*Journal of Mammalogy*, 104(1):146–158, 2023
https://doi.org/10.1093/jmammal/gyac098
Published online November 11, 2022

# Mycoplasmataceae dominate microbial community differences between gut regions in mammals with a simple gut architecture

Mark T. Swanson,[1,*] Michael W. Henson,[2] Heru Handika,[1] Anang S. Achmadi,[3] Syahfitri Anita,[3] Kevin C. Rowe,[4,5] and Jacob A. Esselstyn[1]

[1]*Museum of Natural Science and Department of Biological Sciences, 119 Foster Hall, Louisiana State University, Baton Rouge, Louisiana 70803, USA*
[2]*Department of Geophysical Sciences, University of Chicago, Illinois 60616, USA*
[3]*Museum Zoologicum Bogoriense, Research Center for Biosystematics and Evolution, Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN), Cibinong 16912, Indonesia*
[4]*Sciences Department, Museums Victoria Research Institute, G.P.O. Box 666, Melbourne, Victoria 3001, Australia*
[5]*School of Biosciences, University of Melbourne, Royal Parade, Parkville, Melbourne, Victoria 3052, Australia*

*Associate Editor was Jorge Ortega.***To whom correspondence should be addressed: mtswanson2017@gmail.com*

Faunivorous mammals with simple guts are thought to rely primarily on endogenously produced enzymes to digest food, in part because they lack fermentation chambers for facilitating mutualistic interactions with microbes. However, variation in microbial community composition along the length of the gastrointestinal tract has yet to be assessed in faunivorous species with simple guts. We tested for differences in bacterial taxon abundances and community compositions between the small intestines and colons of 26 individuals representing four species of shrew in the genus *Crocidura*. We sampled these hosts from a single locality on Sulawesi Island, Indonesia, to control for potential geographic and temporal variation. Bacterial community composition differed significantly between the two gut regions and members of the family Mycoplasmataceae contributed substantially to these differences. Three operational taxonomic units (OTUs) of an unclassified genus in this family were more abundant in the small intestine, whereas 1 OTU of genus *Ureaplasma* was more abundant in the colon. Species of *Ureaplasma* encode an enzyme that degrades urea, a metabolic byproduct of protein catabolism. Additionally, a *Hafnia–Obesumbacterium* OTU, a genus known to produce chitinase in bat gastrointestinal tracts, was also more abundant in the colon compared to the small intestine. The presence of putative chitinase- and urease-producing bacteria in shrew guts suggests mutualisms with microorganisms play a role in facilitating the protein-rich, faunivorous diets of simple gut mammals.

Key words: Eulipotyphla, *Hafnia*, host–microbe, microbial ecology, Soricidae, *Ureaplasma*

Mamalia faunivora dengan usus sederhana diperkirakan mengandalkan sejumlah enzim yang diproduksi secara endogen untuk mencerna makanan, salah satu alasannya di satu sisi karena mereka tidak memiliki ruang fermentasi untuk memfasilitasi interaksi mutualistik dengan mikroba. Namun begitu, variasi komposisi komunitas mikroba di sepanjang saluran pencernaan dari spesies-spesies faunivora dengan usus sederhana masih perlu dipelajari lebih lanjut. Kami menguji perbedaan-perbedaan dalam hal kelimpahan taksa dan komposisi komunitas bakteri antara usus halus dengan usus besar pada 26 individu yang merepresentasikan 4 spesies celurut genus *Crocidura*. Kami mengambil sampel inang dari satu lokasi tunggal di Sulawesi, Indonesia, demi mengendalikan variasi geografis dan temporal yang mungkin terjadi. Komposisi komunitas bakteri berbeda secara signifikan antara dua bagian usus, dan anggota-anggota dari keluarga famili Mycoplasmataceae berkontribusi besar terhadap perbedaan ini. Dalam famili ini, tiga OTU yang genusnya belum terklasifikasikan ternyata lebih melimpah di usus halus, sementara satu OTU dari genus *Ureaplasma* lebih melimpah di usus besar. Spesies *Ureaplasma* mengkodekan enzim yang mendegradasi urea, produk sampingan metabolik dari katabolisme protein. Selain itu, *Hafnia–Obesumbacterium* OTU, suatu genus yang diketahui memproduksi kitinase pada saluran pencernaan kelelawar ternyata juga lebih melimpah di usus besar dibandingkan dengan di usus halus. Keberadaan bakteri

yang terduga menghasilkan kitinase dan urease di saluran pencernaan celurut tersebut mengindikasikan bahwasannya mutualisme dengan mikroorganisme berperan penting dalam memfasilitasi asupan faunivora yang kaya protein pada mamalia berusus sederhana.

Kata kunci: Eulipotyphla, *Hafnia*, interaksi mamalia–mikroba inang–mikroba, ekologi mikrobial mikroba, celurut, *Ureaplasma*

It is unclear whether faunivorous mammals with simple guts obtain nutritional benefits from symbioses with microbial communities to the same extent as herbivorous species with more complex guts. Mammals cannot endogenously digest cellulose, the most abundant carbohydrate in nature (Karasov and Douglas 2013). Rather, herbivorous mammals rely on microbes to both hydrolyze complex carbohydrates and ferment the resultant sugars into readily absorbable short-chain fatty acids (SCFAs; Van Soest 1994; Karasov and Martinez del Rio 2007). Modeling animal gastrointestinal tracts as chemical reactors has shown that distinct chambers that increase the time microbes have to break down plant materials optimizes digestive efficiency in systems that rely on symbioses (Penry and Jumars 1987). This theoretical work provides an explanation for the evolution of fermentation chambers that facilitate microbial growth. These include the rumen in artiodactyls and the cecum in rodents and nonhuman primates (Karasov and Douglas 2013; Langer 2017). The same models predict that plug-flow reactors similar to the tubular intestine in other mammals are ideal for enhancing reactions between endogenously produced digestive enzymes and the digesta (Penry and Jumars 1987). Primarily faunivorous mammals such as carnivorans (e.g., cats, dogs, seals) and eulipotyphlans (e.g., moles, hedgehogs, shrews) have simple tubular intestines without distinct chambers for facilitating interactions with microbial communities (Langer 2017). In these animals, endogenously produced proteases, peptidases, and lipases break down animal material into readily absorbable substances. As such, the contribution of microbes to digestion is less obvious (Karasov and Douglas 2013).

