Structure and culture of the gut microbiome of the Mormon cricket *Anabrus simplex* (Orthoptera: Tettigoniidae)

Chad C. Smith
Department of Integrative Biology
University of Texas at Austin
1 University Station C0990
Austin, TX 78724
chad.smith123@gmail.com

Robert B. Srygley
USDA-Agricultural Research Service
Northern Plains Area Research Laboratory
1500 N. Central Avenue
Sidney MT 59270

Frank Healy
Department of Biology
Trinity University
1 Trinity Pl.
San Antonio, TX 78212

Karthikeyan Swaminath

Department of Integrative Biology

University of Texas at Austin

1 University Station C0990

Austin, TX 78724

Header: Structure of the Mormon cricket gut microbiome

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ABSTRACT

The gut microbiome of insects plays an important role in their ecology and evolution, participating in nutrient acquisition, immunity, and behavior. Microbial community structure within the gut is thought to be heavily influenced by differences among gut regions in morphology and physiology, which determine the niches available for microbes to colonize. We present a high-resolution analysis of the structure of the gut microbiome in the Mormon cricket *Anabrus simplex,* an insect known for its periodic outbreaks in the Western United States and nutrition-dependent mating system. The Mormon cricket microbiome was dominated by eleven bacterial phylotypes from the Lactobacillaceae, Enterobacteriaceae, and Streptococcaeae. While most of these were represented in all gut regions, there were marked differences in their relative abundance, with lactic-acid bacteria (Lactobacillaceae) more common in the foregut and midgut and enteric (Enterobacteriaceae) bacteria more common in the hindgut. Differences in community structure were driven by variation in the relative prevalence of three groups: a *Lactobacillus* phylotype in the foregut, *Pediococcus* lactic-acid bacteria in the midgut, and *Pantoea agglomerans*, an enteric bacterium, in the hindgut. These taxa have been shown to have beneficial effects on their hosts in insects and other animals by improving nutrition, increasing resistance to pathogens, and modulating social behavior. Phylogenetic analysis of 16s rRNA sequences from cultured isolates indicated low levels of divergence from sequences derived from plants and other insects, suggesting that these bacteria are likely to be exchanged between Mormon crickets and the environment. Our study provides the foundation for future work on an economically important insect and emerging model for the study of how social interaction influence host-microbe symbiosis.

Insects are the most speciose and abundant taxa in the animal kingdom, playing a key ecological role in many of the world's ecosystems. Symbioses between insects and their microbial associates has undoubtedly contributed to their success, providing the capability to degrade recalcitrant food items, supplementing nutrient-deficient diets, protecting them from their natural enemies, and modulating the expression of social behavior (Moran *et al.*, 2008; Engel and Moran, 2013; Douglas, 2015). Among the niches available to occupy within the host, the gut houses the largest and most diverse microbiome in insects (Engel and Moran, 2013; Douglas, 2015) and other animals (Ley *et al.*, 2008; Cho and Blaser, 2012). Gut morphology and physiology vary markedly along the alimentary tract in insects, resulting in an environmental gradient that influences, and is influenced by, the microbial communities that populate it (Dillon and Dillon, 2004; Engel and Moran, 2013; Brune and Dietrich, 2015).

The insect gut consists of three regions that are analogous to that in mammals, the foregut, the midgut and the hindgut, each of which contributes to a different aspect of gut function (Douglas, 2013). The foregut serves as the entry point for food, where it is stored in the crop before passing through the proventriculus, a valve that can also be modified to mechanically filter food (Woodring and Lorenz, 2007; Douglas, 2013) and even microbes (Lanan *et al.*, 2016). Digestion and absorption of nutrients begins at the midgut, which, in some species, contains specialized crypts that house microbes that aid in insect nutrition (Kikuchi *et al.*, 2005; Bistolas *et al.*, 2014). Host immune factors have been shown to play an important role in regulation of commensal microbes in the midgut (Ryu *et al.*, 2010; Buchon *et al.*, 2013), some of which protect the host from pathogens (Forsgren *et al.*, 2010). Following the midgut is the hindgut, which is comprised of the ileum, colon, and rectum. Malphigian tubules permeate the anterior hindgut, excreting nitrogenous waste and other solutes from the hemocoel that can provide

nutrients for dense populations of microbes (Bignell, 1984). In some species, dense bristle-like structures in the ileum (Woodring and Lorenz, 2007) and rectal papillae (Hunt and Charnley, 1981) provide attachment sites for microbes, some of which fix nitrogen (Tai *et al.*, 2016), degrade recalcitrant plant polymers (Kaufman and Klug, 1991; Engel and Moran, 2013; Brune and Dietrich, 2015), and prevent infection (Dillon and Charnley, 2002).

The Mormon crickets *Anabrus simplex* (Orthoptera: Tettigoniidae) is an economically important shield-backed katydid distributed throughout the Western United States. Mormon crickets can form dense aggregations of millions of individuals spread over 10 kilometers long and several kilometers wide, feeding on forbes, grasses, and agricultural crops as they march in migratory bands across the landscape (Wakeland, 1959; MacVean, 1987). Mormon crickets are also emerging as a model for the study of how social interactions and diet influence the microbiome (Smith *et al.*, 2016). Differences in population density are linked to reproductive behavior, as in high density populations, protein-limited females compete for access to males to gain access a proteinaceous “nuptial gift” males produce for females during copulation (Gwynne, 1984)While consumption of male nuptial gifts by females does not influence the composition of the microbiome, sexually inactive females experience a dramatic decline in *Pediococcus* lactic-acid gut bacteria compared to sexually active females (Smith *et al.*, 2016). Lactic-acid bacteria are common associates of the alimentary tract and regarded for their beneficial effects on immune function and nutrition in animals, including insects (Forsgren *et al.*, 2010; Storelli *et al.*, 2011; Erkosar *et al.*, 2015).

We characterize the structure of the gut microbiome of Mormon crickets and infer their evolutionary relationships using a combination of culture-dependent and culture-independent approaches. Our aims are to determine whether gut microbial communities vary along the

105 alimentary tract in the Mormon cricket and to infer their potential to influence host function
106 based on their taxonomy and known associations with other insects. We also establish methods
107 for isolating Mormon cricket gut microbiota in culture to permit future experimental
108 manipulations of the gut microbiome and build genomic resources to infer their evolution and
109 function.
110

111 # METHODS

112 *Spatial structure of the gut microbiome*

113 Mormon crickets were obtained from field (n=5) and laboratory-raised (n=8) collections. Wild
114 females were caught in EK Mountain (43°47'58"N, 106°50'31"W, 1752 m) near Kaycee,
115 Wyoming in the summer of 2014, immediately preserved in 100% ethanol, and stored at -80°C
116 until dissection. Laboratory-raised Mormon crickets were derived from eggs collected from
117 individuals caught in EK Mountain and fed a mixture of wheat bran, wheat germ, sunflower,
118 mixed bird seeds, tropical fish flakes, fresh Romaine lettuce (added daily), and water *ad libitum*.
119 Mormon crickets were dissected using flame-sterilized tools after rinsing in 1% bleach
120 for 3 minutes followed by two rinses in autoclaved distilled water to remove bacteria on the
121 exoskeleton. DNA from the foregut (crop and proventriculus), midgut (ventriculus), ileum, and
122 rectum (Fig. 1) of laboratory-raised crickets was extracted with MoBio Powersoil© as in Smith et
123 al. (2016). Foregut (crop and proventriculus), midgut (ventriculus), and hindgut tissue (ileum and
124 rectum combined) from field-collected animals were bead-beat in a sterile 2ml screw cap tube
125 with 750 µl of 0.1mm silica-zircon beads (Biospec Products), two 3.2mm stainless steel beads
126 (Biospec Products) and 1ml of sterile CTAB buffer (0.1M Tris, 1.4M NaCl, 20mM EDTA, 2%

PVP, 2 µl beta-mercaptanol, 20µl of 20mg/ml Proteinase-K). Tubes were bead beat at maximum speed for 2 minutes (Mini Beadbeater-96, Biospec Products) and incubated overnight at 55°C. RNAse (400µg, Qiagen) was added to the lysate and incubated at 37°C for 30 minutes. A phenol-chloroform extraction with isopropanol precipitation was performed to isolate the DNA. DNA extraction methods can influence the representation of bacterial taxa in 16s rRNA metagenomic studies (Yuan *et al.*, 2012), however our aim here is not to make inferences about differences between field and laboratory-raised animals. We include the source of the animal (field or laboratory) and its interaction with tissue type in all statistical analyses to assess how the microbiome differs among gut regions after accounting for variation due to source/DNA extraction method (see Statistics).

Sequencing and Bioinformatics

Library preparation was done by the Genome Sequencing and Analysis Facility at the University of Texas at Austin using the NEBNext kit for Illumina. The variable V4 region of 16s rRNA gene was amplified with universal primers (Hyb515F: 5'-GTGYCAGCMGCCGCGGTA -3', Hyb806R: 5'-GGACTACHVGGGTWTCTAAT-3') and sequenced on the Illumina Miseq V3 platform. DADA2 1.1.5 (Callahan *et al.*, 2016, 2) was used to process the raw sequencing data, truncating reads when Illumina quality scores were less than two, removing sequences with a maximum expected error of one, and removing sequences flagged as chimeric. Clustering was then performed with DADA2 (Callahan *et al.*, 2016), specifying joint inference of sample composition and sequence error rates (selfConsist=T). Taxonomy was then assigned with the Greengenes 13.8 database at 97% identity. OTUs that were classified as unassigned, mitochondria, or chloroplast, and those that comprised an average of less than 1% of the reads

149 recovered within a given Mormon cricket, were removed for analysis using phyloseq 1.16.2
150 (McMurdie and Holmes, 2013).

