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.71
Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.8
Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.9
Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Termite_cluster_III;Termite_cluster_III_unclassified;Termite_cluster_III_unclassified;
Proteobacteria;Deltaproteobacteria;Rs-K70;Insect_cluster;Insect_cluster_unclassified;Insect_cluster_unclassified;Insect_cluster_unclassified;
Synergistetes;Synergistia;Synergistales;Synergistaceae;Candidatus_Tammella;Candidatus_Tammella_unclassified;Candidatus_Tammella_unclassified;.1
Synergistetes;Synergistia;Synergistales;Synergistaceae;Candidatus_Tammella;Candidatus_Tammella_unclassified;Candidatus_Tammella_unclassified;.4
Synergistetes;Synergistia;Synergistales;Synergistaceae;Synergistaceae_unclassified;Synergistaceae_unclassified;Synergistaceae_unclassified;
Synergistetes;Synergistia;Synergistales;Synergistaceae;Termite_cockroach_cluster;Termite_cockroach_cluster_unclassified;Termite_cockroach_cluster_unclassified;
Tenericutes;Mollicutes;Acholeplasmatales;Acholeplasmataceae;Acholeplasma_2;Acholeplasma_2_unclassified;Acholeplasma_2_unclassified;
Tenericutes;Mollicutes;Acholeplasmatales;Acholeplasmataceae;Acholeplasma_2;Acholeplasma_2_unclassified;Acholeplasma_2_unclassified;.1

*OTUS found in all insect gut microbiota

Supplemental Table 4.6. Core OTUs found in the gut microbiota of all *T. sinensis*

Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Acidobacteriaceae_unclassified;Acidobacteriaceae_unclassified;Acidobacteriaceae_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Candidatus_Solibacter;Candidatus_Solibacter_unclassified;Candidatus_Solibacter_unclassified;
6
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.1
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_11;Uncultured_11_unclassified;Uncultured_11_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_18;Uncultured_18_unclassified;Uncultured_18_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_18;Uncultured_18_unclassified;Uncultured_18_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_22;Uncultured_22_unclassified;Uncultured_22_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_26;Uncultured_26_unclassified;Uncultured_26_unclassified;.2
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_27;Uncultured_27_unclassified;Uncultured_27_unclassified;.1
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;.1
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;.10
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;.11
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;.14
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;.7
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;.8
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;.9
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_9;Uncultured_9_unclassified;Uncultured_9_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_9;Uncultured_9_unclassified;Uncultured_9_unclassified;.2
Acidobacteria;Holophagae;Cluster_32-20;Cluster_32-20_unclassified;Cluster_32-20_unclassified;Cluster_32-20_unclassified;Cluster_32-20_unclassified;
Acidobacteria;RB25;RB25_unclassified;RB25_unclassified;RB25_unclassified;RB25_unclassified;RB25_unclassified;
Acidobacteria;RB25;RB25_unclassified;RB25_unclassified;RB25_unclassified;RB25_unclassified;RB25_unclassified;.1
Actinobacteria;Actinobacteria;Acidimicrobiales;Acidimicrobiales_unclassified;Acidimicrobiales_unclassified;Acidimicrobiales_unclassified;Acidimicrobiales_u
nclassified;
Actinobacteria;Actinobacteria;Acidimicrobiales;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;Uncultured_6_unclassified;
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;.1
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;.12
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;.13

Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;.2
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;.3
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;.4
Actinobacteria;Actinobacteria;Coriobacteriales;Coriobacteriaceae;Uncultured_10;Uncultured_10_unclassified;Uncultured_10_unclassified;
Actinobacteria;Actinobacteria;Coriobacteriales;Coriobacteriaceae;Uncultured_10;Uncultured_10_unclassified;Uncultured_10_unclassified;.1
Actinobacteria;Actinobacteria;Coriobacteriales;Coriobacteriaceae;Uncultured_10;Uncultured_10_unclassified;Uncultured_10_unclassified;
Actinobacteria;Actinobacteria;Corynebacteriales;Corynebacteriaceae;Corynebacterium_1;Corynebacterium_1_unclassified;Corynebacterium_1_unclassified;
Actinobacteria;Actinobacteria;Corynebacteriales;Mycobacteriaceae;Mycobacterium;Mycobacterium_unclassified;Mycobacterium_unclassified;
Actinobacteria;Actinobacteria;Corynebacteriales;Nocardiaceae_1;Gordonia;Gordonia_unclassified;Gordonia_unclassified;
Actinobacteria;Actinobacteria;Frankiales;Nakamurellaceae;Nakamurella;Nakamurella_unclassified;Nakamurella_unclassified;
Actinobacteria;Actinobacteria;MB-A2-108;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;.2
Actinobacteria;Actinobacteria;MB-A2-108;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;.3
Bacteroidetes;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;.61
Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;Bacteroides_unclassified;Bacteroides_unclassified;.14*
Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;Bacteroides_unclassified;Bacteroides_unclassified;.2
Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;Bacteroides_unclassified;Bacteroides_unclassified;.5
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_1;Dysgonomonas;Dysgonomonas_unclassified;Dysgonomonas_unclassified;
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;.3
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Cluster_V;Porphyromonadaceae_Cluster_V_unclassified;Porphyromonadaceae_Cluster_V_unclassified;Porphyromonadaceae_Cluster_V_unclassified;.11
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Gut_group;Mixed_gut_cluster;Mixed_gut_cluster_unclassified;Mixed_gut_cluster_unclassified;.6
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Gut_group;Termite_cluster_III;Termite_cluster_III_unclassified;Termite_cluster_III_unclassified;*
Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;gut_cluster_c;gut_cluster_c_unclassified;gut_cluster_c_unclassified;
Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae_1;Flavobacterium_1;Flavobacterium_1_unclassified;Flavobacterium_1_unclassified;
Bacteroidetes;Sphingobacteria;Sphingobacteriales_2;Chitinophagaceae;Chitinophagaceae_unclassified;Chitinophagaceae_unclassified;Chitinophagaceae_unclassified;
Bacteroidetes;Sphingobacteria;Sphingobacteriales_2;Chitinophagaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;
Bacteroidetes;Sphingobacteria;Sphingobacteriales_2;Saprospiraceae;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;
Bacteroidetes;Sphingobacteria;Sphingobacteriales_3;Cytophagaceae_1;Flexibacter_1;Flexibacter_1_unclassified;Flexibacter_1_unclassified;
Bacteroidetes;Sphingobacteria;Sphingobacteriales_3;Cytophagaceae_1;Flexibacter_1;Flexibacter_1_unclassified;Flexibacter_1_unclassified;.1
Bacteroidetes;Sphingobacteria;Sphingobacteriales_3;Cytophagaceae_1;Flexibacter_1;Flexibacter_1_unclassified;Flexibacter_1_unclassified;.17

Bacteroidetes;Sphingobacteria;Sphingobacteriales_3;Cytophagaceae_1;Flexibacter_1;Flexibacter_1_unclassified;Flexibacter_1_unclassified;.2
 Bacteroidetes;Sphingobacteria;Sphingobacteriales_3;Cytophagaceae_1;Flexibacter_1;Flexibacter_1_unclassified;Flexibacter_1_unclassified;.9
 Candidate_phylum_WS3;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;.1
 Candidate_phylum_WS3;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;.1
 Candidate_phylum_WS3;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;.4
 Chlorobi;Chlorobia;Chlorobiales;SJA-28;SJA-28_unclassified;SJA-28_unclassified;SJA-28_unclassified;.4
 Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;Uncultured_11;Uncultured_11_unclassified;Uncultured_11_unclassified;.1
 Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;.1
 Chloroflexi;Caldilineae;Caldilineales;Caldilineaceae;Uncultured_5;Uncultured_5_unclassified;Uncultured_5_unclassified;.1
 Chloroflexi;Caldilineae;Caldilineales;Caldilineaceae;Uncultured_5;Uncultured_5_unclassified;Uncultured_5_unclassified;.1
 Chloroflexi;KD4-96;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;.4
 Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus_11;Bacillus_11_unclassified;Bacillus_11_unclassified;.1
 Firmicutes;Bacilli;Lactobacillales;Enterococcaceae;Enterococcus;Enterococcus_unclassified;Enterococcus_unclassified;*.1
 Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillaceae_unclassified;Lactobacillaceae_unclassified;Lactobacillaceae_unclassified;.1
 Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_1;Lactobacillus_1_unclassified;Lactobacillus_1_unclassified;.1
 Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_2;Lactobacillus_2_unclassified;Lactobacillus_2_unclassified;.1
 Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_sp_a;Lactobacillus_sp_a_unclassified;Lactobacillus_sp_a_unclassified;.1
 Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Streptococcaceae_unclassified;Streptococcaceae_unclassified;Streptococcaceae_unclassified;.1
 Firmicutes;Clostridia_1;Clostridiales;Clostridiaceae_1;Arthropod_cluster;Arthropod_cluster_unclassified;Arthropod_cluster_unclassified;.1
 Firmicutes;Clostridia_1;Clostridiales;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;.4
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Gut_cluster_1;Gut_cluster_1_unclassified;Gut_cluster_1_unclassified;.1
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Gut_cluster_13;Gut_cluster_13_unclassified;Gut_cluster_13_unclassified;.1
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Gut_cluster_13;Gut_cluster_13_unclassified;Gut_cluster_13_unclassified;.3
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Gut_cluster_13;Gut_cluster_13_unclassified;Gut_cluster_13_unclassified;.7
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Gut_cluster_13;Gut_cluster_13_unclassified;Gut_cluster_13_unclassified;.9
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Lachnospiraceae_unclassified;Lachnospiraceae_unclassified;Lachnospiraceae_unclassified;.24
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Lachnospiraceae_unclassified;Lachnospiraceae_unclassified;Lachnospiraceae_unclassified;.40
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Lachnospiraceae_unclassified;Lachnospiraceae_unclassified;Lachnospiraceae_unclassified;.1
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Robinsoniella_Insects;Robinsoniella_Insects_unclassified;Robinsoniella_Insects_unclassified;.2
 Firmicutes;Clostridia_1;Clostridiales;Peptostreptococcaceae;Peptostreptococcaceae_unclassified;Peptostreptococcaceae_unclassified;Peptostreptococcaceae_unclassified;.2

Firmicutes;Clostridia_1;Clostridiales;Peptostreptococcaceae;Peptostreptococcaceae_unclassified;Peptostreptococcaceae_unclassified;Peptostreptococcaceae_unclassified;1

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Incertae_Sedis_6;Incertae_Sedis_6_unclassified;Incertae_Sedis_6_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Insect_cluster;Insect_cluster_unclassified;Insect_cluster_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Insect_cluster;Insect_cluster_unclassified;Insect_cluster_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Ruminococcaceae_unclassified;Ruminococcaceae_unclassified;Ruminococcaceae_unclassified;48

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Ruminococcaceae_unclassified;Ruminococcaceae_unclassified;Ruminococcaceae_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Termite_cockroach_cluster;Termite_cockroach_cluster_unclassified;Termite_cockroach_cluster_unclassified;*

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Termite_cockroach_cluster;Termite_cockroach_cluster_unclassified;Termite_cockroach_cluster_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Uncultured_28;Uncultured_28_unclassified;Uncultured_28_unclassified;1