Research characterizing the microbial communities of mammals with simple guts has shown that they generally have limited taxonomic diversity and lower abundances compared to herbivorous mammals with fermentation chambers (Ley et al. 2008; Milani et al. 2020). Bacterial communities in mammals with complex gastrointestinal tracts primarily consist of members of the phyla Bacteroidota (also known as Bacteroidetes) and Firmicutes, with smaller proportions of Proteobacteria and Actinobacteria (Ley et al. 2008; Sanders et al. 2015; Milani et al. 2020). In contrast, mammals with simple guts such as bats (Youngblut et al. 2019; Song et al. 2020), anteaters and armadillos (Delsuc et al. 2014), as well as raccoons and skunks (Zhu et al. 2018) generally have much higher proportions of Proteobacteria and fewer members of Firmicutes and Bacteroidota. Proteobacteria are particularly prominent components of insect gut microbial communities (Hu et al. 2018), obscuring whether the high proportions observed in insectivorous mammals are residents of mammalian gastrointestinal tracts or digesta from the guts of their insect prey. This has been documented in arctic shorebirds where the Proteobacteria in their intestines is primarily composed of *Ricketsiella*, an obligate arthropod symbiont, suggesting that the Proteobacteria are derived from the food of the birds (Grond et al. 2017).

In addition to Proteobacteria, faunivorous mammals with simple guts also harbor large proportions of the phylum Fusobacteria, which are almost absent in mammals with fermentation chambers (Milani et al. 2020). Fusobacteria make up about 29% of the reads recovered from domestic dogs and cats (Zhu et al. 2018), 26% in wild seals (Pacheco-Sandoval et al. 2019), and are also found in wild fossas and hedgehogs (Perofsky et al. 2019; Youngblut et al. 2019). However, this has not so far been found to be the case for shrews, faunivorous small mammals in the same order as hedgehogs (Eulipotyphla; Sato et al. 2019). Shrews prey on a broad array of metazoans including worms, insects, isopods, snails, and small vertebrates (Churchfield 1990).

Soricidae (shrews) is one of the most species-rich families of mammals with well over 400 described species (Burgin et al. 2018) that are divided into three subfamilies that differ in their metabolic traits and geographic distributions. Soricines are known as “hot shrews” for their exceptionally fast metabolisms while Crocidurines and Myosorines are known as “cold shrews” for having lower activity levels and being able to survive longer without food (Vogel 1976). To date, the gut microbial communities of shrews have been examined in two species of *Sorex* from Lithuania (Knowles et al. 2019) and another two species of *Sorex* from the western United States (Greiman et al. 2020). Similar to other faunivores with simple guts, all of these shrews had high proportions of Firmicutes, Proteobacteria, and Tenericutes. However, none had a substantial proportion (>1%) of Fusobacteria. Gut microbial communities have not been examined in either crocidurines or myosoricines, which, given their slower metabolism, may have distinct gut microbial communities.

A rarely examined aspect of bacterial diversity in mammals with simple guts is how microbial community composition might vary along the length of the gastrointestinal tract. As a microbial habitat, the small intestine differs substantially from the colon (Donaldson et al. 2015). The small intestine absorbs the greatest volume and diversity of nutrients, whereas water and SCFAs are absorbed primarily in the colon (Borgstrom et al. 1957; Karasov and Martinez del Rio 2007). To prevent pathogens from exploiting the highly permeable epithelium and nutrient-rich environment in the small intestine, Paneth cells secrete lysozyme and defensins that disrupt bacterial peptidoglycan cell walls (Porter et al. 2002; Karasov and Douglas 2013). The small intestine also differs from other regions of the gut in that urea, a metabolic byproduct of protein catabolism, is secreted into the small intestine in greater quantities (Fuller and Reeds 1998). In contrast, the colon has fewer readily available

nutrients and the colonic epithelium secretes fewer antimicrobial peptides or urea (Fuller and Reeds 1998; Karasov and Douglas 2013). Despite these stark differences, almost all studies of mammalian gut microbial communities use fecal samples, which do not necessarily contain residents of the mucosal layer of either the small intestine or colon (but see Ingala et al. 2018). Thus far, the only mammals with simple guts to have their bacterial communities characterized at different points in the gastrointestinal tract are black bears (Gillman et al. 2020). This effort found small differences in bacterial community structure between the small intestine and colon. Both gut regions housed primarily Firmicutes and Proteobacteria taxa but higher abundances of Actinobacteria in the small intestine and more Campilobacterota in the colon. Additionally, operational taxonomic units (OTUs) of *Helicobacter* and an unclassified genus in Enterobacteriaceae were more abundant in the colon compared to the small intestine. However, black bears alone cannot be considered representative of mammals with simple guts because they consume much more plant material than most carnivorans or eulipotyphlans.

Crocidurine shrews are diverse and abundant in the rainforests on Sulawesi Island in Indonesia, with local communities commonly containing several co-occurring species from a single endemic radiation (Esselstyn et al. 2019, 2021). We leveraged the high abundances of these animals to obtain a reasonable sample size and perform the most detailed characterization yet of shrew gut bacterial communities. Our goals were to test whether bacterial communities differed between the small intestine and colon and whether host species taxonomy affects bacterial community composition without geography or season as a confounding variable (Linnenbrink et al. 2013). We hypothesized that there are differences in bacterial community composition between the small intestine and colon of crocidurine shrews and that there would be differences between communities sampled from different co-occurring host species.

## Materials and Methods

*Sampling.*—We collected shrew specimens from Mount Ambang on Sulawesi Island in Indonesia over a single 2-week period (16 February to 1 March 2016) according to IACUC-approved and American Society of Mammalogists-recommended protocols (Sikes et al. 2016). We collected specimens using pitfall traps consisting of lines of 20-liter buckets sunk into the ground and spaced about 3 m apart. A tarp was used as a drift fence to direct shrews into the buckets. We euthanized each specimen and immediately removed the gastrointestinal tract using bleached forceps and a sterile scalpel blade. Prior to removal, a green string was tied below the stomach at the start of the small intestine and a red string was tied at the end of the colon just before the anus to provide orientation during subsampling. We placed whole gastrointestinal tracts in Nunc Cryogenic Tubes (ThermoFisher Scientific, Waltham, Massachusetts), immediately froze those tubes in liquid nitrogen, and later deposited them in the Louisiana State University Museum of Natural Science (LSUMZ; Supplementary Data SD1). We prepared liver tissue, study skin, and skeletal specimens of each animal and deposited them in the Museum Zoologicum Bogoriense (MZB), Museums Victoria (MV), and LSUMZ.

*Bacterial 16S rRNA gene sequencing.*—We sampled microbial communities from both the small intestine and colon of 29 shrews representing five species by thawing the intestinal tract section in a biosafety cabinet (The Baker Company, Stanford, Maine) and making an incision down the length of the tissue with autoclaved tools. Splayed intestinal tracts were then swabbed using sterile single-use cotton swabs with wood stems (Cardinal Health, Waukegan, Illinois) to collect microbes from both the gut tissue and contents using a single swab. This strategy minimized the number of host cells collected and ensured consistency in contents being sampled from each gut region. We placed each swab tip in a bead-beating tube and used a DNeasy PowerSoil Kit (QIAGEN Inc., Germantown, Maryland) to extract DNA according to the manufacturer's instructions. DNA extractions were performed on two unused swabs as blanks for negative controls. DNA was then quantified using the Qubit 2.0 Broad Range dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, Massachusetts). The Argonne Biosciences Division Environmental Sample Preparation and Sequencing Facility (ESPSF) amplified the V4 16S rRNA gene from the DNA extractions using the 515f and 806r primer set with PNA clamps to block the amplification of host 16S sequences (Caporaso et al. 2012; Lundberg et al. 2013). ESPSF prepared barcoded libraries from the resulting amplicons and sequenced them on an Illumina MiSeq using 250-bp paired-end reads and VX chemistry.