151 *Bacterial Abundance*

152 The abundance of bacteria was estimated using qPCR following Powell et. al (2014). Universal
153 16S rRNA gene primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 355R (5'-
154 CTGCTGCCTCCCGTAGGAGT-3') were used to amplify all copies of the 16S rRNA gene in
155 tissue samples from laboratory (n=8) and field caught individuals (n=8) on an Applied
156 Biosystems ViiA7 (Life Technologies). Triplicate 20 ul reactions were used with 10 ul of 2x
157 PowerSYBR master mix (Applied Biosystems), 0.4 ul of each 10 mM primer and 5 ng of
158 template DNA. PCR amplification was performed at 95°C for 10 minutes followed by 40 cycles
159 of 95°C for 15 seconds and 1 minute at 60°C. Quantification of copy number was based on
160 standard curves from amplification of the cloned target sequence in a pGEM-T (Promega,
161 Madison, WI, USA) vector (Powell *et al.*, 2014).

162 *Culturing*

163 Five female Mormon crickets were surface sterilized in 1% bleach for three minutes, rinsed
164 twice in sterile water and dissected using flame-sterilized tools. Gut tissue was homogenized for
165 10 seconds with a bead beater using autoclaved 3.2mm stainless steel beads in sterile PBS.
166 Homogenates were plated onto trypsin soy agar, brain heart infusion agar, nutrient agar, or Man–
167 Rogosa–Sharpe agar (BD), cultured in anaerobic or Campy (low O_2) Gaspak pouches (Becton,
168 Dickinson and Company, Franklin Lakes, NJ) at 37°C for 24-48 hours, and individual colonies
169 passaged three times to obtain pure isolates.

170 DNA was extracted by boiling cells for 15 minutes in lysis buffer (100mM NaCl and
171 0.5% sarcosyl), adding an equal volume of 20% chelex, and boiling for 15 additional minutes.

16s rRNA amplicons were amplified with 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') primers using Apex PCR master mix (Genesee Scientific, San Diego, CA) with 35 cycles (95°C for 20 s, 52°C for 1 min 30 s and 72°C for 40 s). PCR products were cleaned up with Sera-mag beads (GE Healthcare Life Sciences, Pittsburgh, PA) or ethanol precipitation and sequenced at the University of Texas at Austin on an Applied Biosystems 3730XL DNA analyzer.

We compiled 16s rRNA sequences greater than 1.2kb long reported as sourced from insect guts from NCBI Genbank, and used BLAST to find the closest matches to our Mormon cricket isolates. *Pediococcus* and *Lactobacillus* sequences were aligned with pyNAST as implemented in Qiime 1.9 (Caporaso *et al.*, 2010) using a curated alignment for *Lactobacillus* (McFrederick *et al.*, 2013) as the reference template. The alignment was then manually edited with Mesquite (Maddison and Maddison, 2016) and filtered to remove characters with less than 80% coverage across sequences using Qiime 1.9 (Caporaso *et al.*, 2010). Sequences from the Enterobacteriaceae were aligned with online implementation of the SILVA release 113 (Pruesse *et al.*, 2012; Quast *et al.*, 2013), manually checked in Mesquite (Maddison and Maddison, 2016), and filtered as above, with the additional removal of the top 10% most entropic (hypervariable) base positions. The phylogenies were constructed using maximum likelihood with a GTR + Gamma model for nucleotide evolution in RaxML 8.2.4 (Stamatakis, 2014), with 1000 bootstraps to assess branch support. Archaeopteryx 0.9916 (Han and Zmasek, 2009) was used to visualize the tree and produce the figures.

Phenotypic assays

Fresh overnight cultures of all isolates were used for microscopic analysis. Lactobacillaceae isolates were cultured in Man–Rogosa–Sharpe medium and Enterobacteriaceae were cultured in

nutrient broth or LB medium. Biochemical tests were done following Bridson (1998). Motility was determined using SIM medium and microscopic examination of culture wet mounts. Man–Rogosa–Sharpe or nutrient broth containing 1 g/L potassium nitrate was used for nitrate reduction tests. Fermentation tests were done anaerobically in Man–Rogosa–Sharpe and nutrient broth media with the addition of indicated sugars to 1% w/v final concentration.

Statistics

Analyses were performed in R 3.3.1 (R Core Development Team, 2013). OTU tables rarified at 1300 reads using phyloseq (McMurdie and Holmes, 2013), resulting in the exclusion of hindgut samples from two field-caught females that had a low number of reads. Alpha diversity was compared among tissue types and between origin of subject (field vs. lab) with a linear mixed model with the lme4 package (Bates *et al.*, 2013), entering the individual ID as a random effect to account for within-subject correlations in diversity. Three metrics were calculated: species richness, the Chao1 species richness estimator, and the Shannon-Weiner diversity index. Post-hoc comparisons among gut regions were performed using a Tukey test as implemented in the multcomp package (Hothorn *et al.*, 2008).

Beta diversity among gut tissue types and between animal source (field vs. lab) was assessed with a distance-based redundancy analysis (db-RDA) as implemented in vegan 2.3 (Oksanen *et al.*, 2015), specifying Bray-Curtis distances. Prior to analysis, the relative abundance of OTUs were calculated for each Mormon cricket and those that comprised less than 1% of the sequences on average were discarded. Statistical significance of the terms in the db-RDA model were determined by 999 permutations of the distance matrix as implemented in vegan, restricting the permutations to within each individual to retain the nested structure of the data.

We assessed the difference in abundance of specific OTUs identified in the db-RDA analysis by fitting the the data to a negative binomial generalized linear mixed model using the lme4 package (Bates *et al.*, 2013), specifying the individual ID as the random effect and the tissue type and animal source (field vs. lab) as fixed effects. A similar procedure was used to assess differences in bacterial 16s rRNA gene copy number between tissue types and animal source except a normal distribution was specified. Likelihood ratio tests were used to determine the statistical significance of each factor using the MASS package (Venables and Ripley, 2002). Goodness-of-fit was assessed by comparing the fit of the data to a negative binomial distribution with a Chi-square test (Faraway, 2006), and homoscedasticity was assessed by examination of residual plots. P-values were adjusted for multiple tests using the false discovery rate (Benjamini and Hochberg, 1995).

RESULTS

Spatial structure of the gut microbiome

We recovered 11 dominant OTUs from field and lab-raised individuals, with the remaining 749 OTUs comprising <1% of the sequences from a given Mormon cricket (Fig. 2). Field and laboratory-raised individuals shared 7 of the 11 OTUS, including the most abundant *Pediococcus acidilactici* phylotype that varied with mating status in a previous study (*P. acidilactici* 102222; Smith *et al.*, 2016). The remaining five shared OTUs were two Lactobacilliaceae (*Lactobacillus sp* and *P. acidilactici* 2), two Enterobacteriaceae (*Pantoea agglomerans* and a *Klebsiella sp*) and one Streptococcaceae (*Lactococcus garvieae*). Field Mormon crickets had three OTUS that were not shared with laboratory-raised individuals, while lab-raised individuals had two OTUs that were not shared with field individuals (Fig. 2). Guts from two laboratory individuals were almost

239 completely comprised of the enterobacterium *Pantoea agglomerans* (99.3% and 80.8% of reads
240 respectively), so we conducted our analysis with and without these individuals.

241 Species richness and diversity differed among gut regions and were higher in field
242 compared to lab-raised animals (Table 1, Fig. 3). There was no significant interaction between
243 collection source and tissue type (Table 1), indicating that differences in alpha diversity among
244 tissue types were shared between lab and field caught animals. We found that the midgut was the
245 most diverse part of the gut with two of the three measures of alpha diversity (species richness
246 and the Chao1 diversity estimator), while the hindgut and foregut had similar levels of richness
247 and diversity. The third metric (Shannon-Weiner) also found the foregut to be the least diverse
248 region, but differed in that the midgut and hindgut had similar levels of species diversity (Table
249 1, Fig 3).

250 The db-RDA analysis revealed that the structure of the gut microbiome also varied
251 among gut regions and between field and laboratory animals (Table 2, Fig. 4). The non-
252 significant interaction in this analysis indicates that the differences in community structure
253 among tissue types were consistent between field and laboratory individuals (Table 2). To
254 determine which members of the gut microbiome varied among gut regions, we ordinated the
255 OTU scores from db-RDA analyses of field and laboratory Mormon crickets (Fig. 5). Three
256 groups of bacteria appeared to separate along the gut axis: a *Lactobacillus sp.* lactic-acid
257 bacterium associated with in the foregut, *Pediococcus* lactic-acid bacteria were associated with
258 the midgut, and *Pantoea agglomerans*, an enterobacterium, was found in association with the
259 hindgut. Inspection of the plots from laboratory animals indicate that *Pantoea agglomerans* is
260 more abundant in the rectum (Fig. 5b). The composition of the ileum, which is separated from
261 the rectum by the colon, closely resembled that of the midgut (Fig. 5b).

Univariate analyses of these three groups largely confirmed the pattern in the ordination (Table 3, Fig. 6). The interaction between tissue type and source was not significant in any of the analyses and dropped to estimate the differences in abundance between tissue types. *Lactobacillus sp*. was 3 and 7 times more common in the foregut than in the midgut ($\beta=1.4 \pm 0.50$, $p=0.02$) and hindgut ($\beta=2.0 \pm 0.51$, $p<0.001$) respectively, *Pediococcus* was similar in abundance in the midgut and hindgut but 4.7 times more common in these areas than the foregut ($\beta=1.1 \pm 0.36$, $p=0.006$), and *Pantoea agglomerans* was 209 and 12 times more abundant in the hindgut compared to the foregut ($\beta=3.8 \pm 0.87$, $p<0.001$) and midgut ($\beta=2.5 \pm 0.82$, $p=0.007$) respectively.