Firmicutes;Clostridia_2;Clostridiales_1;Veillonellaceae;Uncultured_8;Uncultured_8_unclassified;Uncultured_8_unclassified;

Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;Erysipelotrichaceae_unclassified;Erysipelotrichaceae_unclassified;Erysipelotrichaceae_unclassified;

Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;Incertae_Sedis_8;Incertae_Sedis_8_unclassified;Incertae_Sedis_8_unclassified;

Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;Uncultured_3;Uncultured_3_unclassified;Uncultured_3_unclassified;1

Firmicutes;Firmicutes_unclassified;Firmicutes_unclassified;Firmicutes_unclassified;Firmicutes_unclassified;Firmicutes_unclassified;Firmicutes_unclassified;10

Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;

Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;1

Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;3

Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;Uncultured_8;Uncultured_8_unclassified;Uncultured_8_unclassified;

Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;

Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;1

Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;2

Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;3

Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;4

Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;6

Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;7

Nitrospirae;Nitrospira;Nitrospirales;Nitrospiraceae;Nitrospira;Nitrospira_unclassified;Nitrospira_unclassified;

Nitrospirae;Nitrospira;Nitrospirales;Nitrospiraceae;Nitrospira;Nitrospira_unclassified;Nitrospira_unclassified;3

NPL-UPA2;NPL-UPA2_unclassified;NPL-UPA2_unclassified;NPL-UPA2_unclassified;NPL-UPA2_unclassified;NPL-UPA2_unclassified;NPL-UPA2_unclassified;

Planctomycetes;OM190;OM190_unclassified;OM190_unclassified;OM190_unclassified;OM190_unclassified;OM190_unclassified;3

Planctomycetes;OM190;OM190_unclassified;OM190_unclassified;OM190_unclassified;OM190_unclassified;OM190_unclassified;8

Planctomycetes;Phycisphaerae;WD2101_soil_group;WD2101_soil_group_unclassified;WD2101_soil_group_unclassified;WD2101_soil_group_unclassified;WD2101_soil_group_unclassified;

Planctomycetes;Pla4_lineage;Pla4_lineage_unclassified;Pla4_lineage_unclassified;Pla4_lineage_unclassified;Pla4_lineage_unclassified;Pla4_lineage_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Pir4_lineage;Pir4_lineage_unclassified;Pir4_lineage_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Planctomyces_1;Planctomyces_1_unclassified;Planctomyces_1_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Planctomyces_2;Planctomyces_2_unclassified;Planctomyces_2_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Planctomycetaceae_unclassified;Planctomycetaceae_unclassified;Planctomycetaceae_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Termite_cockroach_cluster_2;Termite_cockroach_cluster_2_unclassified;Termite_cockroach_cluster_2_unclassified;.2

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Uncultured_18;Uncultured_18_unclassified;Uncultured_18_unclassified;.1

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Uncultured_5;Uncultured_5_unclassified;Uncultured_5_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Uncultured_5;Uncultured_5_unclassified;Uncultured_5_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;

Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;.1

Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;.32

Proteobacteria;Alphaproteobacteria;Rhizobiales_1;Rhizobiaceae;Rhizobium-Agrobacterium;Rhizobium-Agrobacterium_unclassified;Rhizobium-Agrobacterium_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Bradyrhizobiaceae;Bradyrhizobium_12;Bradyrhizobium_12_unclassified;Bradyrhizobium_12_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Xanthobacteraceae;Labrys;Labrys_unclassified;Labrys_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Xanthobacteraceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Xanthobacteraceae;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Xanthobacteraceae;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Xanthobacteraceae;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;

assified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_3;Hyphomicrobiaceae;Pedomicrobium;Pedomicrobium_unclassified;Pedomicrobium_unclassified;
Proteobacteria;Alphaproteobacteria;Rhizobiales_3;Hyphomicrobiaceae;Pedomicrobium;Pedomicrobium_unclassified;Pedomicrobium_unclassified;. 1
Proteobacteria;Alphaproteobacteria;Rhizobiales;Methylocystaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;. 2
Proteobacteria;Alphaproteobacteria;Rhizobiales;Methylocystaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;. 4
Proteobacteria;Alphaproteobacteria;Rhodospirillales_1;Rhodospirillaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;JG37-AG-20;JG37-AG-20_unclassified;JG37-AG-20_unclassified;JG37-AG-20_unclassified;
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;JG37-AG-20;JG37-AG-20_unclassified;JG37-AG-20_unclassified;JG37-AG-20_unclassified;. 1
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;wr0007;wr0007_unclassified;wr0007_unclassified;wr0007_unclassified;
Proteobacteria;Betaproteobacteria;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;. 1
Proteobacteria;Betaproteobacteria;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;. 2
Proteobacteria;Betaproteobacteria;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;. 7
Proteobacteria;Betaproteobacteria;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;. 8
Proteobacteria;Betaproteobacteria;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;. 9
Proteobacteria;Betaproteobacteria;Burkholderiales;Alcaligenaceae_1;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;
Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Chitinimonas;Chitinimonas_unclassified;Chitinimonas_unclassified;
Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Comamonadaceae_unclassified;Comamonadaceae_unclassified;Comamonadaceae_unclassified;. 1
Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Comamonadaceae_unclassified;Comamonadaceae_unclassified;Comamonadaceae_unclassified;. 1
Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Comamonadaceae_unclassified;Comamonadaceae_unclassified;Comamonadaceae_unclassified;. 1
Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Rhodoferax;Rhodoferax_unclassified;Rhodoferax_unclassified;*
Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Uncultured_23;Uncultured_23_unclassified;Uncultured_23_unclassified;
Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;
Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;. 2
Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;. 3
Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;. 4

Proteobacteria;Deltaproteobacteria;Myxococcales;Myxococcales_unclassified;Myxococcales_unclassified;Myxococcales_unclassified;Myxococcales_unclassified;
 Proteobacteria;Deltaproteobacteria;Myxococcales;Myxococcales_unclassified;Myxococcales_unclassified;Myxococcales_unclassified;Myxococcales_unclassified;
 Proteobacteria;Deltaproteobacteria;Myxococcales;Myxococcales_unclassified;Myxococcales_unclassified;Myxococcales_unclassified;Myxococcales_unclassified;
 Proteobacteria;Deltaproteobacteria;Myxococcales;Phaselicystidaceae;Phaselicystis;Phaselicystis_unclassified;Phaselicystis_unclassified;
 Proteobacteria;Gammaproteobacteria_1;Pseudomonadales;Moraxellaceae;Acinetobacter;Acinetobacter_unclassified;Acinetobacter_unclassified;.1
 Proteobacteria;Gammaproteobacteria_2;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;
 Proteobacteria;Gammaproteobacteria_2;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;.3
 Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Sinobacteraceae_unclassified;Sinobacteraceae_unclassified;Sinobacteraceae_unclassified;.1
 Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;
 Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;.1
 Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;.2
 Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;.3
 Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;.5
 Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;
 Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_8;Uncultured_8_unclassified;Uncultured_8_unclassified;
 Spirochaetes;Spirochaetes;Spirochaetales;Spirochaetaceae_Treponema_I;Spirochaetaceae_Treponema_I_unclassified;Spirochaetaceae_Treponema_I_unclassified;Spirochaetaceae_Treponema_I_unclassified;
 Synergistetes;Synergistia;Synergistales;Synergistaceae;Candidatus_Tammella;Candidatus_Tammella_unclassified;Candidatus_Tammella_unclassified;.1
 Synergistetes;Synergistia;Synergistales;Synergistaceae;Synergistaceae_unclassified;Synergistaceae_unclassified;Synergistaceae_unclassified;
 Synergistetes;Synergistia;Synergistales;Synergistaceae;Termite_cockroach_cluster;Termite_cockroach_cluster_unclassified;Termite_cockroach_cluster_unclassified;
 Verrucomicrobia;OPB35_soil_group;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;
 Verrucomicrobia;OPB35_soil_group;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;.1
 Verrucomicrobia;OPB35_soil_group;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;.13
 Verrucomicrobia;OPB35_soil_group;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;.3

Verrucomicrobia;OPB35_soil_group;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;6

Verrucomicrobia;Opitutae;Opitales;Opitutaceae;Opitutus;Opitutus_unclassified;Opitutus_unclassified;.2

Verrucomicrobia;Opitutae;Opitales;Opitutaceae;Opitutus;Opitutus_unclassified;Opitutus_unclassified;.3

Verrucomicrobia;Opitutae;Opitales;Opitutaceae;Opitutus;Opitutus_unclassified;Opitutus_unclassified;.6

Verrucomicrobia;S-BQ2-57_soil_group;S-BQ2-57_soil_group_unclassified;S-BQ2-57_soil_group_unclassified;S-BQ2-57_soil_group_unclassified;S-BQ2-57_soil_group_unclassified;S-BQ2-57_soil_group_unclassified;

Verrucomicrobia;Spartobacteria;Chthoniobacter;Chthoniobacter_unclassified;Chthoniobacter_unclassified;Chthoniobacter_unclassified;Chthoniobacter_unclassified;

Verrucomicrobia;Spartobacteria;Chthoniobacter;Chthoniobacter_unclassified;Chthoniobacter_unclassified;Chthoniobacter_unclassified;Chthoniobacter_unclassified;.1

Verrucomicrobia;Spartobacteria;Chthoniobacterales;Xiphinematobacteraceae;Candidatus_Xiphinematobacter;Candidatus_Xiphinematobacter_unclassified;Candidatus_Xiphinematobacter_unclassified;

Verrucomicrobia;Spartobacteria;DA101_soil_group;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;

Verrucomicrobia;Spartobacteria;DA101_soil_group;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;.1

Verrucomicrobia;Spartobacteria;DA101_soil_group;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;.2

*OTUS found in all insect gut microbiota

Supplemental Table 4.7. Core OTUs found in the gut microbiota of all *H. venosa*

Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Acidobacteriaceae_unclassified;Acidobacteriaceae_unclassified;Acidobacteriaceae_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Candidatus_Solibacter;Candidatus_Solibacter_unclassified;Candidatus_Solibacter_unclassified;.6
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.1
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_11;Uncultured_11_unclassified;Uncultured_11_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_18;Uncultured_18_unclassified;Uncultured_18_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_18;Uncultured_18_unclassified;Uncultured_18_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_22;Uncultured_22_unclassified;Uncultured_22_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_26;Uncultured_26_unclassified;Uncultured_26_unclassified;.2
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_27;Uncultured_27_unclassified;Uncultured_27_unclassified;.1
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;.1
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;.10
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;.11
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;.14
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;.7
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;.8
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_31;Uncultured_31_unclassified;Uncultured_31_unclassified;.9
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_9;Uncultured_9_unclassified;Uncultured_9_unclassified;
Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Uncultured_9;Uncultured_9_unclassified;Uncultured_9_unclassified;.2
Acidobacteria;Holophagae;Cluster_32-20;Cluster_32-20_unclassified;Cluster_32-20_unclassified;Cluster_32-20_unclassified;Cluster_32-20_unclassified;
Acidobacteria;RB25;RB25_unclassified;RB25_unclassified;RB25_unclassified;RB25_unclassified;RB25_unclassified;
Acidobacteria;RB25;RB25_unclassified;RB25_unclassified;RB25_unclassified;RB25_unclassified;RB25_unclassified;.1
Actinobacteria;Actinobacteria;Acidimicrobiales;Acidimicrobiales_unclassified;Acidimicrobiales_unclassified;Acidimicrobiales_unclassified;Acidimicrobiales_unclassified;
Actinobacteria;Actinobacteria;Acidimicrobiales;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;Uncultured_6_unclassified;
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;.1
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;.12
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;.13

Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;.2
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;.3
Actinobacteria;Actinobacteria;AKIW543;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;AKIW543_unclassified;.4
Actinobacteria;Actinobacteria;Coriobacteriales;Coriobacteriaceae;Uncultured_10;Uncultured_10_unclassified;Uncultured_10_unclassified;
Actinobacteria;Actinobacteria;Coriobacteriales;Coriobacteriaceae;Uncultured_10;Uncultured_10_unclassified;Uncultured_10_unclassified;.1
Actinobacteria;Actinobacteria;Coriobacteriales;Coriobacteriaceae;Uncultured_10;Uncultured_10_unclassified;Uncultured_10_unclassified;
Actinobacteria;Actinobacteria;Corynebacteriales;Corynebacteriaceae;Corynebacterium_1;Corynebacterium_1_unclassified;Corynebacterium_1_unclassified;
Actinobacteria;Actinobacteria;Corynebacteriales;Mycobacteriaceae;Mycobacterium;Mycobacterium_unclassified;Mycobacterium_unclassified;
Actinobacteria;Actinobacteria;Corynebacteriales;Nocardiaceae_1;Gordonia;Gordonia_unclassified;Gordonia_unclassified;
Actinobacteria;Actinobacteria;Frankiales;Nakamurellaceae;Nakamurella;Nakamurella_unclassified;Nakamurella_unclassified;
Actinobacteria;Actinobacteria;MB-A2-108;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;.2
Actinobacteria;Actinobacteria;MB-A2-108;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;.3
Bacteroidetes;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;.61
Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;Bacteroides_unclassified;Bacteroides_unclassified;.14*
Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;Bacteroides_unclassified;Bacteroides_unclassified;.2
Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;Bacteroides_unclassified;Bacteroides_unclassified;.5
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_1;Dysgonomonas;Dysgonomonas_unclassified;Dysgonomonas_unclassified;
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_3;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;Porphyromonadaceae_3_unclassified;.3
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Cluster_V;Porphyromonadaceae_Cluster_V_unclassified;Porphyromonadaceae_Cluster_V_unclassified;Porphyromonadaceae_Cluster_V_unclassified;.11
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Gut_group;Mixed_gut_cluster;Mixed_gut_cluster_unclassified;Mixed_gut_cluster_unclassified;.6
Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae_Gut_group;Termite_cluster_III;Termite_cluster_III_unclassified;Termite_cluster_III_unclassified;.6*
Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;gut_cluster_c;gut_cluster_c_unclassified;gut_cluster_c_unclassified;
Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae_1;Flavobacterium_1;Flavobacterium_1_unclassified;Flavobacterium_1_unclassified;
Bacteroidetes;Sphingobacteria;Sphingobacteriales_2;Chitinophagaceae;Chitinophagaceae_unclassified;Chitinophagaceae_unclassified;Chitinophagaceae_unclassified;.3
Bacteroidetes;Sphingobacteria;Sphingobacteriales_2;Chitinophagaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;
Bacteroidetes;Sphingobacteria;Sphingobacteriales_2;Saprospiraceae;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;
Bacteroidetes;Sphingobacteria;Sphingobacteriales_3;Cytophagaceae_1;Flexibacter_1;Flexibacter_1_unclassified;Flexibacter_1_unclassified;
Bacteroidetes;Sphingobacteria;Sphingobacteriales_3;Cytophagaceae_1;Flexibacter_1;Flexibacter_1_unclassified;Flexibacter_1_unclassified;.1
Bacteroidetes;Sphingobacteria;Sphingobacteriales_3;Cytophagaceae_1;Flexibacter_1;Flexibacter_1_unclassified;Flexibacter_1_unclassified;.17

Bacteroidetes;Sphingobacteria;Sphingobacteriales_3;Cytophagaceae_1;Flexibacter_1;Flexibacter_1_unclassified;Flexibacter_1_unclassified;.2
 Bacteroidetes;Sphingobacteria;Sphingobacteriales_3;Cytophagaceae_1;Flexibacter_1;Flexibacter_1_unclassified;Flexibacter_1_unclassified;.9
 Candidate_phylum_WS3;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;.1
 Candidate_phylum_WS3;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;.1
 Candidate_phylum_WS3;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;Candidate_phylum_WS3_unclassified;.4
 Chlorobi;Chlorobia;Chlorobiales;SJA-28;SJA-28_unclassified;SJA-28_unclassified;SJA-28_unclassified;.4
 Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;Uncultured_11;Uncultured_11_unclassified;Uncultured_11_unclassified;.1
 Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;.1
 Chloroflexi;Caldilineae;Caldilineales;Caldilineaceae;Uncultured_5;Uncultured_5_unclassified;Uncultured_5_unclassified;.1
 Chloroflexi;Caldilineae;Caldilineales;Caldilineaceae;Uncultured_5;Uncultured_5_unclassified;Uncultured_5_unclassified;.1
 Chloroflexi;KD4-96;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;.4
 Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus_11;Bacillus_11_unclassified;Bacillus_11_unclassified;.1
 Firmicutes;Bacilli;Lactobacillales;Enterococcaceae;Enterococcus;Enterococcus_unclassified;Enterococcus_unclassified;*.
 Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillaceae_unclassified;Lactobacillaceae_unclassified;Lactobacillaceae_unclassified;.1
 Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_1;Lactobacillus_1_unclassified;Lactobacillus_1_unclassified;.1
 Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_2;Lactobacillus_2_unclassified;Lactobacillus_2_unclassified;.1
 Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_sp_a;Lactobacillus_sp_a_unclassified;Lactobacillus_sp_a_unclassified;.1
 Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Streptococcaceae_unclassified;Streptococcaceae_unclassified;Streptococcaceae_unclassified;.1
 Firmicutes;Clostridia_1;Clostridiales;Clostridiaceae_1;Arthropod_cluster;Arthropod_cluster_unclassified;Arthropod_cluster_unclassified;.1
 Firmicutes;Clostridia_1;Clostridiales;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;.4
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Gut_cluster_1;Gut_cluster_1_unclassified;Gut_cluster_1_unclassified;.1
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Gut_cluster_13;Gut_cluster_13_unclassified;Gut_cluster_13_unclassified;.1
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Gut_cluster_13;Gut_cluster_13_unclassified;Gut_cluster_13_unclassified;.3
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Gut_cluster_13;Gut_cluster_13_unclassified;Gut_cluster_13_unclassified;.7
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Gut_cluster_13;Gut_cluster_13_unclassified;Gut_cluster_13_unclassified;.9
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Lachnospiraceae_unclassified;Lachnospiraceae_unclassified;Lachnospiraceae_unclassified;.24
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Lachnospiraceae_unclassified;Lachnospiraceae_unclassified;Lachnospiraceae_unclassified;.40
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Lachnospiraceae_unclassified;Lachnospiraceae_unclassified;Lachnospiraceae_unclassified;.1
 Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Robinsoniella_Insects;Robinsoniella_Insects_unclassified;Robinsoniella_Insects_unclassified;.2
 Firmicutes;Clostridia_1;Clostridiales;Peptostreptococcaceae;Peptostreptococcaceae_unclassified;Peptostreptococcaceae_unclassified;Peptostreptococcaceae_unclassified;.2

Firmicutes;Clostridia_1;Clostridiales;Peptostreptococcaceae;Peptostreptococcaceae_unclassified;Peptostreptococcaceae_unclassified;Peptostreptococcaceae_unclassified;1

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Incertae_Sedis_6;Incertae_Sedis_6_unclassified;Incertae_Sedis_6_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Insect_cluster;Insect_cluster_unclassified;Insect_cluster_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Insect_cluster;Insect_cluster_unclassified;Insect_cluster_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Ruminococcaceae_unclassified;Ruminococcaceae_unclassified;Ruminococcaceae_unclassified;48

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Ruminococcaceae_unclassified;Ruminococcaceae_unclassified;Ruminococcaceae_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Termite_cockroach_cluster;Termite_cockroach_cluster_unclassified;Termite_cockroach_cluster_unclassified;*

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Termite_cockroach_cluster;Termite_cockroach_cluster_unclassified;Termite_cockroach_cluster_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Uncultured_28;Uncultured_28_unclassified;Uncultured_28_unclassified;1

Firmicutes;Clostridia_2;Clostridiales_1;Veillonellaceae;Uncultured_8;Uncultured_8_unclassified;Uncultured_8_unclassified;

Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;Erysipelotrichaceae_unclassified;Erysipelotrichaceae_unclassified;Erysipelotrichaceae_unclassified;

Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;Incertae_Sedis_8;Incertae_Sedis_8_unclassified;Incertae_Sedis_8_unclassified;

Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;Uncultured_3;Uncultured_3_unclassified;Uncultured_3_unclassified;1

Firmicutes;Firmicutes_unclassified;Firmicutes_unclassified;Firmicutes_unclassified;Firmicutes_unclassified;Firmicutes_unclassified;Firmicutes_unclassified;10

Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;

Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;1

Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;3

Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;Uncultured_8;Uncultured_8_unclassified;Uncultured_8_unclassified;

Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;

Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;1

Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;2

Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;3

Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;4

Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;6

Nitrospirae;Nitrospira;Nitrospirales;Cluster_319-6A21;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;Cluster_319-6A21_unclassified;7

Nitrospirae;Nitrospira;Nitrospirales;Nitrospiraceae;Nitrospira;Nitrospira_unclassified;Nitrospira_unclassified;

Nitrospirae;Nitrospira;Nitrospirales;Nitrospiraceae;Nitrospira;Nitrospira_unclassified;Nitrospira_unclassified;3

NPL-UPA2;NPL-UPA2_unclassified;NPL-UPA2_unclassified;NPL-UPA2_unclassified;NPL-UPA2_unclassified;NPL-UPA2_unclassified;NPL-UPA2_unclassified;

Planctomycetes;OM190;OM190_unclassified;OM190_unclassified;OM190_unclassified;OM190_unclassified;OM190_unclassified;3

Planctomycetes;OM190;OM190_unclassified;OM190_unclassified;OM190_unclassified;OM190_unclassified;OM190_unclassified;8

Planctomycetes;Phycisphaerae;WD2101_soil_group;WD2101_soil_group_unclassified;WD2101_soil_group_unclassified;WD2101_soil_group_unclassified;WD2101_soil_group_unclassified;