*Bacterial 16S rRNA gene sequence processing.*—ESPSF demultiplexed sequences using bcl2fastq (Illumina Inc., San Diego, California). We conducted subsequent processing of amplicon sequences on the demultiplexed fastq files with mothur v. 1.44.3 (Schloss et al. 2009). Chimeric sequences were removed using UCHIME (Edgar et al. 2011) and taxonomic classifications assigned to sequences using the Silva reference database version 138 (Quast et al. 2013; Yilmaz et al. 2014; Glockner et al. 2017). We removed all sequences classified as Archaea, chloroplast, Eukaryote, mitochondria, or unknown using the *remove.lineage*() command in mothur. Archaea were removed because the 16S rRNA V4 region primers are optimized for Bacteria leading to severe amplification bias against Archaea. We clustered the remaining sequences into 97% OTUs using the *cluster.split*() function in mothur, which first splits files into distinct groupings using the "classify" method and then clusters sequences with the default *OptiClust* algorithm (Westcott and Schloss 2017). This is an open-reference clustering method. While 97% OTUs can slightly underestimate bacterial diversity relative to amplicon sequencing variants (ASVs; Edgar 2018), we chose to use 97% OTUs in our analyses because processing sequences as ASVs can overestimate taxonomic diversity by an even greater degree (Schloss 2021). This overestimation is caused by artificially splitting multiple copies of the 16S rRNA gene from a single genome into separate ASVs (Schloss 2021). ASV-based estimates can be further

inflated by PCR and sequencing errors. Aligned representative sequences for each 16S OTU were collected with *get.oturep*() in mothur and then used to infer the bacterial phylogeny using FastTree (Price et al. 2010). We used the *phytools* package v. 0.7-70 (Revell 2012) to midpoint root this tree in R v. 3.6.3 (R Core Team 2019). We considered the 19 OTUs with more than five reads in our two blank extractions to be contaminants and removed them from all samples. After trimming contaminants, three specimens had fewer than 1,000 sequenced reads in either the small intestine or colon and were excluded, leaving 26 specimens in our analysis.

*Characterizing bacterial diversity and differentially abundant taxa by gut region.*—Bacterial diversity analyses were performed using the *phyloseq* package (v. 1.28.0; McMurdie and Holmes 2013). We first examined the bacterial diversity in the small intestine and colon samples of only the best-represented ($n$ = 17) species (*Crocidura pseudorhoditis*) in order to avoid host species as a confounding variable. We used the *estimate_richness*() function to calculate the number of observed OTUs in each sample and Shannon's $H$ diversity index. Since small intestine and colon samples were taken from the same individuals and not independent, we used a Wilcoxon signed rank test conducted in R to test whether the observed number of OTUs and Shannon's $H$ differed between gut regions. We then created two data sets of 16S amplicon counts, one merged by bacterial phylum and one merged by bacterial family. We converted these counts to relative abundance within each sample and generated box and whisker plots of the 10 most abundant bacterial phyla and families in the small intestine and colon using the *ampvis2* package (v. 2.6.1; Albertsen et al. 2015).

We prepared data for testing differential abundance of bacterial taxa between the small intestine and colon by merging amplicons at each higher taxonomic level (phylum, class, order, family, and genus). For a test at the OTU level we first filtered out amplicons that did not appear more than twice in 20% of the data (Henson et al. 2020), resulting in a data set of 68 OTUs. We used the *DeSeq2* package (v. 1.24.0) to convert data sets at each taxonomic level to a deseq object using a design formula that incorporated paired sampling by individual specimen and gut region (Love et al. 2014). *DeSeq2* tests for differential abundance of taxa between groups of samples using *normalized amplicon counts* and a negative binomial distribution model to fit a generalized linear model (Love et al. 2014). The coefficients of this model are then tested for significance using the Wald test. We adjusted $P$-values for the false discovery rate using the Benjamini–Hochberg procedure and considered taxa with $P$-values of less than 0.05 to be differentially abundant between gut regions.

We further tested hypotheses that individual bacterial taxa identified by *DeSeq2* are differentially abundant between the small intestine and colon in *C. pseudorhoditis* using the *corncob* package (v. 0.2.0; Martin et al. 2020). This software uses a beta-binomial distribution model and the Wald test to test for differences in relative abundance of individual bacterial taxa between groups of samples. Additionally, *corncob* can model the absence of taxa in a sample without using a pseudocount. We conducted differential abundance testing using data sets that merged 16S rRNA gene amplicons at the order, family, and genus levels. We then tested 8 OTUs that *DeSeq2* determined differed significantly between gut regions using *corncob* on a data set that had not been filtered for rare taxa.

*Small intestine and colon beta diversity in* C. pseudorhoditis.—We tested for community composition differences between the small intestine and colon using *DeSeq2* to perform a variance-stabilizing transformation (VST) on our filtered OTU deseq object in which sequences that did not appear more than twice in more than 20% of the data were removed. We then used the VST values to calculate Bray–Curtis dissimilarities with *phyloseq* and ordinate them in a nonmetric multidimensional scaling (NMDS) plot. We tested whether the microbial communities were distinguishable by gut region using the analysis of similarities test (ANOSIM) with 999 permutations implemented in the *vegan* package (v. 2.5; Oksanen et al. 2020). We also tested whether gut region affected the taxonomic composition of our samples using permutational multivariate analysis of variance (PERMANOVA) using 999 permutations with the *adonis* function in *vegan* (Anderson 2017; Oksanen et al. 2020). The function *betadisper* in *vegan* was used to test whether treatment groups had significantly different variances in order to satisfy the PERMANOVA assumption that sample groups have homogenous variances (Anderson 2017; Oksanen et al. 2020). We then used the coefficients from the *adonis* PERMANOVA test to determine the 20 strongest bacterial contributors to community composition differences between the small intestine and colon in the well-sampled *C. pseudorhoditis*.

*Small intestine and colon beta diversity in other* Crocidura *species.*—We used the same analytical methods to determine if differences between the small intestine and colon observed in *C. pseudorhoditis* carry over to other shrew species. We created a data set with only samples from *C. elongata* (4), *C. rhoditis* (3), and *C. tenebrosa* (2). We filtered, normalized with a VST, and ordinated this data set as described above for *C. pseudorhoditis*. We also performed ANOSIM and PERMANOVA testing, as above. To test for an effect of host species on bacterial community composition in each gut region, we repeated these steps for two additional data sets comprising (1) the small intestine samples and (2) the colon samples for all four species.