Bacterial abundance

The number of copies of bacterial 16s rRNA genes was significantly different among tissue types, as indicated by the significant interaction between tissue type and the source of the Mormon crickets (Analysis of deviance: Source, $F_{1,14}=25.9$, $p<0.001$; tissue type, $F_{3,161}=7.8$, $p<0.001$; Interaction, $F_{3,161}=2.8$, $p=0.04$, Fig. 7). We decomposed the interaction to determine how the total number of 16s rRNA copies differed among tissue types within field and laboratory-raised animals. The major difference between the two sources was that in wild Mormon crickets, the midgut had the lowest abundance of all gut regions, while in laboratory-raised individuals, both the midgut and the ileum had the lowest abundance of bacterial 16s rRNA genes (Table 4, Fig. 7).

Culturing

Ten bacterial phylotypes were cultured from the Mormon cricket gut based on 99% sequence similarity of their near full-length 16s rRNA genes (mean $\pm$ sd: 1406 $\pm$ 30bp). Two of the

284 phylotypes were lactic-acid bacteria (Lactobacillaceae) and eight were enteric bacteria
285 (Enterobacteriaceae).

286 The lactic-acid bacteria fell into two clades in our phylogenetic analysis (Figure 8). The
287 first clade was comprised of *Pediococcus* isolates derived from environmental sources, such as
288 plants and various human foodstuffs, as well as strains from the human gut. Similarity to
289 sequences from the BLAST search was high (>99.5%) and branch lengths were short, indicating
290 *Pediococcus acidilactici* H11 from the Mormon cricket gut is not highly derived from its
291 relatives, as has been found for *Lactobacillus* species isolated from bees (Fig. 8; McFrederick *et*
292 *al.*, 2013). Our search for *Pediococcus* sequences from insect guts in Genbank recovered
293 sequences from the termites *Macrotermes bellicosus* and *M. subhyalinus*, which formed their
294 own well-supported clade. *P. acidilactici* H11 shared 100% sequence identity in the V4 region
295 with the *P. acidilactici 1* phylotype sequenced using the Illumina platform in this study and with
296 the *P. acidilactici* (102222) phylotype associated with variation in mating status in Mormon
297 crickets (Smith *et al.*, 2016). Morphologically, *P. acidilactici* H11 is nonmotile and spherical
298 (0.8 – 1.0 μm), often dividing to form pairs as described for other *Pediococcus*. As other
299 members of the genus, the *P. acidilactici* H11 is gram-positive, non-motile, faculatively
300 anaerobic, grows at low pH, and produces lactic acid from lactose (Table S1).

301 The second clade of lactic-acid bacteria was comprised primarily of plant-associated
302 *Lactobacillus.* Unlike *P. acidilactici* H11, *Lactobacillus* H09 formed a distinct clade with high
303 branch support, indicating it is genetically distinct enough at the 16s rRNA locus to distinguish
304 itself from other clades in the phylogeny. Similar to *P. acidilacitici* H11, *Lactobacillus* H09 had
305 high sequence similarity (>99.5%) to other members of the clade and a short branch length,
306 indicating that while it is distinct enough to form its own clade, it is not highly derived from its

307 relatives at the 16s rRNA locus. Our Genbank search for *Lactobacillus* isolated from insect guts
308 found sequences from ants, bees, and termites, and fruit flies, all of which fell into a different
309 clade than *Lactobacillus* H09. *Lactobacillus* from these taxa thus appear to have a different
310 evolutionary history than *Lactobacillus* H09. *Lactobacillus* H09 shared 100% sequence identity
311 in the V4 region with the Lactobacillaceae 2 phylotype sequenced using the Illumina platform in
312 this study. Morphologically, *Lactobacillus* H09 appear as non-motile straight rods,
313 approximately 1.3-2 μm in length and 0.8-1.0 μm wide. *Lactobacillus* H09 is gram-positive, non-
314 motile, faculatively anaerobic, grows at low pH, and produces lactic acid from lactose (Table
315 S1).

316 The eight Enterobacteriaceae strains were most similar to *Enterobacter* strains in our
317 BLAST search, which recovered sequences from a variety of plant and animal sources (sequence
318 similarity=98.7-99.8%). Our survey of Genbank found *Enterobacter* from alimentary tracts of a
319 diverse group of insects, including termites, cockroaches, flies, beetles, stink bugs, bees, ants,
320 and moths. Like other studies (Brenner *et al.*, 2005), however, the 16s rRNA gene did not have
321 enough signal to resolve relationships among *Enterobacter* and its relatives (data not shown) so
322 we present a simpler phylogeny with the Mormon cricket isolates and type strains from the
323 family (Figure 9). We found that our Mormon cricket isolates formed their own clade with
324 moderate statistical support. A multilocus sequencing approach, however, is needed to improve
325 the inference (Brenner *et al.*, 2005). All five strains isolated from Mormon crickets had 100%
326 identity at the V4 region with the *Klebsiella* phylotype sequenced on the Illumina platform,
327 however the phylogenetic (Fig. 9) and phenotypic data (Table S2) suggests that it is unlikely to
328 be a correct taxonomic assignment. Morphologically, all isolates were straight rods,
329 approximately 0.8-1.0 μm in length and 0.6-0.8 μm wide. Unlike most *Klebsiella*, these strains

were motile, which is typical of *Enterobacter* and other Enterobacteriaceae (Brenner *et al.*, 2005). Strains were gram-negative, and facultatively anaerobic (Table S2).

DISCUSSION

We found striking differences in the diversity and structure of the gut microbiome in the Mormon cricket *Anabrus simplex*. While most OTUs were represented in the foregut, midgut and hindgut, there were dramatic differences in relative abundance within the Lactobacillaceae and between the Lactobacillaceae and Enterobacteriaceae, the main families recovered in our culture and culture-independent studies. Our phylogenetic analysis of cultured isolates found that Mormon cricket gut bacteria are not highly derived from related bacteria associated with plants or the guts of other animals, suggesting that gut bacteria are either acquired from the environment in each generation or have not been restricted to Mormon crickets over appreciable periods of evolutionary time. Our findings have important implications for our understanding of the ecological and evolutionary processes that influence the assembly and function of gut microbial communities in orthopterans and other insects, as it suggests that host-microbe and microbe-microbe interactions shape the abundance and distribution of the gut microbiome.

Our finding that bacterial abundance is lower in the midgut is in agreement with reports from other orthopterans (Hunt and Charnley, 1981; Ulrich *et al.*, 1981) and other insects (Köhler *et al.*, 2012), and has been attributed to characteristics that make the midgut less hospitable to bacteria than other regions of the alimentary tract (Douglas, 2015). The midgut in insects secretes a host of digestive enzymes, is immunologically active, and lined by the peritrophic membrane, which acts as a protective barrier that restricts microbes to the lumen and protects the epithelium (Douglas, 2015). In the two orthopterans that have been studied in detail, bacteria are found in the midgut lumen but not in association with the epithelium (Hunt and Charnley, 1981;

353 Mead *et al.*, 1988). As a consequence, midgut bacteria might need to be continually replenished
354 from ingested food (Blum *et al.*, 2013) because the peritrophic membrane is continually shed
355 into the hindgut. In some insects, specialized midgut crypts provide niches that microbes
356 colonize (Kikuchi *et al.*, 2005; Bistolas *et al.*, 2014), however we did not observe analogous
357 structures in Mormon crickets (Fig. 1).

358 The midgut is particularly vulnerable to pathogens because the lack of an endocuticle
359 leaves the epithelium exposed once the peritrophic membrane is penetrated (Lehane and
360 Billingsley, 1996; Copping and Menn, 2000; Ruud A. de Maagd *et al.*, 2003; Nehme *et al.*,
361 2007). The Mormon cricket midgut was populated by lactic-acid bacteria, with *Pediococcus*
362 specifically exhibiting greater abundance in the midgut (and hindgut) than in the foregut. Lactic-
363 acid bacteria are known for their beneficial effects in insects, increasing resistance to parasites in
364 bees (Forsgren *et al.*, 2010) and promoting development in fruit flies by enhancing proteolytic
365 activity (Erkosar *et al.*, 2015) and upregulating host ecdysone and insulin-like peptides (Storelli
366 *et al.*, 2011). Lactic-acid bacteria are also known to suppress pathogenic bacteria by reducing pH
367 and producing a number of antimicrobial compounds, such as hydrogen peroxide and
368 bacteriocins (Cintas *et al.*, 2001).

369 A previous study found that sexual interactions in Mormon crickets influences the
370 abundance of three *Pediococcus* phylotypes (Smith *et al.*, 2016), however spatial information on
371 where in the gut *Pediococcus* is located has been unavailable until now. *Pediococcus* in the
372 midgut could provide immunological or nutritional benefits to Mormon crickets, as has been
373 shown for *P. acidilactici* in other animals (Castex *et al.*, 2009, 2009). The cultured isolates of *P.*
374 *acidilactici* obtained from Mormon crickets in this study will enable future experimental and
375 comparative genomic approaches to evaluate these hypotheses.