Planctomycetes;Pla4_lineage;Pla4_lineage_unclassified;Pla4_lineage_unclassified;Pla4_lineage_unclassified;Pla4_lineage_unclassified;Pla4_lineage_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Pir4_lineage;Pir4_lineage_unclassified;Pir4_lineage_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Planctomyces_1;Planctomyces_1_unclassified;Planctomyces_1_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Planctomyces_2;Planctomyces_2_unclassified;Planctomyces_2_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Planctomycetaceae_unclassified;Planctomycetaceae_unclassified;Planctomycetaceae_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Termite_cockroach_cluster_2;Termite_cockroach_cluster_2_unclassified;Termite_cockroach_cluster_2_unclassified;.2

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Uncultured_18;Uncultured_18_unclassified;Uncultured_18_unclassified;.1

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Uncultured_5;Uncultured_5_unclassified;Uncultured_5_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Uncultured_5;Uncultured_5_unclassified;Uncultured_5_unclassified;

Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;

Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;.1

Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;.32

Proteobacteria;Alphaproteobacteria;Rhizobiales_1;Rhizobiaceae;Rhizobium-Agrobacterium;Rhizobium-Agrobacterium_unclassified;Rhizobium-Agrobacterium_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Bradyrhizobiaceae;Bradyrhizobium_12;Bradyrhizobium_12_unclassified;Bradyrhizobium_12_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;Rhizobiales_2_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Xanthobacteraceae;Labrys;Labrys_unclassified;Labrys_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Xanthobacteraceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Xanthobacteraceae;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Xanthobacteraceae;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Xanthobacteraceae;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;Xanthobacteraceae_unclassified;

assified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_3;Hyphomicrobiaceae;Pedomicrobium;Pedomicrobium_unclassified;Pedomicrobium_unclassified;
Proteobacteria;Alphaproteobacteria;Rhizobiales_3;Hyphomicrobiaceae;Pedomicrobium;Pedomicrobium_unclassified;Pedomicrobium_unclassified;.1
Proteobacteria;Alphaproteobacteria;Rhizobiales;Methylocystaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.2
Proteobacteria;Alphaproteobacteria;Rhizobiales;Methylocystaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.4
Proteobacteria;Alphaproteobacteria;Rhodospirillales_1;Rhodospirillaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;JG37-AG-20;JG37-AG-20_unclassified;JG37-AG-20_unclassified;JG37-AG-20_unclassified;
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;JG37-AG-20;JG37-AG-20_unclassified;JG37-AG-20_unclassified;JG37-AG-20_unclassified;.1
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;wr0007;wr0007_unclassified;wr0007_unclassified;wr0007_unclassified;
Proteobacteria;Betaproteobacteria;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;.1
Proteobacteria;Betaproteobacteria;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;.2
Proteobacteria;Betaproteobacteria;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;.7
Proteobacteria;Betaproteobacteria;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;.8
Proteobacteria;Betaproteobacteria;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;Betaproteobacteria_unclassified;.9
Proteobacteria;Betaproteobacteria;Burkholderiales;Alcaligenaceae_1;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;
Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Chitinimonas;Chitinimonas_unclassified;Chitinimonas_unclassified;
Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Comamonadaceae_unclassified;Comamonadaceae_unclassified;Comamonadaceae_unclassified;.1
Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Comamonadaceae_unclassified;Comamonadaceae_unclassified;Comamonadaceae_unclassified;.1
Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Comamonadaceae_unclassified;Comamonadaceae_unclassified;Comamonadaceae_unclassified;.1
Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Comamonadaceae_unclassified;Comamonadaceae_unclassified;Comamonadaceae_unclassified;.1
Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Rhodoferax;Rhodoferax_unclassified;Rhodoferax_unclassified;*
Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Uncultured_23;Uncultured_23_unclassified;Uncultured_23_unclassified;
Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;
Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.2
Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.3
Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.4

Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.6
 Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.8
 Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.8
 Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.8
 Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;.1
 Proteobacteria;Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;.1
 Proteobacteria;Betaproteobacteria;SC-I-84;SC-I-84_unclassified;SC-I-84_unclassified;SC-I-84_unclassified;SC-I-84_unclassified;.1
 Proteobacteria;Betaproteobacteria;SC-I-84;SC-I-84_unclassified;SC-I-84_unclassified;SC-I-84_unclassified;SC-I-84_unclassified;.2
 Proteobacteria;Betaproteobacteria;SC-I-84;SC-I-84_unclassified;SC-I-84_unclassified;SC-I-84_unclassified;SC-I-84_unclassified;.7
 Proteobacteria;Betaproteobacteria;TRA3-20;TRA3-20_unclassified;TRA3-20_unclassified;TRA3-20_unclassified;TRA3-20_unclassified;.7
 Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobacteraceae;Desulfatiferula;Desulfatiferula_unclassified;Desulfatiferula_unclassified;.1
 Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobacteraceae;Desulfobacteraceae_unclassified;Desulfobacteraceae_unclassified;Desulfobacteraceae_unclassified;.1*
 Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobulbaceae;Desulfobulbus;Desulfobulbus_unclassified;Desulfobulbus_unclassified;.1
 Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.1
 Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.10
 Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.11
 Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.16
 Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.38
 Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.6
 Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.65
 Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.66
 Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.70
 Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;.8
 Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Desulfovibrionaceae_unclassified;Desulfovibrionaceae_unclassified;Desulfovibrionaceae_unclassified;.1
 Proteobacteria;Deltaproteobacteria;Desulfurellales;Desulfurellaceae;Uncultured;Uncultured_unclassified;Uncultured_unclassified;.1
 Proteobacteria;Deltaproteobacteria;GR-WP33-30;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;Uncultured_1_unclassified;.1
 Proteobacteria;Deltaproteobacteria;GR-WP33-30;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;Uncultured_2_unclassified;.1
 Proteobacteria;Deltaproteobacteria;Myxococcales;Cystobacteraceae;Anaeromyxobacter;Anaeromyxobacter_unclassified;Anaeromyxobacter_unclassified;.1
 Proteobacteria;Deltaproteobacteria;Myxococcales;JG37-AG-15;JG37-AG-15_unclassified;JG37-AG-15_unclassified;JG37-AG-15_unclassified;.1

Proteobacteria;Deltaproteobacteria;Myxococcales;Myxococcales_unclassified;Myxococcales_unclassified;Myxococcales_unclassified;Myxococcales_unclassified;
Proteobacteria;Deltaproteobacteria;Myxococcales;Myxococcales_unclassified;Myxococcales_unclassified;Myxococcales_unclassified;Myxococcales_unclassified;
Proteobacteria;Deltaproteobacteria;Myxococcales;Myxococcales_unclassified;Myxococcales_unclassified;Myxococcales_unclassified;Myxococcales_unclassified;
Proteobacteria;Deltaproteobacteria;Myxococcales;Phaselicystidaceae;Phaselicystis;Phaselicystis_unclassified;Phaselicystis_unclassified;
Proteobacteria;Gammaproteobacteria_1;Pseudomonadales;Moraxellaceae;Acinetobacter;Acinetobacter_unclassified;Acinetobacter_unclassified;.1
Proteobacteria;Gammaproteobacteria_2;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;
Proteobacteria;Gammaproteobacteria_2;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;
Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Sinobacteraceae_unclassified;Sinobacteraceae_unclassified;Sinobacteraceae_unclassified;.1
Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;
Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;.1
Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;.2
Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;.3
Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;.5
Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_6;Uncultured_6_unclassified;Uncultured_6_unclassified;
Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Uncultured_8;Uncultured_8_unclassified;Uncultured_8_unclassified;
Spirochaetes;Spirochaetes;Spirochaetales;Spirochaetaceae_Treponema_I;Spirochaetaceae_Treponema_I_unclassified;Spirochaetaceae_Treponema_I_unclassified;
Synergistetes;Synergistia;Synergistales;Synergistaceae;Candidatus_Tammella;Candidatus_Tammella_unclassified;Candidatus_Tammella_unclassified;.1
Synergistetes;Synergistia;Synergistales;Synergistaceae;Synergistaceae_unclassified;Synergistaceae_unclassified;Synergistaceae_unclassified;
Synergistetes;Synergistia;Synergistales;Synergistaceae;Termite_cockroach_cluster;Termite_cockroach_cluster_unclassified;Termite_cockroach_cluster_unclassified;
Verrucomicrobia;OPB35_soil_group;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;
Verrucomicrobia;OPB35_soil_group;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;.1
Verrucomicrobia;OPB35_soil_group;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;.13
Verrucomicrobia;OPB35_soil_group;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;.3

Verrucomicrobia;OPB35_soil_group;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;6

Verrucomicrobia;Opitutae;Opitales;Opitutaceae;Opitutus;Opitutus_unclassified;Opitutus_unclassified;2

Verrucomicrobia;Opitutae;Opitales;Opitutaceae;Opitutus;Opitutus_unclassified;Opitutus_unclassified;3

Verrucomicrobia;Opitutae;Opitales;Opitutaceae;Opitutus;Opitutus_unclassified;Opitutus_unclassified;6

Verrucomicrobia;S-BQ2-57_soil_group;S-BQ2-57_soil_group_unclassified;S-BQ2-57_soil_group_unclassified;S-BQ2-57_soil_group_unclassified;S-BQ2-57_soil_group_unclassified;S-BQ2-57_soil_group_unclassified;

Verrucomicrobia;Spartobacteria;Chthoniobacter;Chthoniobacter_unclassified;Chthoniobacter_unclassified;Chthoniobacter_unclassified;Chthoniobacter_unclassified;

Verrucomicrobia;Spartobacteria;Chthoniobacter;Chthoniobacter_unclassified;Chthoniobacter_unclassified;Chthoniobacter_unclassified;Chthoniobacter_unclassified;.1

Verrucomicrobia;Spartobacteria;Chthoniobacterales;Xiphinematobacteraceae;Candidatus_Xiphinematobacter;Candidatus_Xiphinematobacter_unclassified;Candidatus_Xiphinematobacter_unclassified;

Verrucomicrobia;Spartobacteria;DA101_soil_group;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;

Verrucomicrobia;Spartobacteria;DA101_soil_group;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;.1

Verrucomicrobia;Spartobacteria;DA101_soil_group;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;DA101_soil_group_unclassified;.2