*Phylogenetic relationships among Mycoplasmataceae taxa.*—OTUs in the family Mycoplasmataceae: Mycoplasmatales that could not be assigned to a known genus emerged as important components of shrew gut bacterial communities. We inferred the relationships of these unassigned taxa to described species by extracting representative 16S sequences for the 11 Mycoplasmataceae OTUs and the single Entomoplasmatales OTU (a relative of the Mycoplasmatales) in our filtered *C. pseudorhoditis* data set from the fasta file produced earlier by mothur's *get.oturep*() command. We enriched this sampling by adding two representatives from each major Mycoplasmatales and Entomoplasmatales clade recovered by Volokhov et al. (2012) in order to put the OTUs we recovered into the context of known phylogenetic diversity of these groups. We used an *Enterococcus* sequence from our data as an outgroup because it is also a member of the Firmicutes phylum. Sequences were

aligned in Geneious (v. 7.1.9) using the default "Geneious Alignment" settings. We then performed DNA sequence evolution model selection (using AIC) on this alignment and inferred a maximum likelihood tree with 10,000 ultrafast bootstrap replicates in IQTree 2.1.2 (Kalyaanamoorthy et al. 2017; Hoang et al. 2018; Minh et al. 2020).

## Results

*Characterizing bacterial diversity and differentially abundant taxa by gut region.*—We collected paired small intestine and colon samples from 4 *C. elongata*, 3 *C. rhoditis*, 17 *C. pseudorhoditis*, and 2 *C. tenebrosa* specimens for a total of 52 samples. We recovered 1,021,131 16S rRNA amplicon reads that clustered into 11,431 bacterial OTUs. However, 5,739 of these OTUs were removed because they were represented by a single read. The remaining 5,692 OTUs represented 42 phyla, 116 classes, 280 orders, and 514 families of Bacteria. The average number of reads in small intestine samples (17,930 ± 14,997) was similar to the results from colon samples (21,344 ± 15,941). For *C. pseudorhoditis*, our best sampled species, there was no difference between small intestine (SI) and colon (C) samples in either number of OTUs (Fig. 1; SI = 231.9 ± 243.4, C = 293.6 ± 218.9, $P = 0.227$) or Shannon's *H* diversity index (Fig. 1; SI = 1.670 ± 0.5941, C = 1.960 ± 0.8073, $P = 0.243$).

Relatively few bacterial phyla and families occurred in high abundances (>1%) across the samples. Within *C. pseudorhoditis*, both small intestine and colon samples were dominated in terms of mean relative read abundance by the phyla Firmicutes (SI = 66.8% ± 35.6, C = 16.8% ± 18.0), Proteobacteria (SI = 6.89% ± 35.6, C = 39.3% ± 34.4), Bacteroidota (SI = 3.30% ± 13.5, C = 1.21% ± 18.8), and Campylobacterota (SI = 1.02% ± 4.09, C = 2.58% ± 25.1; Fig. 2A). Primary contributors to bacterial family-level composition in both gut regions were Mycoplasmataceae (SI = 64.9% ± 37.0, C = 8.84% ± 17.5), Enterobacteriaceae (SI = 0.815% ± 13.0, C = 0.445% ± 15.7), Helicobacteraceae (SI = 0.220% ± 4.05, C = 2.58% ± 24.7), and Amoebophilaceae (SI = 2.69% ± 13.5, C = 0.136% ± 18.8; Fig. 2B).

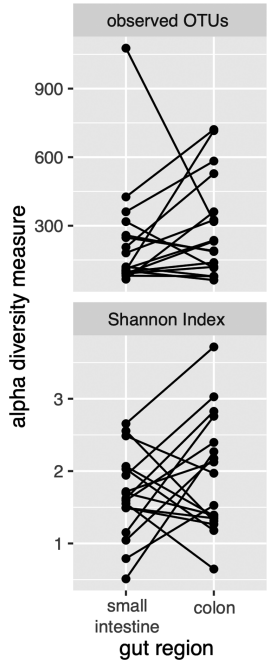


**Fig. 1.**—Plots of observed operational taxonomic units (OTUs) and Shannon's *H* alpha diversity index from the small intestine and colon of *Crocidura pseudorhoditis*. Small intestine and colon samples from the same individual specimen are connected by lines.

Testing with *DeSeq2* showed that none of the 42 bacterial phyla or 116 classes were differentially abundant between the small intestine and colon in *C. pseudorhoditis*. However, of the 280 orders, Mycoplasmatales was significantly more abundant in the small intestine relative to the colon (Table 1). This was also true for bacterial families, with Mycoplasmataceae more abundant in the small intestine but Enterococcaceae and an unclassified family of Enterobacteriales more abundant in the colon out of 514 tested families (Table 1). Among 1,076 bacterial genera tested, *Mycoplasma*: Mycoplasmataceae, *Ureaplasma*: Mycoplasmataceae, and three unclassified genera in families Mycoplasmataceae, Pasteurellaceae, and Amoebophilaceae were more abundant in the small intestine. In the colon, *Enterococcus*: Enterococcaceae, *Hafnia–Obesumbacterium*: Hafniaceae, *Wolbachia*: Anaplasmataceae, and one unclassified genus in order Enterobacteriales were more abundant (Table 1). Finally, using our filtered data set of 68 OTUs in which OTUs that did not appear more than twice in 20% of the data were removed (Henson et al. 2020), *DeSeq2* showed that a *Mycoplasma* OTU, three unclassified Mycoplasmataceae OTUs, and an unclassified Pasteurellaceae OTU were more abundant in the small intestine while a different Mycoplasmataceae OTU, a *Hafnia–Obesumbacterium* OTU, and an *Enterococcus* OTU were all more abundant in the colon samples (Table 1).

Results of differential abundance testing with *corncob* were mostly concordant with those from *DeSeq2* with four exceptions. *corncob* did not find significant differences between the small intestine and colon in *C. pseudorhoditis* for the genus *Mycoplasma*, an unclassified genus in Amoebophilaceae, or *Mycoplasma* OTU 2 (Table 1). *corncob* is unable to test for differential abundance when there are no amplicons in one of the categories, as was the case for *Wolbachia* in the small intestine.