376 Lactic-acid bacteria were also common in the foregut, which was dominated by a
377 *Lactobacillus sp.* that averaged 73.9% of the sequences recovered from this region. Bignell
378 (1984) noted that the foregut of insects tends to be the most acidic compartment, however studies
379 that measure the physiochemical environment and characterize microbiome composition of the
380 foregut are rare (but see Köhler *et al.*, 2012). This is because the endocuticle, lack of
381 differentiated cells for absorption of nutrients, and frequent purging of consumed material into
382 the midgut provides little opportunity for foregut microbes to contribute to host nutrition. The
383 large differences in community structure between the foregut and the rest of the alimentary tract
384 in our study does illustrate the dramatic transition in microbial communities between what is
385 ingested and what can colonize the more distal regions of the gut.
386 In contrast to the foregut and midgut, the hindgut was characterized by a dramatic
387 increase in enteric bacteria (Enterobacteriaceae). Ordination of the laboratory Mormon cricket
388 samples indicated that the rectum, not the ileum, was primarily responsible for the difference in
389 community structure in the hindgut. Enterobacteriaceae comprised 83.5% of the sequences from
390 the rectum compared to 57.5% from the ileum, which was more similar to the midgut in
391 community structure (Fig. 5). This distinction is potentially important because higher digestive
392 efficiency in conventional compared to germ-free crickets has been attributed to microbial
393 colonization of the ileum in the orthopteran *Achetus domesticus* (Kaufman and Klug, 1991).
394 Detailed taxonomic information on the gut microbiota of *A. domesticus* or are not yet available
395 for comparison to our study (but see Santo Domingo *et al.*, 1998).
396 Of the three enteric bacteria represented in this study, *Pantoea agglomerans* was common
397 to both field and lab individuals and increased in abundance in the hindgut. *Pantoea* are known
398 plant pathogens and have been associated with a variety of medical conditions in humans

399 (Walterson and Stavrinides, 2015). In insects, however, *Pantoea* have been shown to have
400 mutualistic associations with their host. They are required for the completion of development in
401 stinkbugs (Hosokawa *et al.*, 2016; but see Dillon and Charnley, 2002), produce compounds that
402 attract insects to their host plants in flies (Robacker *et al.*, 2004; Maccollom *et al.*, 2009), and in
403 the orthopteran *Schistocerca gregaria*, produce a key component of the locust aggregation
404 pheromone (Dillon *et al.*, 2000, 2002) and reduce susceptibility to entomopathogens (Dillon and
405 Charnley, 1986, 1995). *P. agglomerans* similarly occurs at its highest abundance in the hindgut
406 of *S. gregaria*, with histological surveys showing that enterobacteria colonize the cuticle within
407 crevices formed by the rectal papillae (Hunt and Charnley, 1981). Whether *P. agglomerans*
408 similarly protects Mormon crickets from their own fungal entomopathogens (MacVean and
409 Capinera, 1991) or influences its aggregation behavior (Wakeland, 1959; MacVean, 1987) is an
410 important direction for future research.

411 *Conclusion*

412 Variation in morphology and physiology is thought to differentiate niches within the gut that
413 influence the organization of the microbiome. Our study describes at high resolution how
414 bacterial communities vary among gut regions, and suggests that host-microbe and/or microbe-
415 microbe interactions have a role in how microbial communities are assembled and maintained.
416 While the taxonomic information gleaned from our study suggests that some of these bacteria
417 might benefit Mormon cricket nutrition, immunity, and perhaps even modulate social behavior,
418 experiments are needed to evaluate this possibility. Our establishment of methods for culturing
419 Mormon cricket gut bacteria will enable experimental and comparative genomic approaches in
420 the future to infer the ecological and evolutionary consequences of host-microbe symbiosis.

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References

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2013) lme4: Linear mixed-effects models using S4 classes.

Benjamini, Y. and Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B Methodol.* **57**: 289–300.

Bignell, D.E. (1984) The arthropod gut as an environment for microorganisms. In, Anderson,J.M., Rayner,A.D.M., and Walton,D.W.H. (eds), *Invertebrate-Microbial Interactions*. Cambridge University Press, Cambridge, pp. 205–227.

Bistolas, K.S.I., Sakamoto, R.I., Fernandes, J.A.M., and Goffredi, S.K. (2014) Symbiont polyphyly, co-evolution, and necessity in pentatomid stinkbugs from Costa Rica. *Microb. Symbioses* **5**: 349.

Blum, J.E., Fischer, C.N., Miles, J., and Handelsman, J. (2013) Frequent Replenishment Sustains the Beneficial Microbiome of Drosophila melanogaster. *mBio* **4**: e00860-13.

Brenner, D.J., Krieg, N.R., Staley, J.T., Garrity, G.M., Boone, D.R., De Vos, P., et al. eds. (2005) Bergey's Manual® of Systematic Bacteriology Springer US, Boston, MA.

Bridson, E.Y. ed. (1998) The Oxoid Manual 8th ed. Oxoid LTD, Basingstoke, United Kingdom.

Brune, A. and Dietrich, C. (2015) The Gut Microbiota of Termites: Digesting the Diversity in the Light of Ecology and Evolution. *Annu. Rev. Microbiol.* **69**: 145–166.

Buchon, N., Broderick, N.A., and Lemaitre, B. (2013) Gut homeostasis in a microbial world: insights from *Drosophila melanogaster*. *Nat. Rev. Microbiol.* **11**: 615–626.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13**: 581–583.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **7**: 335–336.

Castex, M., Lemaire, P., Wabete, N., and Chim, L. (2009) Effect of dietary probiotic *Pediococcus acidilactici* on antioxidant defences and oxidative stress status of shrimp *Litopenaeus stylirostris*. *Aquaculture* **294**: 306–313.

Cho, I. and Blaser, M.J. (2012) The human microbiome: at the interface of health and disease. *Nat. Rev. Genet.* **13**: 260–270.

Cintas, L.M., Casaus, M.P., Herranz, C., Nes, I.F., and Hernández, P.E. (2001) Review: Bacteriocins of Lactic Acid Bacteria. *Food Sci. Technol. Int.* **7**: 281–305.

Copping, L.G. and Menn, J.J. (2000) Biopesticides: a review of their action, applications and efficacy. *Pest Manag. Sci.* **56**: 651–676.

Dillon, R. and Charnley, A. (1986) Inhibition of Metarhizium anisopliae by the gut bacterial flora of the desert locust, Schistocerca gregaria: Evidence for an antifungal toxin. *J. Invertebr. Pathol.* **47**: 350–360.

Dillon, R. and Charnley, K. (2002) Mutualism between the desert locust *Schistocerca gregaria* and its gut microbiota. *Res. Microbiol.* **153**: 503–509.

Dillon, R. j., Vennard, C. t., and Charnley, A. k. (2002) A Note: Gut bacteria produce components of a locust cohesion pheromone. *J. Appl. Microbiol.* **92**: 759–763.

Dillon, R.J. and Charnley, A.K. (1995) Chemical Barriers to Gut Infection in the Desert Locust: In Vivo Production of Antimicrobial Phenols Associated with the Bacterium Pantoea agglomerans. *J. Invertebr. Pathol.* **66**: 72–75.

473 Dillon, R.J. and Dillon, V.M. (2004) The gut bacteria of insects: nonpathogenic interactions.
474 *Annu. Rev. Entomol.* **49**: 71–92.
475 Dillon, R.J., Vennard, C.T., and Charnley, A.K. (2000) Pheromones: Exploitation of gut bacteria
476 in the locust. *Nature* **403**: 851–851.
477 Douglas, A.E. (2013) Alimentary canal, digestion and absorption. In, Simpson,S.. J.,
478 Douglas,A.E., and Chapman,R.F. (eds), *The Insects: Structure and Function*. Cambridge
479 University Press, Cambridge, pp. 46–80.
480 Douglas, A.E. (2015) Multiorganismal Insects: Diversity and Function of Resident
481 Microorganisms. *Annu. Rev. Entomol.* **60**: 17–34.
482 Engel, P. and Moran, N.A. (2013) The gut microbiota of insects – diversity in structure and
483 function. *FEMS Microbiol. Rev.* **37**: 699–735.
484 Erkosar, B., Storelli, G., Mitchell, M., Bozonnet, L., Bozonnet, N., and Leulier, F. (2015)
485 Pathogen virulence impedes mutualist-mediated enhancement of host juvenile growth via
486 inhibition of protein digestion. *Cell Host Microbe* **18**: 445–455.
487 Faraway, J.J. (2006) Extending the linear model with R: generalized linear, mixed effects and
488 nonparametric regression models Chapman & Hall/CRC, New York.
489 Forsgren, E., Olofsson, T.C., Vásquez, A., and Fries, I. (2010) Novel lactic acid bacteria
490 inhibiting *Paenibacillus* larvae in honey bee larvae. *Apidologie* **41**: 99–108.
491 Gwynne, D.T. (1984) Sexual selection and sexual differences in Mormon crickets (Orthoptera:
492 Tettigoniidae, *Anabrus simplex*). *Evolution* **38**: 1011–1022.
493 Han, M.V. and Zmasek, C.M. (2009) phyloXML: XML for evolutionary biology and
494 comparative genomics. *BMC Bioinformatics* **10**: 356.
495 Hosokawa, T., Ishii, Y., Nikoh, N., Fujie, M., Satoh, N., and Fukatsu, T. (2016) Obligate
496 bacterial mutualists evolving from environmental bacteria in natural insect populations.
497 *Nat. Microbiol.* **1**: 15011.
498 Hothorn, T., Bretz, F., and Westfall, P. (2008) Simultaneous Inference in General Parametric
499 Models. *Biom. J.* **50**: 346–363.
500 Hunt, J. and Charnley, A.K. (1981) Abundance and distribution of the gut flora of the desert
501 locust, Schistocerca gregaria. *J. Invertebr. Pathol.* **38**: 378–385.
502 Kaufman, M.G. and Klug, M.J. (1991) The contribution of hindgut bacteria to dietary
503 carbohydrate utilization by crickets (Orthoptera: Gryllidae). *Comp. Biochem. Physiol. A*
504 *Physiol.* **98**: 117–123.
505 Kikuchi, Y., Meng, X.-Y., and Fukatsu, T. (2005) Gut Symbiotic Bacteria of the Genus
506 Burkholderia in the Broad-Headed Bugs Riptortus clavatus and Leptocorisa chinensis
507 (Heteroptera: Alydidae). *Appl. Environ. Microbiol.* **71**: 4035–4043.
508 Köhler, T., Dietrich, C., Scheffrahn, R.H., and Brune, A. (2012) High-Resolution Analysis of
509 Gut Environment and Bacterial Microbiota Reveals Functional Compartmentation of the
510 Gut in Wood-Feeding Higher Termites (Nasutitermes spp.). *Appl. Environ. Microbiol.*
511 **78**: 4691–4701.
512 Lanan, M.C., Rodrigues, P.A.P., Agellon, A., Jansma, P., and Wheeler, D.E. (2016) A bacterial
513 filter protects and structures the gut microbiome of an insect. *ISME J.*
514 Lehane, M.J. and Billingsley, P.F. eds. (1996) Biology of the Insect Midgut Springer
515 Netherlands, Dordrecht.
516 Ley, R.E., Lozupone, C.A., Hamady, M., Knight, R., and Gordon, J.I. (2008) Worlds within
517 worlds: evolution of the vertebrate gut microbiota. *Nat. Rev. Microbiol.* **6**: 776–788.