*OTUS found in all insect gut microbiota

Actinobacteria;Actinobacteria;Coriobacteriales;Coriobacteriaceae;Uncultured_10;Uncultured_10_unclassified;Uncultured_10_unclassified;
Actinobacteria;Actinobacteria;Corynebacteriales;Corynebacteriaceae;Corynebacterium_1;Corynebacterium_1_unclassified;Corynebacterium_1_unclassified;
Actinobacteria;Actinobacteria;Corynebacteriales;Corynebacteriales_unclassified;Corynebacteriales_unclassified;Corynebacteriales_unclassified;Corynebacteriales_unclassified;
Actinobacteria;Actinobacteria;Corynebacteriales;Mycobacteriaceae;Mycobacterium;Mycobacterium_unclassified;Mycobacterium_unclassified;
Actinobacteria;Actinobacteria;Corynebacteriales;Mycobacteriaceae;Mycobacterium;Mycobacterium_unclassified;Mycobacterium_unclassified;.1
Actinobacteria;Actinobacteria;Corynebacteriales;Nocardiaceae_1;Gordonia;Gordonia_unclassified;Gordonia_unclassified;
Actinobacteria;Actinobacteria;Corynebacteriales;Nocardiaceae;Rhodococcus_1;Rhodococcus_1_unclassified;Rhodococcus_1_unclassified;
Actinobacteria;Actinobacteria;Frankiales;Acidothermaceae;Acidothermus;Acidothermus_unclassified;Acidothermus_unclassified;.1
Actinobacteria;Actinobacteria;Frankiales;Nakamurellaceae;Nakamurella;Nakamurella_unclassified;Nakamurella_unclassified;
Actinobacteria;Actinobacteria;Frankiales;Nakamurellaceae;Humicoccus;Humicoccus_unclassified;Humicoccus_unclassified;
Actinobacteria;Actinobacteria;MB-A2-108;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;
Actinobacteria;Actinobacteria;MB-A2-108;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;.1
Actinobacteria;Actinobacteria;MB-A2-108;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;.14
Actinobacteria;Actinobacteria;MB-A2-108;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;.2
Actinobacteria;Actinobacteria;MB-A2-108;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;.3
Actinobacteria;Actinobacteria;MB-A2-108;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;.4
Actinobacteria;Actinobacteria;MB-A2-108;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;.5
Actinobacteria;Actinobacteria;MB-A2-108;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;MB-A2-108_unclassified;.8
Actinobacteria;Actinobacteria;Micrococcales_1;Brevibacteriaceae;Brevibacterium;Brevibacterium_unclassified;Brevibacterium_unclassified;
Actinobacteria;Actinobacteria;Micrococcales_1;Micrococcaceae;Arthrobacter_14;Arthrobacter_14_unclassified;Arthrobacter_14_unclassified;
Actinobacteria;Actinobacteria;Micrococcales_3;Cellulomonadaceae;Cellulomonadaceae_unclassified;Cellulomonadaceae_unclassified;Cellulomonadaceae_unclassified;
Actinobacteria;Actinobacteria;Micrococcales_3;Microbacteriaceae;Leucobacter;Leucobacter_unclassified;Leucobacter_unclassified;
Actinobacteria;Actinobacteria;Micrococcales_3;Microbacteriaceae;Leucobacter;Leucobacter_unclassified;Leucobacter_unclassified;
Actinobacteria;Actinobacteria;Micrococcales_3;Microbacteriaceae;Microbacterium;Microbacterium_unclassified;Microbacterium_unclassified;
Actinobacteria;Actinobacteria;Micrococcales_4;Intrasporangiaceae_1;Intrasporangiaceae_1_unclassified;Intrasporangiaceae_1_unclassified;Intrasporangiaceae_1_unclassified;
Actinobacteria;Actinobacteria;Micromonosporales;Micromonosporaceae;Micromonosporaceae_unclassified;Micromonosporaceae_unclassified;Micromonosporaceae_unclassified;
Actinobacteria;Actinobacteria;Micromonosporales;Micromonosporaceae;Micromonosporaceae_unclassified;Micromonosporaceae_unclassified;Micromonosporaceae_unclassified;

Actinobacteria;Actinobacteria;Micromonosporales;Micromonosporaceae;Micromonosporaceae_unclassified;Micromonosporaceae_unclassified;Micromonosporaceae_unclassified;

Actinobacteria;Actinobacteria;PeM15;PeM15_unclassified;PeM15_unclassified;PeM15_unclassified;PeM15_unclassified;

Actinobacteria;Actinobacteria;Propionibacteriales;Nocardioideae;Marmoricola;Marmoricola_unclassified;Marmoricola_unclassified;

Actinobacteria;Actinobacteria;Propionibacteriales;Nocardioideae;Nocardioides;Nocardioides_unclassified;Nocardioides_unclassified;

Actinobacteria;Actinobacteria;Propionibacteriales;Nocardioideae;Nocardioides;Nocardioides_unclassified;Nocardioides_unclassified;.8

Actinobacteria;Actinobacteria;Pseudonocardiales_2;Pseudonocardiaceae_2;Pseudonocardia;Pseudonocardia_unclassified;Pseudonocardia_unclassified;.1

Actinobacteria;Actinobacteria;Pseudonocardiales_2;Pseudonocardiaceae_2;Pseudonocardia;Pseudonocardia_unclassified;Pseudonocardia_unclassified;.2

Actinobacteria;Actinobacteria;Pseudonocardiales_2;Pseudonocardiaceae_2;Pseudonocardia;Pseudonocardia_unclassified;Pseudonocardia_unclassified;.3

Actinobacteria;Actinobacteria;Solirubrobacterales;Cluster_319-6M6;Cluster_319-6M6_unclassified;Cluster_319-6M6_unclassified;Cluster_319-6M6_unclassified;.1

Actinobacteria;Actinobacteria;Solirubrobacterales;Cluster_480-2;Cluster_480-2_unclassified;Cluster_480-2_unclassified;Cluster_480-2_unclassified;

Actinobacteria;Actinobacteria;Solirubrobacterales;Cluster_480-2;Cluster_480-2_unclassified;Cluster_480-2_unclassified;Cluster_480-2_unclassified;.1

Actinobacteria;Actinobacteria;Solirubrobacterales;Patulibacteraceae;Patulibacter;Patulibacter_unclassified;Patulibacter_unclassified;

Actinobacteria;Actinobacteria;Solirubrobacterales;Patulibacteraceae;Patulibacter;Patulibacter_unclassified;Patulibacter_unclassified;.2

Actinobacteria;Actinobacteria;Solirubrobacterales;Solirubrobacteriaceae;Solirubrobacter;Solirubrobacter_unclassified;Solirubrobacter_unclassified;

Actinobacteria;Actinobacteria;Solirubrobacterales;TM146;TM146_unclassified;TM146_unclassified;TM146_unclassified;

Armatimonadetes;Armatimonadetes_unclassified;Armatimonadetes_unclassified;Armatimonadetes_unclassified;Armatimonadetes_unclassified;Armatimonadetes_unclassified;.29

Bacteroidetes;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;

Bacteroidetes;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;.3

Bacteroidetes;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;.5

Bacteroidetes;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;.53

Bacteroidetes;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;.57

Bacteroidetes;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;.61

Bacteroidetes;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;Bacteroidetes_unclassified;.71

Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Alistipes_III;Alistipes_III_unclassified;Alistipes_III_unclassified;
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Alistipes_III;Alistipes_III_unclassified;Alistipes_III_unclassified;.21
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;gut_cluster_c;gut_cluster_c_unclassified;gut_cluster_c_unclassified;
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;RC9_gut_group;RC9_gut_group_unclassified;RC9_gut_group_unclassified;.1
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;RC9_gut_group;RC9_gut_group_unclassified;RC9_gut_group_unclassified;.3
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;RC9_gut_group;RC9_gut_group_unclassified;RC9_gut_group_unclassified;.9
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Rikenellaceae_unclassified;Rikenellaceae_unclassified;Rikenellaceae_unclassified;
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Rikenellaceae_unclassified;Rikenellaceae_unclassified;Rikenellaceae_unclassified;.125
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Rikenellaceae_unclassified;Rikenellaceae_unclassified;Rikenellaceae_unclassified;.27
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Rikenellaceae_unclassified;Rikenellaceae_unclassified;Rikenellaceae_unclassified;
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Rikenella_1;Rikenella_1_unclassified;Rikenella_1_unclassified;
 Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Rikenellaceae_unclassified;Rikenellaceae_unclassified;Rikenellaceae_unclassified;
 Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae_1;Flavobacterium_1;Flavobacterium_1_unclassified;Flavobacterium_1_unclassified;
 Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae_1;Flavobacterium_1;Flavobacterium_1_unclassified;Flavobacterium_1_unclassified;.2
 Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae_1;Flavobacterium_1;Flavobacterium_1_unclassified;Flavobacterium_1_unclassified;.3
 Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae_1;Myroides;Myroides_unclassified;Myroides_unclassified;
 Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae_1;Myroides;Myroides_unclassified;Myroides_unclassified;.1
 Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae_1;Myroides;Myroides_unclassified;Myroides_unclassified;.2
 Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriales_unclassified;Flavobacteriales_unclassified;Flavobacteriales_unclassified;Flavobacteriales_unclassified;
 Bacteroidetes;Flavobacteria;Flavobacteriales;NS9_marine_group;NS9_marine_group_unclassified;NS9_marine_group_unclassified;NS9_marine_group_unclassified;
 Bacteroidetes;Sphingobacteria;Sphingobacteria_unclassified;Sphingobacteria_unclassified;Sphingobacteria_unclassified;Sphingobacteria_unclassified;Sphingobacteria_unclassified;
 Bacteroidetes;Sphingobacteria;Sphingobacteria_unclassified;Sphingobacteria_unclassified;Sphingobacteria_unclassified;Sphingobacteria_unclassified;Sphingobacteria_unclassified;.1
 Bacteroidetes;Sphingobacteria;Sphingobacteria_unclassified;Sphingobacteria_unclassified;Sphingobacteria_unclassified;Sphingobacteria_unclassified;Sphingobacteria_unclassified;.2
 Bacteroidetes;Sphingobacteria;Sphingobacteriales_1;env_OPS_17;env_OPS_17_unclassified;env_OPS_17_unclassified;env_OPS_17_unclassified;.1
 Bacteroidetes;Sphingobacteria;Sphingobacteriales_1;env_OPS_17;env_OPS_17_unclassified;env_OPS_17_unclassified;env_OPS_17_unclassified;.10
 Bacteroidetes;Sphingobacteria;Sphingobacteriales_1;NS11-12_marine_group;NS11-12_marine_group_unclassified;NS11-12_marine_group_unclassified;NS11-12_marine_group_unclassified;.2
 Bacteroidetes;Sphingobacteria;Sphingobacteriales_1;PHOS-HE51;PHOS-HE51_unclassified;PHOS-HE51_unclassified;PHOS-HE51_unclassified;.1