*Small intestine and colon beta diversity in* C. pseudorhoditis.—Two separate and mechanistically distinct methods revealed that gut region had a significant effect on gut bacterial community composition in *C. pseudorhoditis* (Fig. 3). An ANOSIM test of Bray–Curtis dissimilarities showed a statistically significant but weak signal of distinguishability between small intestine and colon samples in *C. pseudorhoditis* ($R = 0.18$, $P = 0.005$). PERMANOVA results in *adonis* were also significant ($R^2 = 0.0918$, $P = 0.003$) and samples from the two gut regions did not have significantly different variances as determined by *betadisper* ($F = 2.68$, $P = 0.11$). Coefficients from the *adonis* PERMANOVA test showed that all eight differentially abundant OTUs from the *DeSeq2* test contributed to structural differences between small intestine and colon bacterial communities in *C. pseudorhoditis*

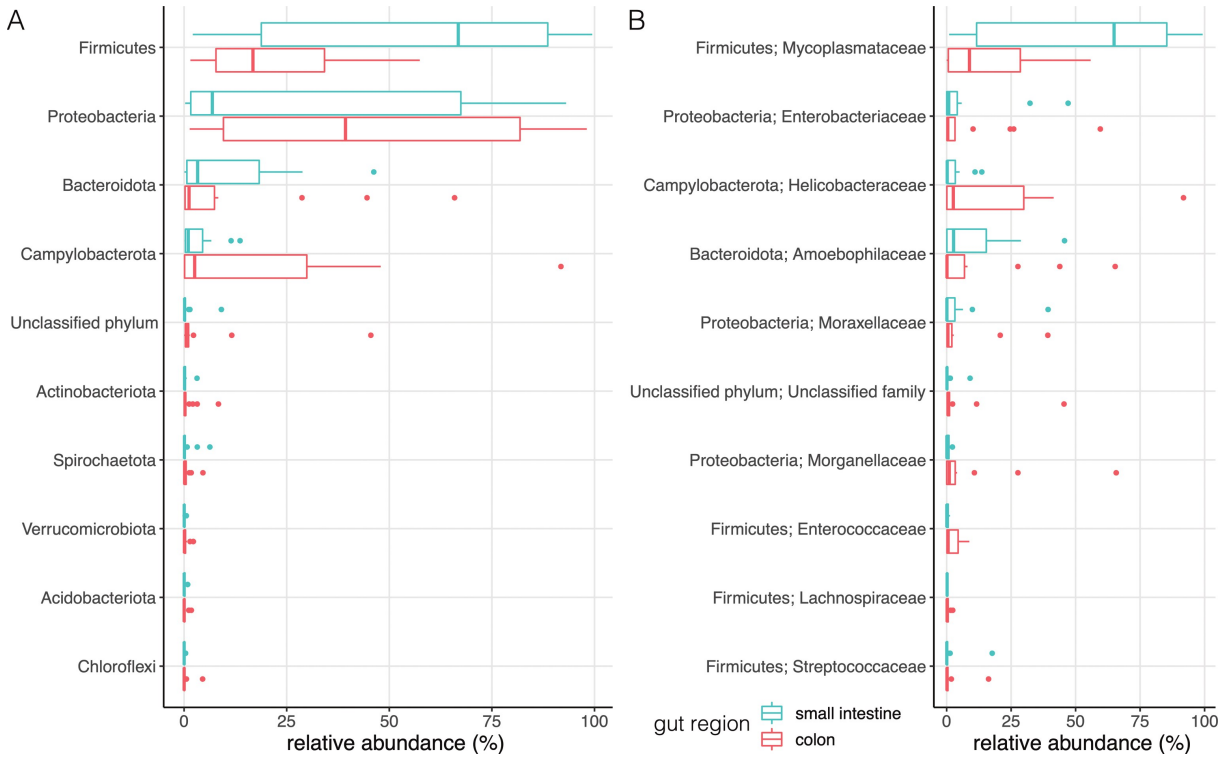


**Fig. 2.**—Box and whisker plot for relative read abundances of the 10 most abundant bacterial phyla (A) and bacterial families (B) in the small intestine and colon of *Crocidura pseudorhoditis*.

**Table 1.**—Bacterial taxa found to be differentially abundant between the small intestine and colon of *Crocidura pseudorhoditis* by the *DeSeq2* package and then tested with *corncob*. BH = Benjamini–Hochberg; OTU = operational taxonomic units; Unc. = unclassified.

| | DeSeq2 | | | | | corncob | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean normalized amplicon counts | $Log_2$-fold change colon-small intestine | $Log_2$-fold change *SE* | Wald statistic | BH-adjusted *P*-value | Coefficient estimate | Coefficient *SE* | *t*-value | *P*-value |
| Order | | | | | | | | | |
| Mycoplasmatales | 6,006 | −3.213 | 0.6197 | −5.186 | <0.0001 | −1.723 | 0.4475 | −3.850 | 0.000575 |
| Family | | | | | | | | | |
| Mycoplasmataceae | 5,657 | −3.166 | 0.6198 | −5.108 | 0.0001314 | −1.723 | 0.4475 | −3.850 | 0.000575 |
| Enterococcaceae | 187.7 | 3.657 | 0.8506 | 4.299 | 0.003468 | 2.022 | 0.5894 | 3.431 | 0.00177 |
| Unc. Enterobacterales | 35.75 | 2.419 | 0.6624 | 3.652 | 0.03506 | 1.490 | 0.3555 | 4.191 | 0.000226 |
| Genus | | | | | | | | | |
| *Mycoplasma* | 224.6 | −2.614 | 0.6814 | −3.836 | 0.001685 | −0.8826 | 0.5505 | −1.603 | 0.119 |
| Unc. Mycoplasmataceae | 5,355 | −3.409 | 0.6842 | −4.983 | <0.0001 | −1.576 | 0.4659 | −3.382 | 0.00201 |
| *Ureaplasma* | 36.69 | −3.359 | 1.165 | −2.882 | 0.03045 | −3.752 | 0.8165 | −4.595 | 0.0000728 |
| *Enterococcus* | 197.2 | 3.694 | 0.8724 | 4.234 | 0.0004131 | 2.022 | 0.5894 | 3.430 | 0.00178 |
| *Hafnia–Obesumbacterium* | 1,017 | 2.707 | 0.8136 | 3.327 | 0.007891 | 2.846 | 0.7536 | 3.776 | 0.000704 |
| Unc. Enterobacterales | 37.03 | 2.391 | 0.7045 | 3.393 | 0.007455 | 1.490 | 0.3555 | 4.191 | 0.000226 |
| Unc. Amoebophilaceae | 648.3 | −2.375 | 0.8632 | −2.751 | 0.03565 | 0.1075 | 0.6739 | 0.1600 | 0.874 |
| Unc. Pasteurellaceae | 513.5 | −2.411 | 0.8595 | −2.805 | 0.03395 | −1.634 | 0.7011 | −2.330 | 0.0267 |
| *Wolbachia* | 23.24 | 18.83 | 2.967 | 6.346 | <0.0001 | N/A | N/A | N/A | N/A |
| OTU | | | | | | | | | |
| Unc. Mycoplasmataceae 8 | 430.2 | −6.033 | 0.6653 | −9.068 | <0.0001 | −3.520 | 0.5388 | −6.533 | <0.0001 |
| Unc. Mycoplasmataceae 6 | 5,279 | −4.211 | 0.5963 | −7.061 | <0.0001 | −2.116 | 0.5559 | −3.806 | 0.00065 |
| Unc. Mycoplasmataceae 5 | 503.9 | −4.651 | 0.7960 | −5.843 | <0.0001 | −1.977 | 0.6635 | −2.979 | 0.00568 |
| Unc. Mycoplasmataceae 4 | 477.9 | 9.184 | 1.012 | 9.076 | <0.0001 | 5.324 | 0.9961 | 5.345 | <0.0001 |
| *Enterococcus* 1 | 240.2 | 3.352 | 0.8419 | 3.982 | 0.0007246 | 2.021 | 0.5893 | 3.429 | 0.00178 |
| *Hafnia–Obesumbacterium* 1 | 1,118 | 2.719 | 0.7948 | 3.420 | 0.004143 | 2.846 | 0.7536 | 3.777 | 0.000702 |
| Unc. Pasteurellaceae 1 | 549.8 | −3.353 | 0.8609 | −3.895 | 0.0008683 | −1.637 | 0.7565 | −2.164 | 0.0386 |
| *Mycoplasma* 2 | 29.39 | −4.122 | 1.093 | −3.770 | 0.001237 | 0.9803 | 2.0167 | 0.4860 | 0.630 |