Maccollom, G.B., Lauzon, C.R., Sjogren, R.E., Meyer, W.L., and Olday, F. (2009) Association and Attraction of Blueberry Maggot Fly Curran (Diptera: Tephritidae) to Pantoea (Enterobacter) agglomerans. *Environ. Entomol.* **38**: 116–120.

MacVean, C.. (1987) Ecology and management of the Mormon cricket, *Anabrus simplex*. In, *Integrated Pest Management on Rangeland: A Shortgrass Prairie Perspective*. Westview Press, Boulder, CO, pp. 116–136.

MacVean, C.M. and Capinera, J.L. (1991) Pathogenicity and transmission potential of Nosema locustae and Vairimorpha n. sp. (Protozoa: Microsporida) in Mormon crickets (Anabrus simplex; Orthoptera: Tettigoniidae): A laboratory evaluation. *J. Invertebr. Pathol.* **57**: 23–36.

Maddison, W.P. and Maddison, D.R. (2016) Mesquite: a modular system for evolutionary analysis.

McFrederick, Q.S., Cannone, J.J., Gutell, R.R., Kellner, K., Plowes, R.M., and Mueller, U.G. (2013) Specificity between *Lactobacilli* and hymenopteran hosts is the exception rather than the rule. *Appl. Environ. Microbiol.* **79**: 1803–1812.

McMurdie, P.J. and Holmes, S. (2013) Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **8**: e61217.

Mead, L.J., Khachatourians, G.G., and Jones, G.A. (1988) Microbial Ecology of the Gut in Laboratory Stocks of the Migratory Grasshopper, Melanoplus sanguinipes (Fab.) (Orthoptera: Acrididae). *Appl. Environ. Microbiol.* **54**: 1174–1181.

Moran, N.A., McCutcheon, J.P., and Nakabachi, A. (2008) Genomics and Evolution of Heritable Bacterial Symbionts. *Annu. Rev. Genet.* **42**: 165–190.

Nehme, N.T., Liégeois, S., Kele, B., Giammarinaro, P., Pradel, E., Hoffmann, J.A., et al. (2007) A Model of Bacterial Intestinal Infections in Drosophila melanogaster. *PLOS Pathog* **3**: e173.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., et al. (2015) vegan: Community Ecology Package.

Powell, J.E., Martinson, V.G., Urban-Mead, K., and Moran, N.A. (2014) Routes of acquisition of the gut microbiota of the honey bee *Apis mellifera*. *Appl. Environ. Microbiol.* **80**: 7378–7387.

Pruesse, E., Peplies, J., and Glockner, F.O. (2012) SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* **28**: 1823–1829.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**: D590–D596.

R Core Development Team (2013) R: A language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria.

Robacker, D.C., Lauzon, C.R., and He, X. (2004) Volatiles Production and Attractiveness to the Mexican Fruit Fly of Enterobacter agglomerans Isolated from Apple Maggot and Mexican Fruit Flies. *J. Chem. Ecol.* **30**: 1329–1347.

Ruud A. de Maagd, Alejandra Bravo, Colin Berry, Neil Crickmore, and Schnepf, H.E. (2003) Structure, Diversity, and Evolution of Protein Toxins from Spore-Forming Entomopathogenic Bacteria. *Annu. Rev. Genet.* **37**: 409–433.

Ryu, J.-H., Ha, E.-M., and Lee, W.-J. (2010) Innate immunity and gut–microbe mutualism in *Drosophila*. *Dev. Comp. Immunol.* **34**: 369–376.

563 Santo Domingo, J.W., Kaufman, M.G., Klug, M.J., Holben, W.E., Harris, D., and Tiedje, J.M.
564 (1998) Influence of diet on the structure and function of the bacterial hindgut community
565 of crickets. *Mol. Ecol.* **7**: 761–767.
566 Smith, C.C., Srygley, R.B., Dietrich, E.I., and Mueller, U. (2016) Mating promotes lactic-acid
567 gut bacteria in a gift-giving insect. *bioRxiv*.
568 Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of
569 large phylogenies. *Bioinformatics* **30**: 1312–1313.
570 Storelli, G., Defaye, A., Erkosar, B., Hols, P., Royet, J., and Leulier, F. (2011) *Lactobacillus*
571 *plantarum* promotes *Drosophila* systemic growth by modulating hormonal signals
572 through TOR-dependent nutrient sensing. *Cell Metab.* **14**: 403–414.
573 Tai, V., Carpenter, K.J., Weber, P.K., Nalepa, C.A., Perlman, S.J., and Keeling, P.J. (2016)
574 Genome Evolution and Nitrogen Fixation in Bacterial Ectosymbionts of a Protist
575 Inhabiting Wood-Feeding Cockroaches. *Appl. Environ. Microbiol.* **82**: 4682–4695.
576 Ulrich, R.G., Buthala, D.A., and Klug, M.J. (1981) Microbiota associated with the
577 gastrointestinal tract of the common house cricket, *Acheta domestica*. *Appl. Environ.*
578 *Microbiol.* **41**: 246–254.
579 Venables, W.N. and Ripley, B.D. (2002) Modern Applied Statistics with S Fourth. Springer,
580 New York.
581 Wakeland, C.C. (1959) Mormon crickets in North America US Dept. of Agriculture.
582 Walterson, A.M. and Stavrinides, J. (2015) Pantoea: insights into a highly versatile and diverse
583 genus within the Enterobacteriaceae. *FEMS Microbiol. Rev.* fuv027.
584 Woodring, J. and Lorenz, M.W. (2007) Feeding, nutrient flow, and functional gut morphology in
585 the cricket Gryllus bimaculatus. *J. Morphol.* **268**: 815–825.
586 Yuan, S., Cohen, D.B., Ravel, J., Abdo, Z., and Forney, L.J. (2012) Evaluation of Methods for
587 the Extraction and Purification of DNA from the Human Microbiome. *PLoS ONE* **7**:
588 e33865.
589

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Table 1. Analysis of deviance comparing alpha diversity between source populations (wild or laboratory) and among tissue types (foregut, midgut, and hindgut). Values represent the F-statistic (p-value) for each term. Statistically significant terms ($p<0.05$) are indicated in bold. Degrees of freedom were estimated using the Kenward-Rogers approximation.

	Reduced Dataset			**Full Dataset**		
	Species Richness	Chao1	Shannon-Weiner	Species Richness	Chao1	Shannon-Weiner
Source	**13.9 (0.003)**	**11.0 (0.007)**	**9.17(0.01)**	**14.0(0.002)**	**10.5 (0.006)**	**8.22 (0.013)**
Tissue type	**5.85 (0.010)**	**4.79 (0.02)**	**7.07 (0.005)**	**6.77 (0.004)**	**5.68 (0.008)**	**8.44 (0.001)**
Interaction	0.51 (0.61)	1.02 (0.38)	0.98 (0.39)	0.66 (0.53)	1.15 (0.33)	1.28 (0.29)

Table 2. Permutation test from distance-based redundancy analysis comparing Bray-Curtis distance between source populations (wild or laboratory) and among tissue types (foregut, midgut and hindgut).

	Reduced Dataset		**Full Dataset**	
	F	*P*	*F*	*P*
Source	**8.99**	**<0.001**	**7.75**	**<0.001**
Tissue type	**9.85**	**<0.001**	**5.49**	**<0.001**
Interaction	0.26	0.47	0.52	0.84

Table 3. Likelihood ratio tests from GLMMs fitting the abundance of OTUs to source population (wild or laboratory) and tissue type (foregut, midgut or hindgut). Values are Chi-square (p-value). LAC1=*Lactobacillus sp.*, PED=*Pediococcus*, PAG=*Pantoea agglomerans*.

	Reduced Dataset			**Full Dataset**		
	LAC1	PED	PAG	LAC1	PED	PAG
Source	1.32 (0.25)	0.80 (0.37)	2.36 (0.12)	0.18 (0.66)	0.51 (0.48)	0.01 (0.96)
Tissue type	**16.3 (<0.001)**	**9.25 (0.10)**	**20.7 (<0.001)**	**12.7 (0.002)**	**16.1 (<0.001)**	**41.9 (<0.001)**
Interaction	0.36 (0.84)	0.84 (0.66)	3.86 (0.15)	0.3 (0.86)	0.95 (0.62)	2.67 (0.26)

Table 4. Posthoc Tukey tests comparing the total number of 16s RNA copies between tissue types in wild and laboratory-raised Mormon crickets. Values are the test statistic with the significance of the test indicated with an asterisk. Comparisons within field-caught individuals are on the bottom diagonal and comparisons within laboratory-raised individuals are on the upper diagonal. FG=foregut; MG=midgut; ILE=ileum; REC=rectum.