Chlamydiae;Chlamydiae;Chlamydiales;Chlamydiales_unclassified;Chlamydiales_unclassified;Chlamydiales_unclassified;Chlamydiales_unclassified;.5
Chlamydiae;Chlamydiae;Chlamydiales;Chlamydiales_unclassified;Chlamydiales_unclassified;Chlamydiales_unclassified;Chlamydiales_unclassified;
Chlamydiae;Chlamydiae;Chlamydiales;cvE6;cvE6_unclassified;cvE6_unclassified;cvE6_unclassified;.12
Chlamydiae;Chlamydiae;Chlamydiales;cvE6;cvE6_unclassified;cvE6_unclassified;cvE6_unclassified;.29
Chlamydiae;Chlamydiae;Chlamydiales;cvE6;cvE6_unclassified;cvE6_unclassified;cvE6_unclassified;.3
Chlamydiae;Chlamydiae;Chlamydiales;cvE6;cvE6_unclassified;cvE6_unclassified;cvE6_unclassified;.6
Chlamydiae;Chlamydiae;Chlamydiales;Simkaniaceae;Candidatus_Rhabdochlamydia;Candidatus_Rhabdochlamydia_unclassified;Candidatus_Rhabdochlamydia_unclassified;
Chlamydiae;Chlamydiae;Chlamydiales;Simkaniaceae;Candidatus_Rhabdochlamydia;Candidatus_Rhabdochlamydia_unclassified;Candidatus_Rhabdochlamydia_unclassified;.1
Chlamydiae;Chlamydiae;Chlamydiales;Simkaniaceae;Candidatus_Rhabdochlamydia;Candidatus_Rhabdochlamydia_unclassified;Candidatus_Rhabdochlamydia_unclassified;.12
Chlamydiae;Chlamydiae;Chlamydiales;Simkaniaceae;Candidatus_Rhabdochlamydia;Candidatus_Rhabdochlamydia_unclassified;Candidatus_Rhabdochlamydia_unclassified;.2
Chlamydiae;Chlamydiae;Chlamydiales;Simkaniaceae;Candidatus_Rhabdochlamydia;Candidatus_Rhabdochlamydia_unclassified;Candidatus_Rhabdochlamydia_unclassified;.21
Chlamydiae;Chlamydiae;Chlamydiales;Simkaniaceae;Candidatus_Rhabdochlamydia;Candidatus_Rhabdochlamydia_unclassified;Candidatus_Rhabdochlamydia_unclassified;.4
Chlamydiae;Chlamydiae;Chlamydiales;Simkaniaceae;Candidatus_Rhabdochlamydia;Candidatus_Rhabdochlamydia_unclassified;Candidatus_Rhabdochlamydia_unclassified;
Chlamydiae;Chlamydiae;Chlamydiales;Simkaniaceae;Candidatus_Rhabdochlamydia;Candidatus_Rhabdochlamydia_unclassified;Candidatus_Rhabdochlamydia_unclassified;
Chlorobi;Chlorobia;Chlorobiales;BSV26;BSV26_unclassified;BSV26_unclassified;BSV26_unclassified;
Chlorobi;Chlorobia;Chlorobiales;BSV26;BSV26_unclassified;BSV26_unclassified;BSV26_unclassified;.1
Chlorobi;Chlorobia;Chlorobiales;BSV26;BSV26_unclassified;BSV26_unclassified;BSV26_unclassified;.2
Chlorobi;Chlorobia;Chlorobiales;BSV26;BSV26_unclassified;BSV26_unclassified;BSV26_unclassified;.3
Chlorobi;Chlorobia;Chlorobiales;Chlorobiales_unclassified;Chlorobiales_unclassified;Chlorobiales_unclassified;Chlorobiales_unclassified;
Chlorobi;Chlorobia;Chlorobiales;OPB56;OPB56_unclassified;OPB56_unclassified;OPB56_unclassified;
Chlorobi;Chlorobia;Chlorobiales;OPB56;Termite_cluster;Termite_cluster_unclassified;Termite_cluster_unclassified;
Chlorobi;Chlorobia;Chlorobiales;SJA-28;SJA-28_unclassified;SJA-28_unclassified;SJA-28_unclassified;
Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;Anaerolinea;Anaerolinea_unclassified;Anaerolinea_unclassified;
Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;Anaerolinea;Anaerolinea_unclassified;Anaerolinea_unclassified;
Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;Anaerolineaceae_unclassified;Anaerolineaceae_unclassified;Anaerolineaceae_unclassified;.1

Chloroflexi;JG30-KF-CM66;JG30-KF-CM66_unclassified;JG30-KF-CM66_unclassified;JG30-KF-CM66_unclassified;JG30-KF-CM66_unclassified;JG30-KF-CM66_unclassified;.4

Chloroflexi;KD4-96;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;.1

Chloroflexi;KD4-96;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;.10

Chloroflexi;KD4-96;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;.14

Chloroflexi;KD4-96;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;.2

Chloroflexi;KD4-96;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;.4

Chloroflexi;KD4-96;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;.5

Chloroflexi;KD4-96;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;.6

Chloroflexi;KD4-96;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;KD4-96_unclassified;.7

Chloroflexi;Ktedonobacteria;Ktedonobacteriales;Ktedonobacteriaceae;Ktedonobacter;Ktedonobacter_unclassified;Ktedonobacter_unclassified;.2

Chloroflexi;P2-11E;P2-11E_unclassified;P2-11E_unclassified;P2-11E_unclassified;P2-11E_unclassified;P2-11E_unclassified;.1

Chloroflexi;S085;S085_unclassified;S085_unclassified;S085_unclassified;S085_unclassified;S085_unclassified;.3

Chloroflexi;TK10;TK10_unclassified;TK10_unclassified;TK10_unclassified;TK10_unclassified;TK10_unclassified;.1

Chloroflexi;TK10;TK10_unclassified;TK10_unclassified;TK10_unclassified;TK10_unclassified;TK10_unclassified;.11

Chloroflexi;TK10;TK10_unclassified;TK10_unclassified;TK10_unclassified;TK10_unclassified;TK10_unclassified;.2

Chloroflexi;TK10;TK10_unclassified;TK10_unclassified;TK10_unclassified;TK10_unclassified;TK10_unclassified;.7

Chloroflexi;TK10;TK10_unclassified;TK10_unclassified;TK10_unclassified;TK10_unclassified;TK10_unclassified;.9

Cyanobacteria;Cyanobacteria;Cyanobacteriales;MLE1-12;MLE1-12_unclassified;MLE1-12_unclassified;MLE1-12_unclassified;.6

Deferribacteres;Deferribacteres;Deferribacterales;Deferribacteraceae;Deferribacteraceae_unclassified;Deferribacteraceae_unclassified;Deferribacteraceae_unclassified;.2

Deferribacteres;Deferribacteres;Deferribacterales;Deferribacteraceae;Mucispirillum;Mucispirillum_unclassified;Mucispirillum_unclassified;.2

Elusimicrobia;Elusimicrobia;Elusimicrobiales;Lineage_Ia;Lineage_Ia_unclassified;Lineage_Ia_unclassified;Lineage_Ia_unclassified;.1

Elusimicrobia;Elusimicrobia;Elusimicrobiales;Lineage_Ia;Lineage_Ia_unclassified;Lineage_Ia_unclassified;Lineage_Ia_unclassified;.1

Elusimicrobia;Elusimicrobia;Elusimicrobiales;Lineage_Ia;Lineage_Ia_unclassified;Lineage_Ia_unclassified;Lineage_Ia_unclassified;.2

Elusimicrobia;Elusimicrobia;Elusimicrobiales;Lineage_Ia;Lineage_Ia_unclassified;Lineage_Ia_unclassified;Lineage_Ia_unclassified;.5

Elusimicrobia;Elusimicrobia;Elusimicrobiales;Lineage_Ia;Lineage_Ia_unclassified;Lineage_Ia_unclassified;Lineage_Ia_unclassified;.6

Elusimicrobia;Elusimicrobia;Elusimicrobiales;Lineage_IIb;Lineage_IIb_unclassified;Lineage_IIb_unclassified;Lineage_IIb_unclassified;.2
Elusimicrobia;Elusimicrobia;Elusimicrobiales;Lineage_IIc;Lineage_IIc_unclassified;Lineage_IIc_unclassified;Lineage_IIc_unclassified;.3
Elusimicrobia;Elusimicrobia;Elusimicrobiales;Lineage_IId;Lineage_IId_unclassified;Lineage_IId_unclassified;Lineage_IId_unclassified;
Elusimicrobia;Elusimicrobia;Elusimicrobiales;Lineage_III;Lineage_III_unclassified;Lineage_III_unclassified;Lineage_III_unclassified;
Elusimicrobia;Elusimicrobia;Elusimicrobiales;Lineage_III;Lineage_III_unclassified;Lineage_III_unclassified;Lineage_III_unclassified;.11
Elusimicrobia;Elusimicrobia;Elusimicrobiales;Lineage_IV;Lineage_IV_unclassified;Lineage_IV_unclassified;Lineage_IV_unclassified;
Elusimicrobia;Elusimicrobia;Elusimicrobiales;Lineage_IV;Lineage_IV_unclassified;Lineage_IV_unclassified;Lineage_IV_unclassified;.2
Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus_11;Bacillus_11_unclassified;Bacillus_11_unclassified;
Firmicutes;Bacilli;Lactobacillales;Cockroach_cluster;Cockroach_cluster_unclassified;Cockroach_cluster_unclassified;Cockroach_cluster_unclassified;
Firmicutes;Bacilli;Lactobacillales;Enterococcaceae;Vagococcus;Vagococcus_unclassified;Vagococcus_unclassified;
Firmicutes;Bacilli;Lactobacillales;Enterococcaceae;Enterococcus;Enterococcus_unclassified;Enterococcus_unclassified;*
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillaceae_unclassified;Lactobacillaceae_unclassified;Lactobacillaceae_unclassified;.4
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillaceae_unclassified;Lactobacillaceae_unclassified;Lactobacillaceae_unclassified;
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_1;Lactobacillus_1_unclassified;Lactobacillus_1_unclassified;
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_2;Lactobacillus_2_unclassified;Lactobacillus_2_unclassified;
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_4;Lactobacillus_4_unclassified;Lactobacillus_4_unclassified;
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_5;Lactobacillus_5_unclassified;Lactobacillus_5_unclassified;
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus_sp_a;Lactobacillus_sp_a_unclassified;Lactobacillus_sp_a_unclassified;
Firmicutes;Bacilli;Lactobacillales;Lactobacillales_unclassified;Lactobacillales_unclassified;Lactobacillales_unclassified;Lactobacillales_unclassified;
Firmicutes;Bacilli;Lactobacillales;Lactobacillales_unclassified;Lactobacillales_unclassified;Lactobacillales_unclassified;Lactobacillales_unclassified;.6
Firmicutes;Bacilli;Lactobacillales;Lactobacillales_unclassified;Lactobacillales_unclassified;Lactobacillales_unclassified;Lactobacillales_unclassified;
Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Lactococcus_3;Lactococcus_3_unclassified;Lactococcus_3_unclassified;.2
Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Streptococcaceae_unclassified;Streptococcaceae_unclassified;Streptococcaceae_unclassified;
Firmicutes;CK-1C4-19;CK-1C4-19_unclassified;CK-1C4-19_unclassified;CK-1C4-19_unclassified;CK-1C4-19_unclassified;CK-1C4-19_unclassified;
Firmicutes;Clostridia_1;Clostridiales;Clostridiaceae_1;Arthropod_cluster;Arthropod_cluster_unclassified;Arthropod_cluster_unclassified;
Firmicutes;Clostridia_1;Clostridiales;Clostridiaceae_1;Clostridiaceae_1_unclassified;Clostridiaceae_1_unclassified;Clostridiaceae_1_unclassified;
Firmicutes;Clostridia_1;Clostridiales;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;
Firmicutes;Clostridia_1;Clostridiales;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;.1
Firmicutes;Clostridia_1;Clostridiales;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;.10
Firmicutes;Clostridia_1;Clostridiales;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;.12
Firmicutes;Clostridia_1;Clostridiales;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;Clostridiales_unclassified;.2

Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Parasporobacterium-Sporobacterium;Parasporobacterium-Sporobacterium_unclassified;Parasporobacterium-Sporobacterium_unclassified;.1

Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Robinsoniella_Insects;Robinsoniella_Insects_unclassified;Robinsoniella_Insects_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Robinsoniella_Insects;Robinsoniella_Insects_unclassified;Robinsoniella_Insects_unclassified;.1

Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Robinsoniella_Insects;Robinsoniella_Insects_unclassified;Robinsoniella_Insects_unclassified;.2

Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Uncultured_58;Uncultured_58_unclassified;Uncultured_58_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Gut_cluster_13;Gut_cluster_13_unclassified;Gut_cluster_13_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Lachnospiraceae_unclassified;Lachnospiraceae_unclassified;Lachnospiraceae_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Catabacter;Catabacter_unclassified;Catabacter_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Lachnospiraceae;Gut_cluster_13;Gut_cluster_13_unclassified;Gut_cluster_13_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Peptostreptococcaceae;Peptostreptococcaceae_unclassified;Peptostreptococcaceae_unclassified;Peptostreptococcaceae_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Peptostreptococcaceae;Peptostreptococcaceae_unclassified;Peptostreptococcaceae_unclassified;Peptostreptococcaceae_unclassified;.1

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Anaerotruncus;Anaerotruncus_unclassified;Anaerotruncus_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Anaerotruncus;Anaerotruncus_unclassified;Anaerotruncus_unclassified;.1

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Anaerotruncus;Anaerotruncus_unclassified;Anaerotruncus_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Gut_cluster_1;Gut_cluster_1_unclassified;Gut_cluster_1_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Gut_cluster_1;Gut_cluster_1_unclassified;Gut_cluster_1_unclassified;.3

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Gut_cluster_3;Gut_cluster_3_unclassified;Gut_cluster_3_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Gut_cluster_4;Gut_cluster_4_unclassified;Gut_cluster_4_unclassified;.2

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Gut_cluster_4;Gut_cluster_4_unclassified;Gut_cluster_4_unclassified;.3

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Gut_cluster_5;Gut_cluster_5_unclassified;Gut_cluster_5_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Gut_cluster_5;Gut_cluster_5_unclassified;Gut_cluster_5_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Gut_cluster_7;Gut_cluster_7_unclassified;Gut_cluster_7_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Gut_cluster_8;Gut_cluster_8_unclassified;Gut_cluster_8_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Hydrogenoanaerobacterium;Hydrogenoanaerobacterium_unclassified;Hydrogenoanaerobacterium_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Incertae_Sedis_5;Incertae_Sedis_5_unclassified;Incertae_Sedis_5_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Incertae_Sedis_5;Incertae_Sedis_5_unclassified;Incertae_Sedis_5_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Incertae_Sedis_6;Incertae_Sedis_6_unclassified;Incertae_Sedis_6_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Incertae_Sedis_6;Intestinimonas;Intestinimonas_unclassified;

Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Uncultured_28;Uncultured_28_unclassified;Uncultured_28_unclassified;.6
Firmicutes;Clostridia_1;Clostridiales;Ruminococcaceae;Uncultured_28;Uncultured_28_unclassified;Uncultured_28_unclassified;
Firmicutes;Clostridia_2;Clostridiales_1;OPB54;OPB54_unclassified;OPB54_unclassified;OPB54_unclassified;.1
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_1;Peptococcaceae_1_unclassified;Peptococcaceae_1_unclassified;Peptococcaceae_1_unclassified;
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_1;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_1;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;.1
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_1;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;.10
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_1;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;.11
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_1;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;.2
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_1;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;.3
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_1;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;.4
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_1;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;.6
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_1;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_1;Uncultured_3;Uncultured_3_unclassified;Uncultured_3_unclassified;.1
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_2;Desulfosporosinus;Desulfosporosinus_unclassified;Desulfosporosinus_unclassified;
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_2;Desulfosporosinus;Desulfosporosinus_unclassified;Desulfosporosinus_unclassified;.1
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_3;Pelotomaculum_3;Pelotomaculum_3_unclassified;Pelotomaculum_3_unclassified;
Firmicutes;Clostridia_2;Clostridiales_1;Peptococcaceae_3;Pelotomaculum_3;Pelotomaculum_3_unclassified;Pelotomaculum_3_unclassified;.1
Firmicutes;Clostridia_2;Clostridiales_1;Veillonellaceae;Acetonema_a;Acetonema_a_unclassified;Acetonema_a_unclassified;
Firmicutes;Clostridia_2;Clostridiales_1;Veillonellaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;.1
Firmicutes;Clostridia_2;Clostridiales_1;Veillonellaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;.2
Firmicutes;Clostridia_2;Clostridiales_1;Veillonellaceae;Uncultured_7;Uncultured_7_unclassified;Uncultured_7_unclassified;.3
Firmicutes;Clostridia_2;Clostridiales_1;Veillonellaceae;Uncultured_8;Uncultured_8_unclassified;Uncultured_8_unclassified;
Firmicutes;Clostridia_2;Clostridiales;Syntrophomonadaceae;Syntrophomonadaceae_unclassified;Syntrophomonadaceae_unclassified;Syntrophomonadaceae_unclassified;
Firmicutes;Clostridia_2;Clostridiales;Syntrophomonadaceae;Syntrophomonadaceae_unclassified;Syntrophomonadaceae_unclassified;Syntrophomonadaceae_unclassified;.3
Firmicutes;Clostridia_2;Clostridiales;Syntrophomonadaceae;Syntrophomonadaceae_unclassified;Syntrophomonadaceae_unclassified;Syntrophomonadaceae_unclassified;.4
Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;Erysipelotrichaceae_unclassified;Erysipelotrichaceae_unclassified;Erysipelotrichaceae_unclassified;

Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;.58

Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;.61

Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;.80

Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;.85

Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;Alphaproteobacteria_unclassified;.97

Proteobacteria;Alphaproteobacteria;Caulobacterales;Caulobacteraceae;Brevundimonas;Brevundimonas_unclassified;Brevundimonas_unclassified;

Proteobacteria;Alphaproteobacteria;Caulobacterales;Caulobacteraceae;Caulobacteraceae_unclassified;Caulobacteraceae_unclassified;Caulobacteraceae_unclassified;.2

Proteobacteria;Alphaproteobacteria;Caulobacterales;Caulobacteraceae;Phenylobacterium;Phenylobacterium_unclassified;Phenylobacterium_unclassified;

Proteobacteria;Alphaproteobacteria;Caulobacterales;Caulobacteraceae;Phenylobacterium;Phenylobacterium_unclassified;Phenylobacterium_unclassified;

Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Hirschia;Hirschia_unclassified;Hirschia_unclassified;

Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;

Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Uncultured_2;Uncultured_2_unclassified;Uncultured_2_unclassified;.7

Proteobacteria;Alphaproteobacteria;Rhizobiales_1;Brucellaceae;Pseudochrobactrum;Pseudochrobactrum_unclassified;Pseudochrobactrum_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_1;Hyphomicrobiaceae;Devosia-Prosthecomicrobium;Devosia-Prosthecomicrobium_unclassified;Devosia-Prosthecomicrobium_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_1;Hyphomicrobiaceae;Hyphomicrobiaceae_unclassified;Hyphomicrobiaceae_unclassified;Hyphomicrobiaceae_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_1;Phyllobacteriaceae;Defluviobacter;Defluviobacter_unclassified;Defluviobacter_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_1;Phyllobacteriaceae;Phyllobacteriaceae_unclassified;Phyllobacteriaceae_unclassified;Phyllobacteriaceae_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_1;Rhizobiaceae;Rhizobium-Agrobacterium;Rhizobium-Agrobacterium_unclassified;Rhizobium-Agrobacterium_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_1;Rhizobiaceae;Rhizobium-Agrobacterium;Rhizobium-Agrobacterium_unclassified;Rhizobium-Agrobacterium_unclassified;.1

Proteobacteria;Alphaproteobacteria;Rhizobiales_1;Rhizobiales_1_unclassified;Rhizobiales_1_unclassified;Rhizobiales_1_unclassified;Rhizobiales_1_unclassified;.1

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;alphaI_cluster_1;alphaI_cluster_1_unclassified;alphaI_cluster_1_unclassified;alphaI_cluster_1_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Bradyrhizobiaceae;Bradyrhizobium_12;Bradyrhizobium_12_unclassified;Bradyrhizobium_12_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_2;Hyphomicrobiaceae;Rhodomicrobium;Rhodomicrobium_unclassified;Rhodomicrobium_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_3;Hyphomicrobiaceae;Hyphomicrobiaceae_unclassified;Hyphomicrobiaceae_unclassified;Hyphomicrobiaceae_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_3;Hyphomicrobiaceae;Hyphomicrobium_1;Hyphomicrobium_1_unclassified;Hyphomicrobium_1_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_3;Hyphomicrobiaceae;Hyphomicrobium_2;Hyphomicrobium_2_unclassified;Hyphomicrobium_2_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_3;Hyphomicrobiaceae;Pedomicrobium;Pedomicrobium_unclassified;Pedomicrobium_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales_3;Hyphomicrobiaceae;Pedomicrobium;Pedomicrobium_unclassified;Pedomicrobium_unclassified;.1

Proteobacteria;Alphaproteobacteria;Rhizobiales;Hypomicrobiaceae;Prosthecomicrobium;Prosthecomicrobium_unclassified;Prosthecomicrobium_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales;Hypomicrobiaceae;Prosthecomicrobium;Prosthecomicrobium_unclassified;Prosthecomicrobium_unclassified;.2

Proteobacteria;Alphaproteobacteria;Rhizobiales;Methylocystaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;

Proteobacteria;Alphaproteobacteria;Rhizobiales;Methylocystaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.1

Proteobacteria;Alphaproteobacteria;Rhizobiales;Methylocystaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.2

Proteobacteria;Alphaproteobacteria;Rhizobiales;Methylocystaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.3

Proteobacteria;Alphaproteobacteria;Rhizobiales;Methylocystaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.4

Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Rhodobium_2;Rhodobium_2_unclassified;Rhodobium_2_unclassified;

Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae_1;Rhodobacteraceae_1_unclassified;Rhodobacteraceae_1_unclassified;Rhodobacteraceae_1_unclassified;.2

Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae_1;Rhodobacteraceae_1_unclassified;Rhodobacteraceae_1_unclassified;Rhodobacteraceae_1_unclassified;

Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae_1;Rhodobacteraceae_1_unclassified;Rhodobacteraceae_1_unclassified;Rhodobacteraceae_1_unclassified;

Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae_1;Rhodobacteraceae_1_unclassified;Rhodobacteraceae_1_unclassified;Rhodobacteraceae_1_unclassified;

Proteobacteria;Alphaproteobacteria;Rhodospirillales_1;Acetobacteraceae;Roseomonas_1;Roseomonas_1_unclassified;Roseomonas_1_unclassified;

Proteobacteria;Alphaproteobacteria;Rhodospirillales_1;Acetobacteraceae;Uncultured_4;Uncultured_4_unclassified;Uncultured_4_unclassified;

Proteobacteria;Alphaproteobacteria;Rhodospirillales_1;Rhodospirillaceae;Thalassospira;Thalassospira_unclassified;Thalassospira_unclassified;.19

Proteobacteria;Alphaproteobacteria;Rhodospirillales_1;Rhodospirillaceae;Thalassospira;Thalassospira_unclassified;Thalassospira_unclassified;.3

Proteobacteria;Alphaproteobacteria;Rhodospirillales_1;Rhodospirillaceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;

Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;DA111;DA111_unclassified;DA111_unclassified;DA111_unclassified;

Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;DA111;DA111_unclassified;DA111_unclassified;DA111_unclassified;.2

Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;DA111;DA111_unclassified;DA111_unclassified;DA111_unclassified;.3

Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;DA111;DA111_unclassified;DA111_unclassified;DA111_unclassified;.31

Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;DA111;DA111_unclassified;DA111_unclassified;DA111_unclassified;.4

Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;DA111;DA111_unclassified;DA111_unclassified;DA111_unclassified;.5
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;DA111;DA111_unclassified;DA111_unclassified;DA111_unclassified;.6
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;DA111;DA111_unclassified;DA111_unclassified;DA111_unclassified;.7
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;DA111;DA111_unclassified;DA111_unclassified;DA111_unclassified;
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;DA111;DA111_unclassified;DA111_unclassified;DA111_unclassified;
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;DA111;DA111_unclassified;DA111_unclassified;DA111_unclassified;.1
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;DA111;DA111_unclassified;DA111_unclassified;DA111_unclassified;
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;I-10;I-10_unclassified;I-10_unclassified;I-10_unclassified;.1
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;JG37-AG-20;JG37-AG-20_unclassified;JG37-AG-20_unclassified;JG37-AG-20_unclassified;
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;JG37-AG-20;JG37-AG-20_unclassified;JG37-AG-20_unclassified;JG37-AG-20_unclassified;.1
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;MNH4;MNH4_unclassified;MNH4_unclassified;MNH4_unclassified;
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;Rhodospirillaceae_2;Skermanella;Skermanella_unclassified;Skermanella_unclassified;
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;.1
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;Rhodospirillales_2_unclassified;.2
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;wr0007;wr0007_unclassified;wr0007_unclassified;wr0007_unclassified;
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;wr0007;wr0007_unclassified;wr0007_unclassified;wr0007_unclassified;.1
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;wr0007;wr0007_unclassified;wr0007_unclassified;wr0007_unclassified;.2
Proteobacteria;Alphaproteobacteria;Rhodospirillales_2;wr0007;wr0007_unclassified;wr0007_unclassified;wr0007_unclassified;.3
Proteobacteria;Alphaproteobacteria;Rickettsiales;Candidatus_Midichloria;Candidatus_Midichloria;Candidatus_Midichloria_unclassified;Candidatus_Midichloria_unclassified;
Proteobacteria;Alphaproteobacteria;Rickettsiales;Candidatus_Odyssella;Candidatus_Odyssella;Candidatus_Odyssella_unclassified;Candidatus_Odyssella_unclassified;
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Novosphingobium_2;Novosphingobium_2_unclassified;Novosphingobium_2_unclassified;
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Novosphingobium_2;Novosphingobium_2_unclassified;Novosphingobium_2_unclassified;
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingomonas_2;Sphingomonas_2_unclassified;Sphingomonas_2_unclassified;
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingomonas_2;Sphingomonas_2_unclassified;Sphingomonas_2_unclassified;.1
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingomonas_2;Sphingomonas_2_unclassified;Sphingomonas_2_unclassified;

Proteobacteria;Deltaproteobacteria;Sh765B-TzT-29;Sh765B-TzT-29_unclassified;Sh765B-TzT-29_unclassified;Sh765B-TzT-29_unclassified;Sh765B-TzT-29_unclassified;.7
 Proteobacteria;Deltaproteobacteria;Syntrophobacteriales;Syntrophorhabdaceae;Syntrophorhabdus;Syntrophorhabdus_unclassified;Syntrophorhabdus_unclassified;
 ;
 Proteobacteria;Epsilonproteobacteria;Campylobacteriales;Campylobacteraceae;Sulfurospirillum;Sulfurospirillum_unclassified;Sulfurospirillum_unclassified;
 Proteobacteria;Gammaproteobacteria_1;Alteromonadales;Alteromonadaceae;BD1-7_clade;BD1-7_clade_unclassified;BD1-7_clade_unclassified;
 Proteobacteria;Gammaproteobacteria_1;Alteromonadales;Alteromonadaceae;OM60_NOR5__clade;Haliea_1;Haliea_1_unclassified;
 Proteobacteria;Gammaproteobacteria_1;Enterobacteriales;Enterobacteriaceae_1;Morganella;Morganella_unclassified;Morganella_unclassified;
 Proteobacteria;Gammaproteobacteria_1;Enterobacteriales;Enterobacteriaceae_1;Proteus;Proteus_unclassified;Proteus_unclassified;
 Proteobacteria;Gammaproteobacteria_1;Enterobacteriales;Enterobacteriaceae_1;Providencia;Providencia_unclassified;Providencia_unclassified;
 Proteobacteria;Gammaproteobacteria_1;Enterobacteriales;Enterobacteriaceae;Enterobacteriaceae_unclassified;Enterobacteriaceae_unclassified;Enterobacteriaceae_unclassified;
 Proteobacteria;Gammaproteobacteria_1;Gammaproteobacteria_1_unclassified;Gammaproteobacteria_1_unclassified;Gammaproteobacteria_1_unclassified;Gammaproteobacteria_1_unclassified;Gammaproteobacteria_1_unclassified;.2
 Proteobacteria;Gammaproteobacteria_1;Oceanospirillales;Alcanivoracaceae;Alcanivorax;Alcanivorax_unclassified;Alcanivorax_unclassified;.1
 Proteobacteria;Gammaproteobacteria_1;Pseudomonadales;Moraxellaceae;Acinetobacter;Acinetobacter_unclassified;Acinetobacter_unclassified;.1
 Proteobacteria;Gammaproteobacteria_1;Pseudomonadales;Pseudomonadaceae;Pseudomonadaceae_unclassified;Pseudomonadaceae_unclassified;Pseudomonadaceae_unclassified;.1
 Proteobacteria;Gammaproteobacteria_2;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;.1
 Proteobacteria;Gammaproteobacteria_2;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;.1
 Proteobacteria;Gammaproteobacteria_2;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;.19
 Proteobacteria;Gammaproteobacteria_2;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;.3
 Proteobacteria;Gammaproteobacteria_2;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;.4
 Proteobacteria;Gammaproteobacteria_2;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;.5
 Proteobacteria;Gammaproteobacteria_2;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;Gammaproteobacteria_2_unclassified;.6
 Proteobacteria;Gammaproteobacteria_2;Legionellales;Coxiellaceae;Aquicella;Aquicella_unclassified;Aquicella_unclassified;.17
 Proteobacteria;Gammaproteobacteria_2;Xanthomonadales;Sinobacteraceae;Sinobacteraceae_unclassified;Sinobacteraceae_unclassified;Sinobacteraceae_unclassified;.

SM2F11;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;1
SM2F11;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;20
SM2F11;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;3
SM2F11;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;SM2F11_unclassified;9
Spirochaetes;Spirochaetes;Spirochaetales;Leptospiraceae;Leptospira;Leptospira_unclassified;Leptospira_unclassified;
Spirochaetes;Spirochaetes;Spirochaetales;Leptospiraceae;Rs-H88_termite_group;Rs-H88_termite_group_unclassified;Rs-H88_termite_group_unclassified;
Spirochaetes;Spirochaetes;Spirochaetales;Leptospiraceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;
Spirochaetes;Spirochaetes;Spirochaetales;Spirochaetaceae_Treponema_I;Spirochaetaceae_Treponema_I_unclassified;Spirochaetaceae_Treponema_I_unclassified;
Spirochaetes;Spirochaetes;Spirochaetales;Spirochaetaceae_Treponema;Animal_cluster_1;Animal_cluster_1_unclassified;Animal_cluster_1_unclassified;
Spirochaetes;Spirochaetes;Spirochaetales;Spirochaetaceae;Spirochaeta;Spirochaeta_unclassified;Spirochaeta_unclassified;
Spirochaetes;Spirochaetes;Spirochaetales;Spirochaetales_unclassified;Spirochaetales_unclassified;Spirochaetales_unclassified;Spirochaetales_unclassified;
Synergistetes;Synergistia;Synergistales;Synergistaceae;Candidatus_Tammella;Candidatus_Tammella_unclassified;Candidatus_Tammella_unclassified;.1
Synergistetes;Synergistia;Synergistales;Synergistaceae;Candidatus_Tammella;Candidatus_Tammella_unclassified;Candidatus_Tammella_unclassified;.2
Synergistetes;Synergistia;Synergistales;Synergistaceae;Candidatus_Tammella;Candidatus_Tammella_unclassified;Candidatus_Tammella_unclassified;.4
Synergistetes;Synergistia;Synergistales;Synergistaceae;Candidatus_Tammella;Candidatus_Tammella_unclassified;Candidatus_Tammella_unclassified;.9
Synergistetes;Synergistia;Synergistales;Synergistaceae;Synergistaceae_unclassified;Synergistaceae_unclassified;Synergistaceae_unclassified;.5
Synergistetes;Synergistia;Synergistales;Synergistaceae;Synergistaceae_unclassified;Synergistaceae_unclassified;Synergistaceae_unclassified;.9
Synergistetes;Synergistia;Synergistales;Synergistaceae;Synergistaceae_unclassified;Synergistaceae_unclassified;Synergistaceae_unclassified;
Synergistetes;Synergistia;Synergistales;Synergistaceae;Synergistaceae_unclassified;Synergistaceae_unclassified;Synergistaceae_unclassified;
Synergistetes;Synergistia;Synergistales;Synergistaceae;Termite_cockroach_cluster;Termite_cockroach_cluster_unclassified;Termite_cockroach_cluster_unclassified;
TA06;TA06_unclassified;TA06_unclassified;TA06_unclassified;TA06_unclassified;TA06_unclassified;TA06_unclassified;
Tenericutes;Mollicutes;Acholeplasmatales;Acholeplasmataceae;Acholeplasma_2;Acholeplasma_2_unclassified;Acholeplasma_2_unclassified;
Tenericutes;Mollicutes;Acholeplasmatales;Acholeplasmataceae;Acholeplasma_2;Acholeplasma_2_unclassified;Acholeplasma_2_unclassified;.5
Tenericutes;Mollicutes;Acholeplasmatales;Acholeplasmataceae;Acholeplasma_2;Acholeplasma_2_unclassified;Acholeplasma_2_unclassified;
Tenericutes;Mollicutes;Acholeplasmatales;Acholeplasmataceae;Acholeplasma_2;Acholeplasma_2_unclassified;Acholeplasma_2_unclassified;
Tenericutes;Mollicutes;Mycoplasmatales;Mycoplasmataceae;Uncultured_1;Uncultured_1_unclassified;Uncultured_1_unclassified;.3
Tenericutes;Mollicutes;RF9;RF9_unclassified;RF9_unclassified;RF9_unclassified;RF9_unclassified;.3
Tenericutes;Mollicutes;RF9;RF9_unclassified;RF9_unclassified;RF9_unclassified;RF9_unclassified;.43
Verrucomicrobia;OPB35_soil_group;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;OPB35_soil_group_unclassified;
lassified;OPB35_soil_group_unclassified;

*OTUS found in all insect gut microbiota