(Fig. 4). Analysis using *adonis* PERMANOVA coefficients found additional taxa supporting compositional differences between gut regions that were not found to be significant using *DeSeq2*. These were OTUs from the genera *Campylobacter*: Campylobacteraceae, *Ureaplasma*: Mycoplasmataceae, unclassified Amoebophilaceae, and unclassified Mycoplasmataceae OTUs in the small

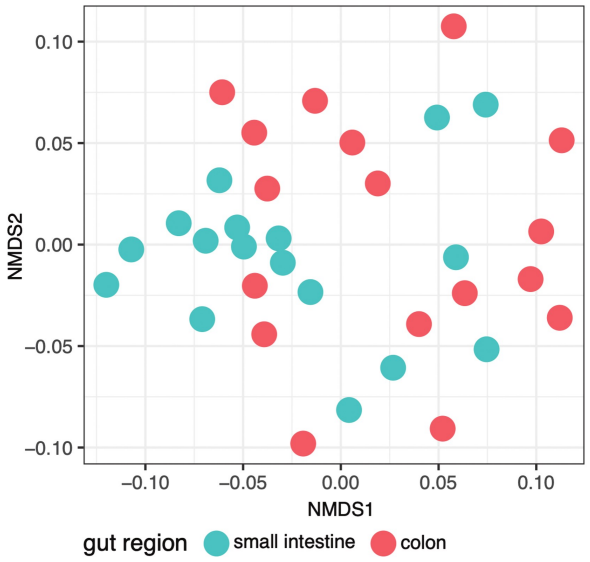


**Fig. 3.**—Nonmetric multidimensional scaling (NMDS) plot of bacterial 16S rRNA gene amplicon Bray–Curtis dissimilarities from the small intestine and colon of *Crocidura pseudorhoditis*.

intestine and *Morganella*: Morganellaceae, Romboutsia: Peptostreptococcaceae, *Helicobacter*: Helicobacteraceae, unclassified Planococcaceae, and an unclassified Mycoplasmataceae OTU in the colon (Fig. 4).

*Small intestine and colon beta diversity in other* Crocidura *species.*—Bacterial community composition was also weakly but significantly different (ANOSIM: $R = 0.19$, $P = 0.034$ and *adonis* PERMANOVA: $R^2 = 0.122$, $P = 0.04$) between the small intestine and colon in the three host species for which we had fewer samples (Fig. 5). In the data used for the PERMANOVA analysis, *betadisper* found the samples did not have significantly different variances ($F = 0.48$, $P = 0.5$).

Sample subsets by gut region that included all host species showed weak but statistically significant effects of host species taxonomy on bacterial community composition. Small intestine samples showed the strongest effect with ANOSIM ($R = 0.278$, $P = 0.008$) and PERMANOVA with *adonis* ($R^2 = 0.191$, $P = 0.03$) yielding significant differences in community composition (Fig. 6). There were not significantly different variances in the data used for this PERMANOVA test as determined by *betadisper* ($F = 0.4758$, $P = 0.7$). For tests for differences in colon bacterial community composition by host taxonomy we found that ANOSIM did not show a significant

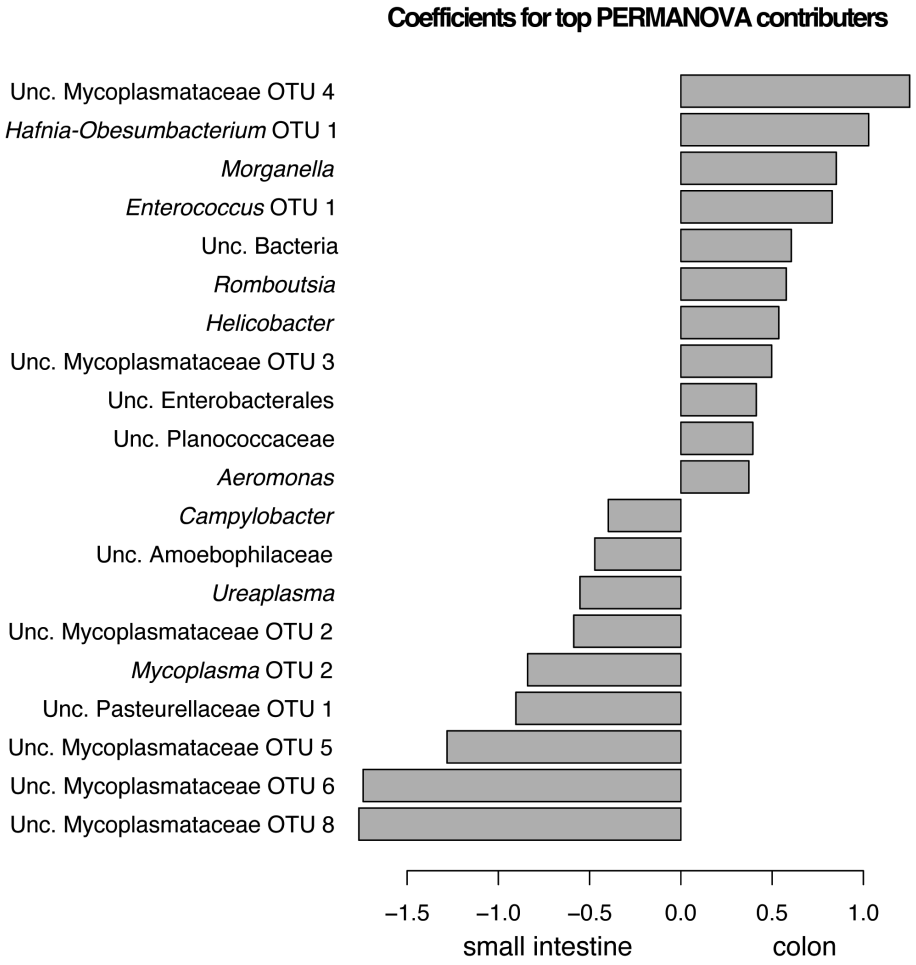


**Fig. 4.**—Coefficients for top 20 bacterial operational taxonomic units (OTUs) responsible for separating *Crocidura pseudorhoditis* small intestine and colon sample groups in permutational multivariate analysis of variance (PERMANOVA) analysis with *adonis*.