	FG	MG	ILE	REC
FG		2.38	2.76*	0.21
MG	3.82***		0.39	2.60*
ILE	1.26	2.61*		2.98**
REC	0.64	3.28**	0.66	

* p=<0.05, ** p<0.01, *** p<0.001

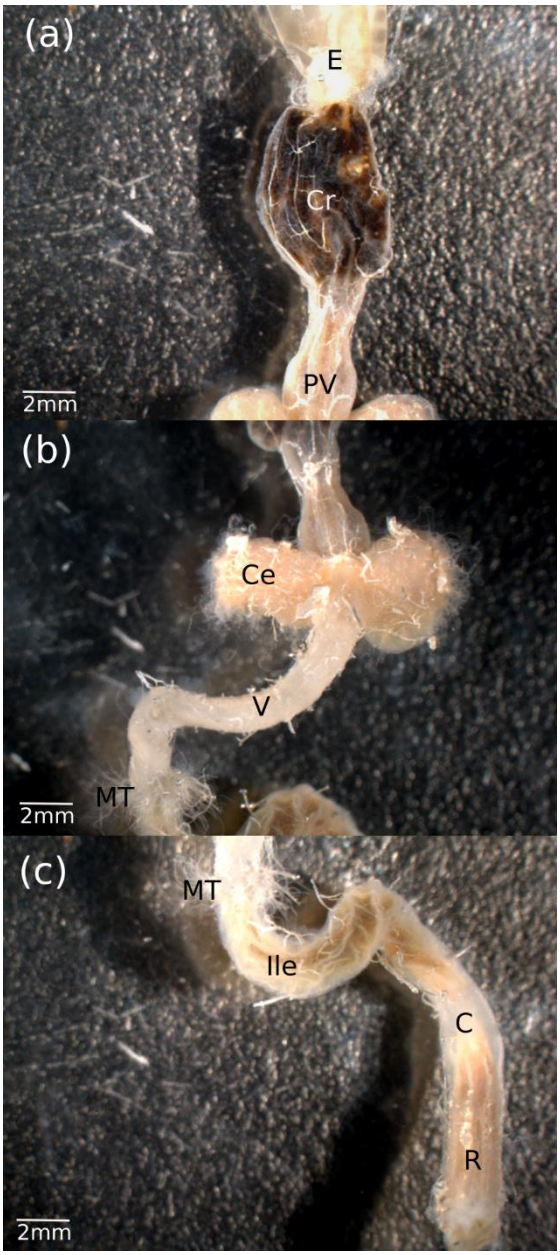

Figure 1. External morphology of the (a) foregut, (b) midgut, and (c) hindgut in the Mormon cricket. E=esophagus, Cr=crop, PV=proventriculus, Ce=cecum, V=ventriculus, MT=Malphigian tubules, Ile=ileum, C=colon, R=rectum. Malphigian tubules have been trimmed to illustrate their entry point into the hindgut.

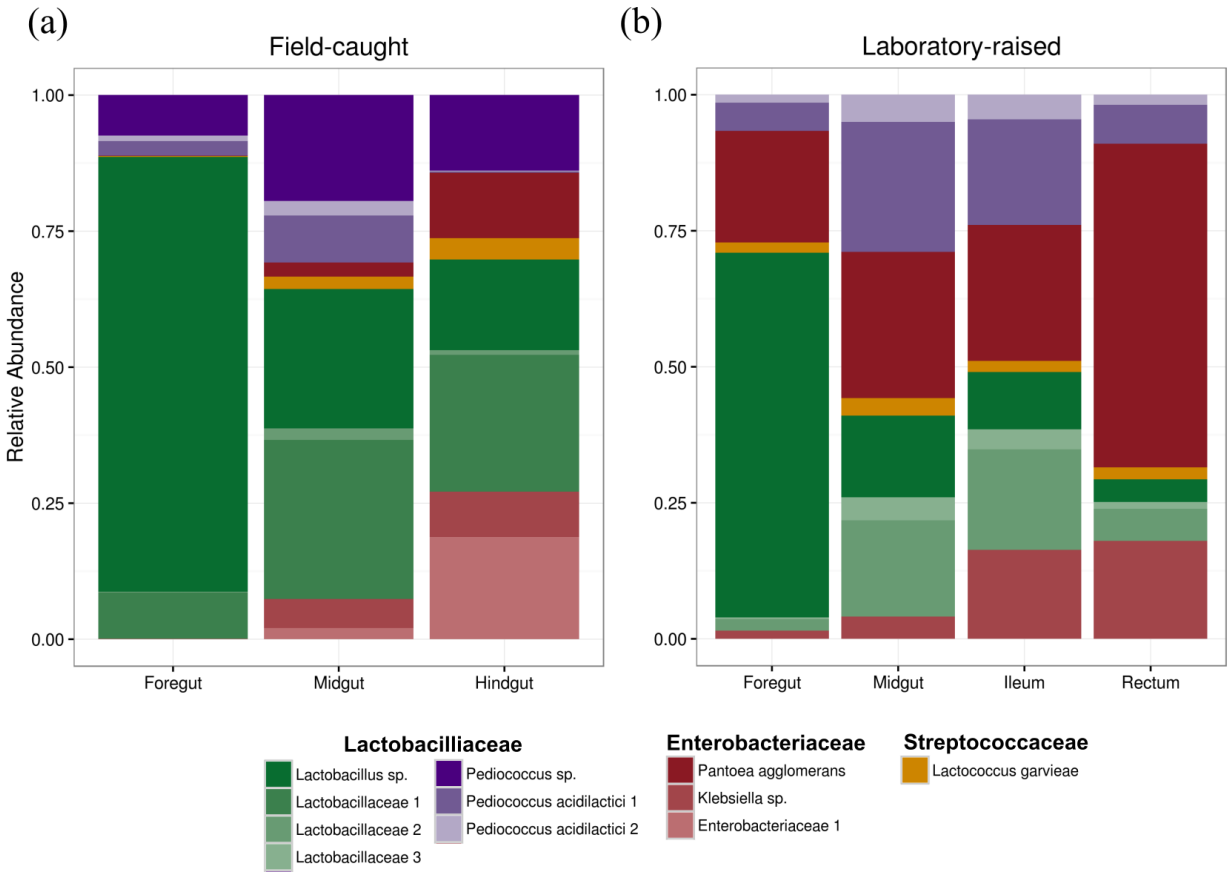

Figure 2. Mean relative abundance of the eleven dominant OTUs from (a) field-caught and (b) laboratory-raised Mormon crickets from 16s rRNA Illumina sequencing.

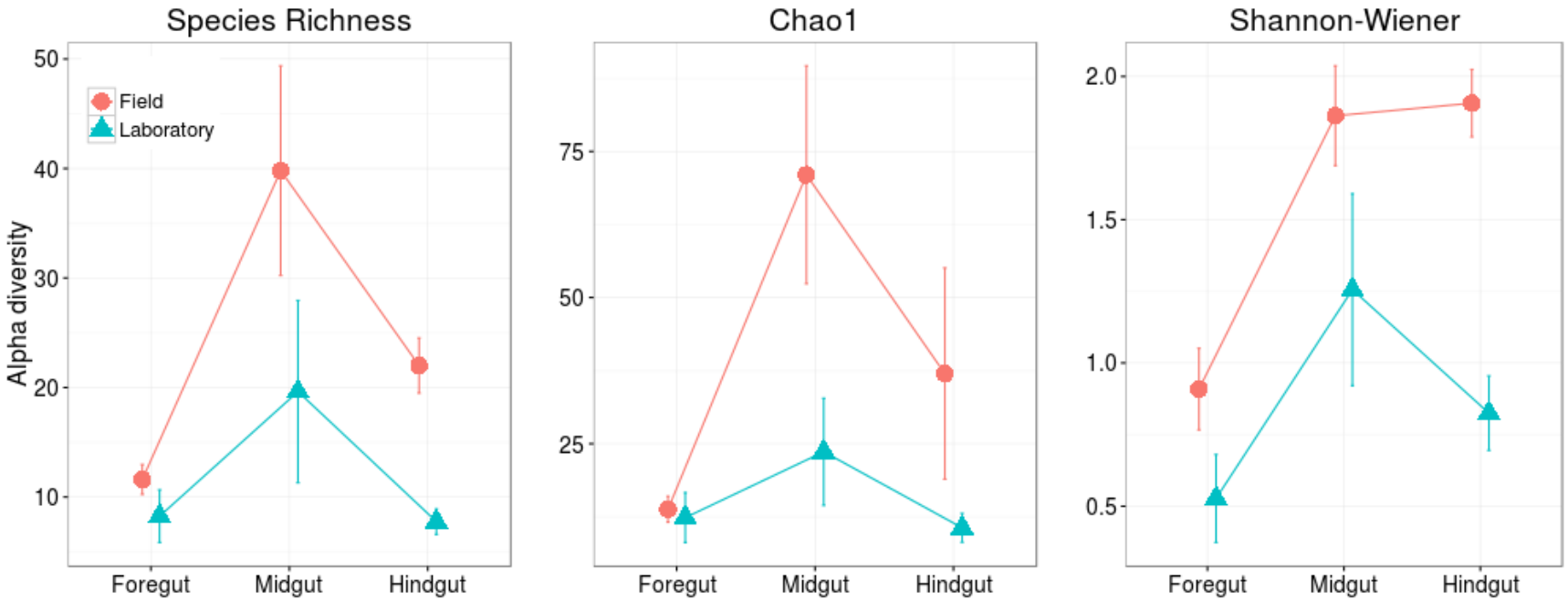

Figure 3. Alpha diversity in field and laboratory-raised Mormon crickets. Means ± SE are depicted for each region of the gut.

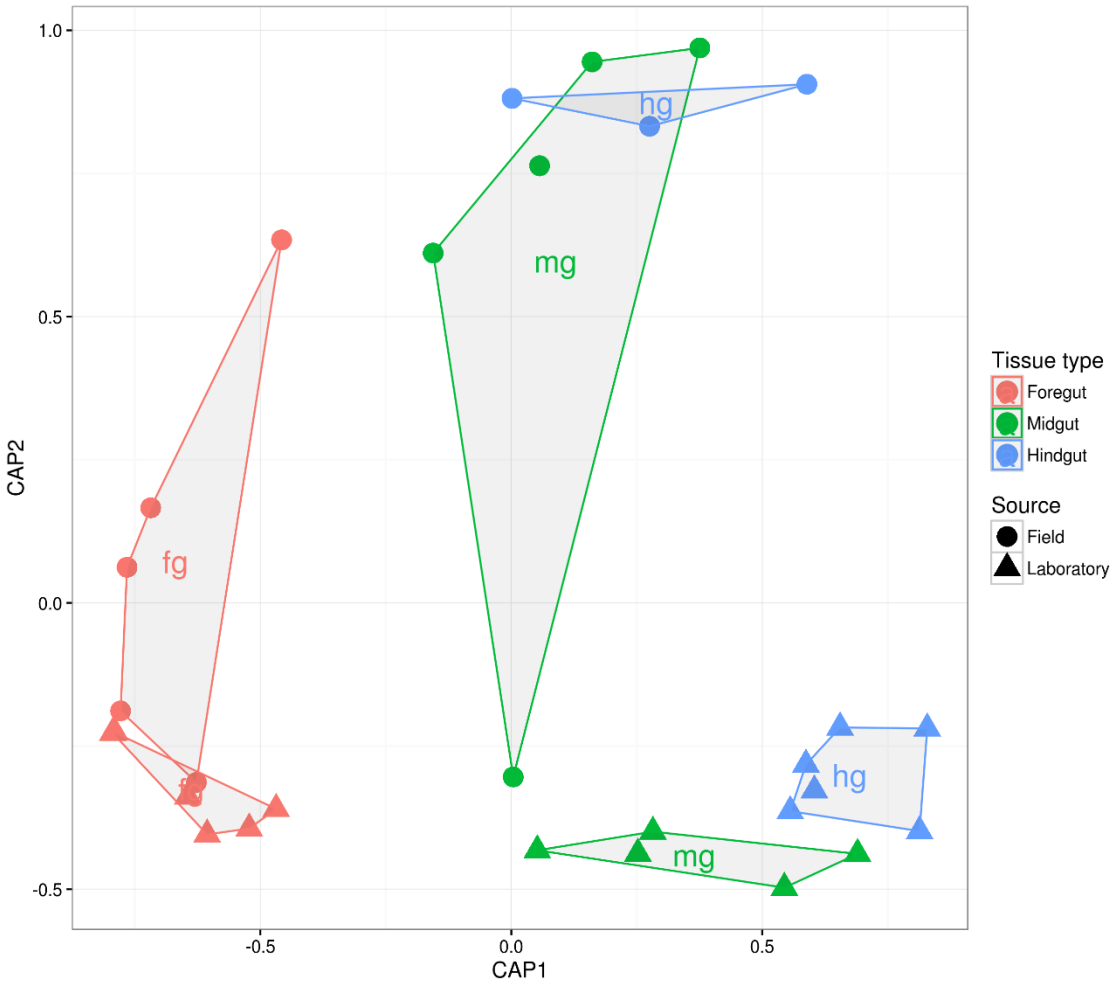

Figure 4. Ordination of sample scores from the db-RDA of the reduced dataset.

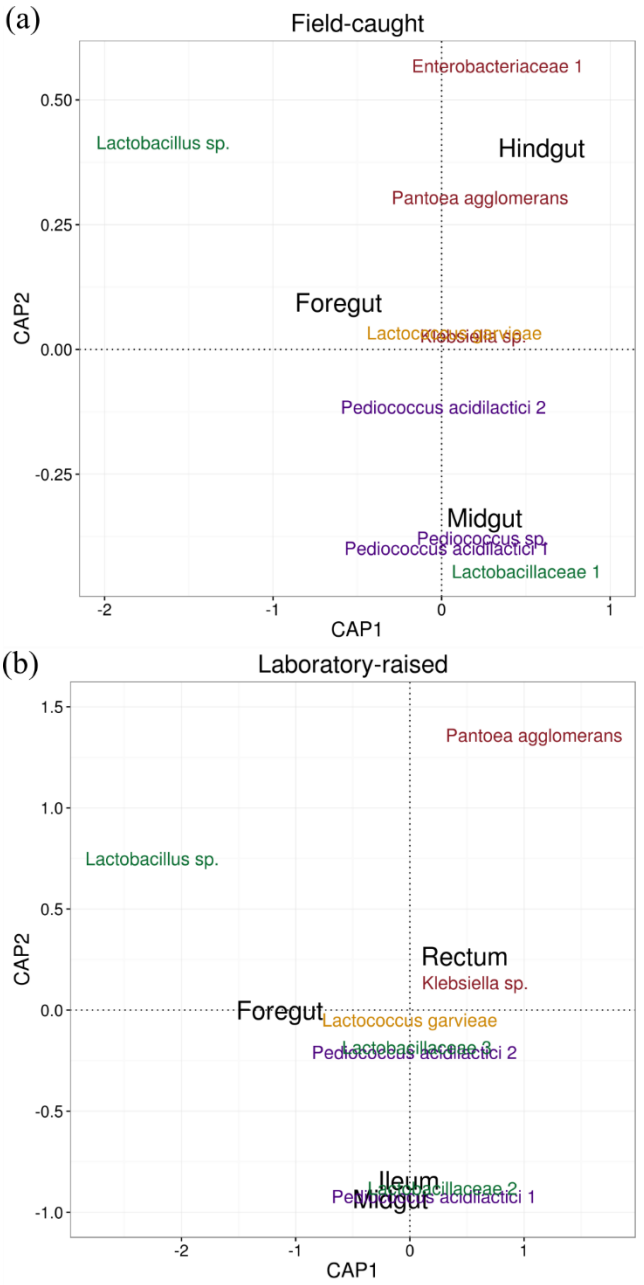

Figure 5. Ordination of OTU scores from db-RDA of (a) field-caught and (b) laboratory-raised (reduced dataset) Mormon crickets. Means of sample scores for each tissue type are indicated. OTUs are colored to represent taxonomic groups as in Figure 1.

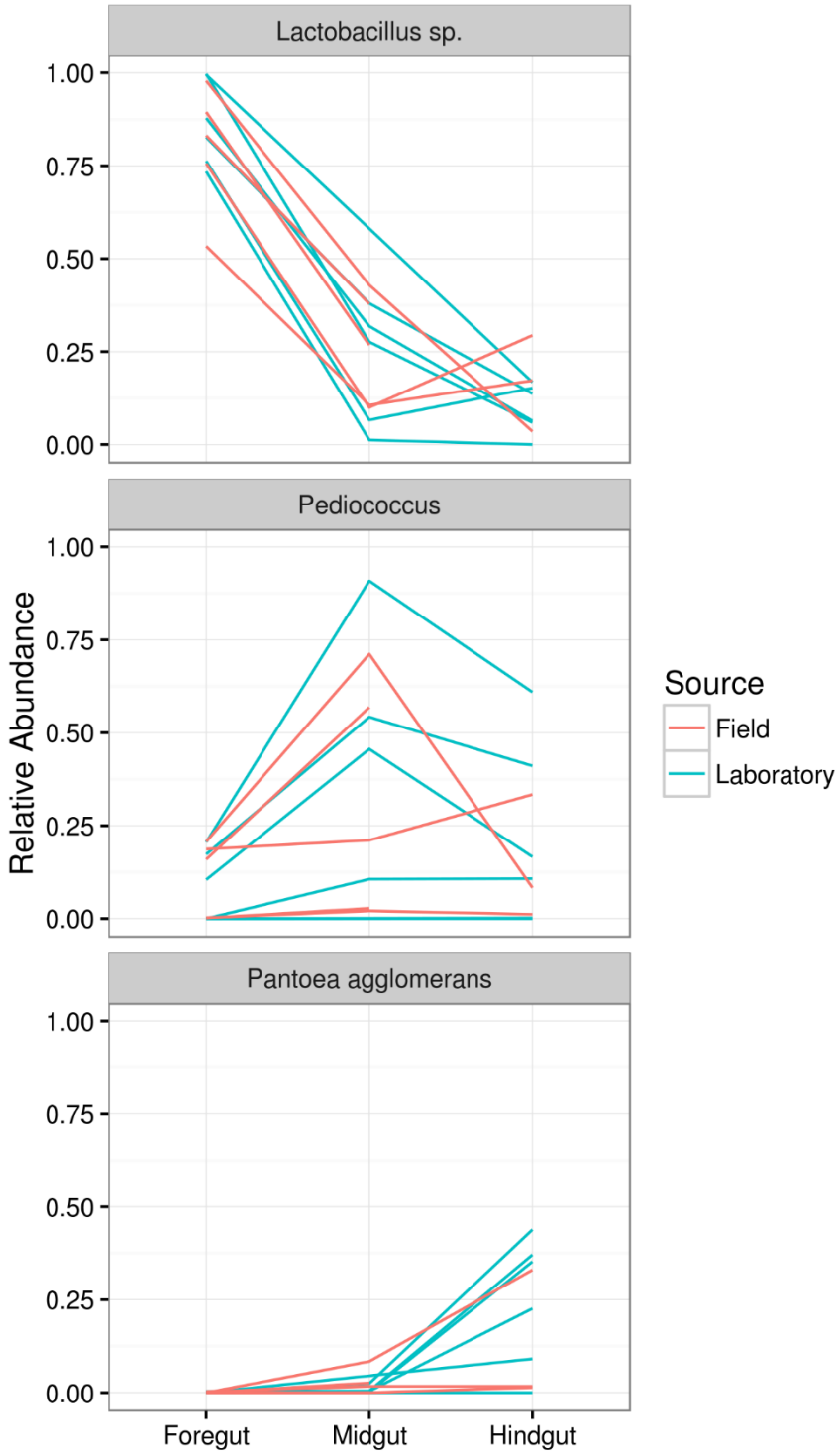

Figure 6. Relative abundance of *Lactobacillus sp.*, *Pediococcus*, and *Pantoea agglomerans* from the field and laboratory (reduced dataset) identified in the ordination (see Fig. 5) as associated with different gut regions. Each line represents an individual Mormon cricket.