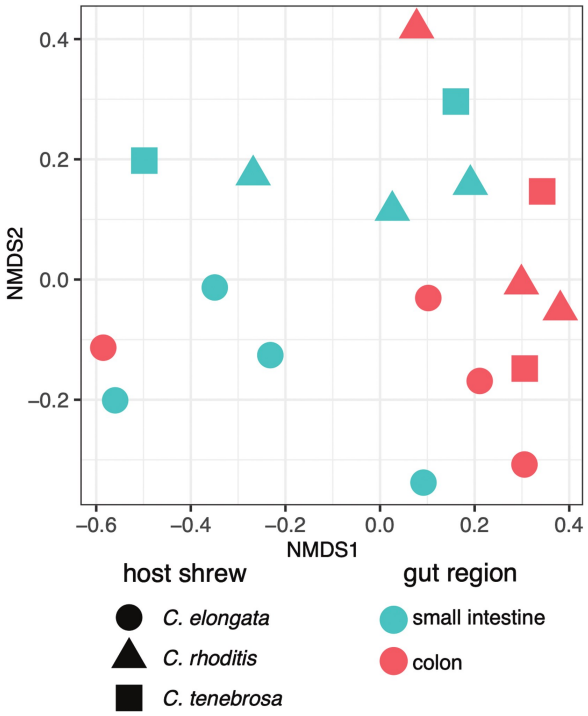


**Fig. 5.**—Nonmetric multidimensional scaling (NMDS) plot of bacterial 16S rRNA gene amplicon Bray–Curtis dissimilarities from the small intestine and colon of *Crocidura elongata*, *C. rhoditis*, and *C. tenebrosa*.

effect ($R$ = 0.126, $P$ = 0.16). PERMANOVA with *adonis* showed that Bray–Curtis distances were significantly different between samples grouped by host taxonomy ($R^2$ = 0.190, $P$ = 0.025) using data that did not show significantly different variances using *betadisper* ($F$ = 2.195, $P$ = 0.12; Fig. 6).

*Phylogenetic relationships among Mycoplasmataceae taxa.*—Several important relationships were supported with ultrafast bootstrapping in the phylogeny of Mycoplasmataceae OTUs despite the limited length of the 16S rRNA sequences (250 bp). First, the 8 unclassified Mycoplasmataceae OTUs formed a well-supported clade (BS = 99) that included *Ureaplasma*, a variety of vertebrate-associated mycoplasmas such as hemoplasmas, and termite-associated mycoplasmas (Fig. 7). Within this clade, we found a sister relationship (BS = 98) between the two unclassified Mycoplasmataceae OTUs 3 and 4 that were more abundant in the colon compared with the small intestine. *Mycoplasma muris* was supported as the sister taxon to Mycoplasmataceae OTUs 3 and 4 (BS = 94). Secondly, our two *Mycoplasma* OTUs were related to taxa in the *Mycoplasma* clade recovered by Volokhov et al. (2012). Specifically, *Mycoplasma* OTU 1 was sister to *M. sualvi* (BS = 100) from the Moatsii–sualvi–mobile group, and *Mycoplasma* OTU 2 formed a clade with *M. iguana* and *M. lagogenitalium* from the Neurolyticum group.

## Discussion

We found the phylum-level bacterial community composition of *Crocidura* intestines to be broadly similar to previous studies on *Sorex* shrews. Only four phyla (Firmicutes, Proteobacteria,

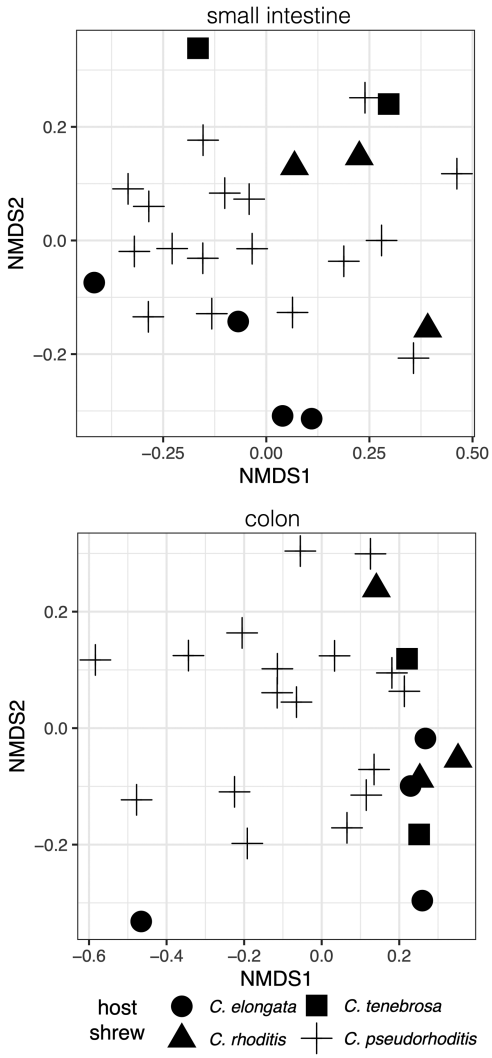


**Fig. 6.**—Nonmetric multidimensional scaling (NMDS) plots of bacterial 16S rRNA gene amplicon Bray–Curtis dissimilarities from the small intestine and colon of *Crocidura elongata*, *C. rhoditis*, *C. pseudorhoditis*, and *C. tenebrosa*.