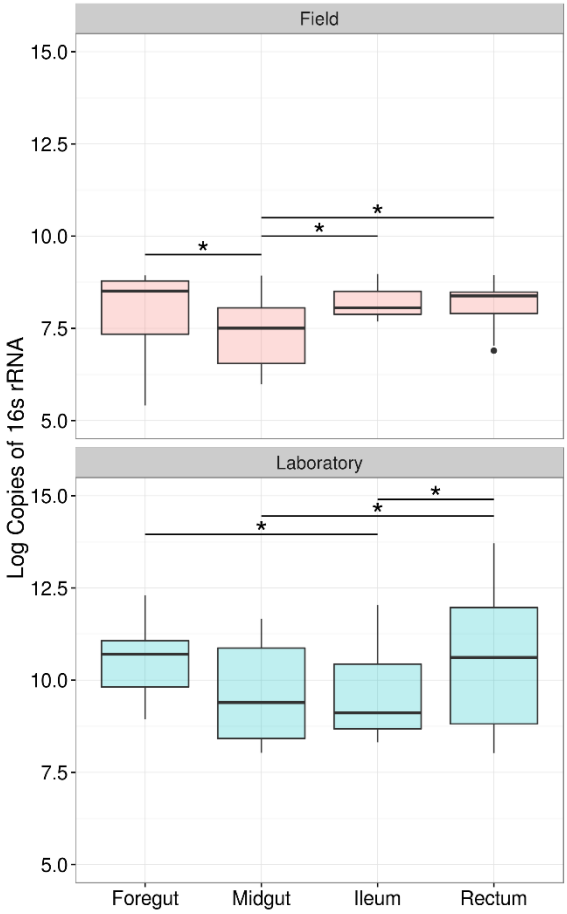

Figure 7. Abundance of bacterial 16s rRNA genes in the Mormon crickets gut. Bars indicate significant differences between regions (* $p<0.05$).

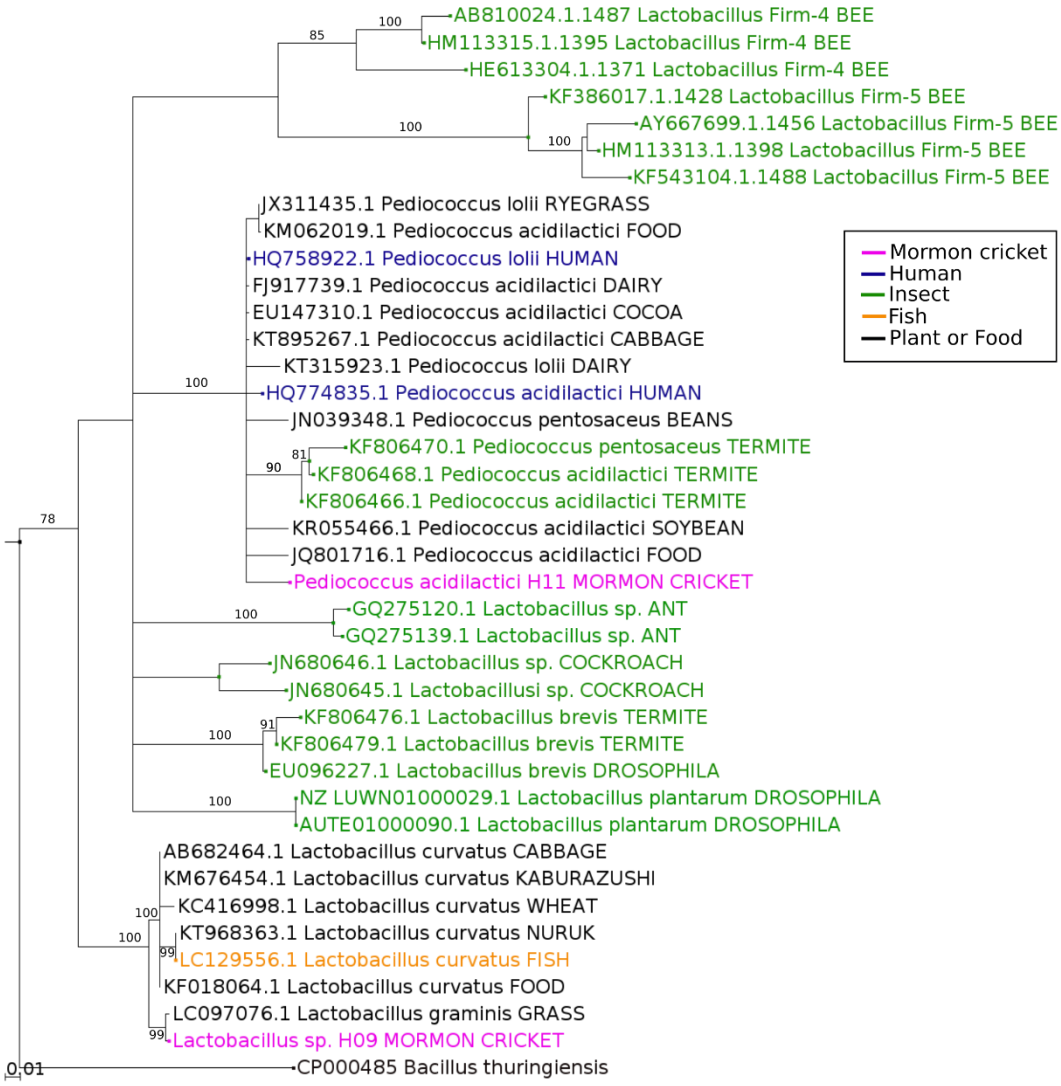

Fig 8. Maximum likelihood estimation of phylogenetic relationships among lactic-acid bacteria 16S rRNA sequences from Mormon cricket gut isolates and their relatives. Branches with bootstrap support <75% are collapsed.

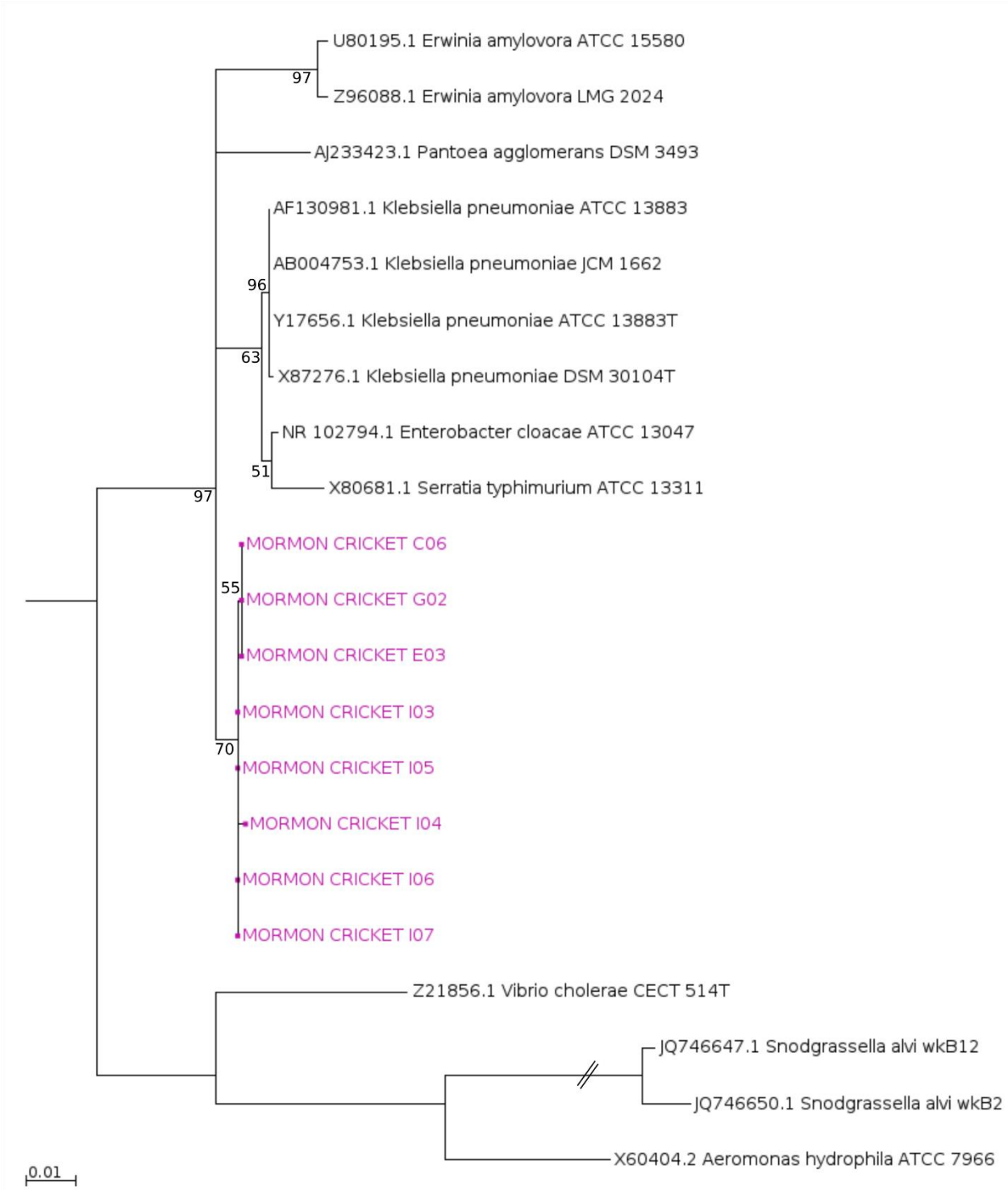

Figure 9. Maximum likelihood estimation of phylogenetic relationships among enteric bacteria 16S rRNA sequences from Mormon cricket gut isolates and type strains. Branches with bootstrap support <50% are collapsed