Bacteroidota, and Campylobacterota) made up the overwhelming majority of our amplicons, as other researchers found in Lithuanian and North American *Sorex* (Knowles et al. 2019; Greiman et al. 2020). Neither *Crocidura* nor *Sorex* intestines harbored members of the phylum Fusobacteria, which are characteristic of other mammals with simple guts like hedgehogs and carnivorans (Zhu et al. 2018; Youngblut et al. 2019). This suggests that high proportions of Fusobacteria are not the result of a simple gut structure, but of a diet mostly consisting of vertebrates. Each of the four primary phyla in our *Crocidura* intestines were represented primarily by single families: Mycoplasmataceae in Firmicutes, Enterobacteriaceae in Proteobacteria, Amoebophilaceae in Bacteroidota, and Helicobacteraceae in Campylobacterota. The large proportion of Mycoplasmataceae and the reclassification of Mycoplasmataceae from Tenericutes to Firmicutes (Parks et al. 2018) likely means we had much smaller proportions of Firmicutes in our samples compared to *Sorex* shrews from the

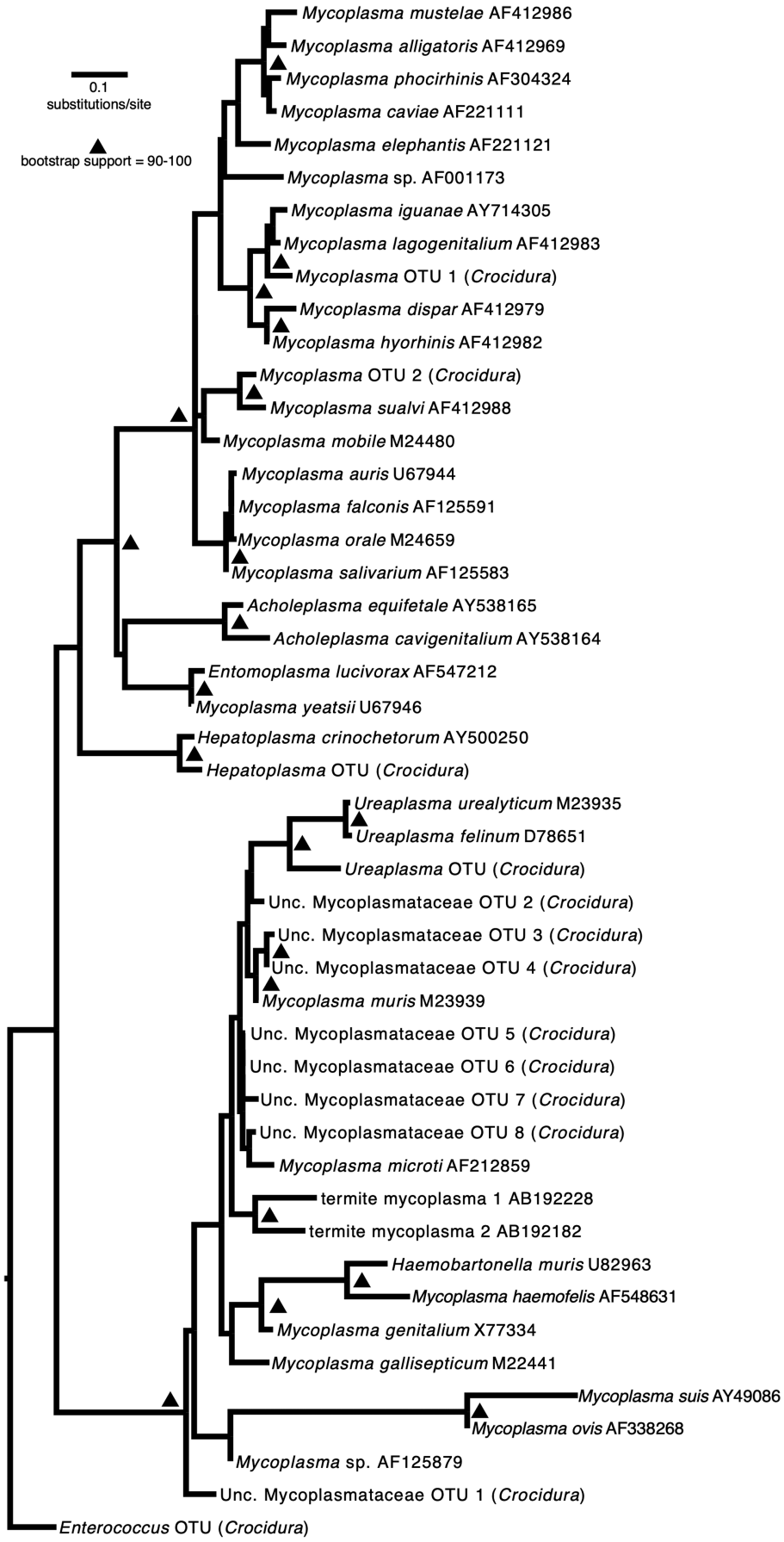


**Fig. 7.**—Maximum likelihood phylogeny of Mycoplasmataceae operational taxonomic units (OTUs) inferred using 16S rRNA gene sequences. Sequences from Volokhov et al. (2012) include GenBank accession numbers in the tip labels.

Knowles et al. (2019) and Greiman et al. (2020) data sets which used a different taxonomy.

Our work demonstrates that gut bacterial community structure differs between gut regions, even in mammals that have simple guts such as eulipotyphlans and carnivorans. Similar to black bears, we found differences in bacterial community composition between the small intestine and colon (Gillman et al. 2020). We found these compositional differences both in tests using a well-sampled host (*C. pseudorhoditis*) and in tests on the other three shrew species, suggesting that the differences are generalizable to species of *Crocidura*. Within our most well-sampled shrew species, members of the order Mycoplasmatales were more abundant in the small intestine than the colon and several OTUs in this order were responsible for differences between the two gut regions. Without sampling from the small intestine and colon separately, we would not have realized how the niches of these bacterial taxa are spatially distinct. Furthermore, sampling from only fecal material or colon swabs would not have revealed the full diversity of Mycoplasmataceae taxa as the most abundant OTU in the small intestine sometimes made up only a negligible proportion of the colon bacterial community. Knowles et al. (2019) and Greiman et al. (2020) both extracted bacterial DNA by digesting the whole gastrointestinal tract, but this would not generally be a feasible solution for crocidurines as most members of this clade have much larger body sizes than soricines. This sampling strategy would have also obscured the gut bacterial community differences between host shrew species that we found among small intestine samples but not among colon samples. In contrast, rodent small intestine bacterial communities sampled from the same forest were not significantly distinguishable by host taxonomy (Swanson M.T., Louisiana State University, Baton Rouge, Louisiana, personal communication, January 2021).

The high abundances of Mycoplasmataceae and their role in driving differences between the small intestine and colon is intriguing because almost all known members are obligate vertebrate symbionts. Both described genera, *Mycoplasma* and *Ureaplasma*, lack cell walls and have reduced genomes that make them dependent on their hosts for amino acids (Razin et al. 1998). The lack of peptidoglycan cell walls in Mycoplasmataceae may explain their relative abundance in the small intestine where lysozymes and defensins produced by the host attack peptidoglycan cell walls of other microbes. Mycoplasmas are also consequential for behavior and physiology in their hosts. House finches (*Haemorhous mexicanus*) infected with *M. gallisepticum* have suppressed immune systems and less oxidative mitochondrial damage in their livers compared with uninfected birds (Zhang et al. 2021). Allenby's gerbils (*Gerbillus andersoni allenbyi*) infected with mycoplasmas were less efficient foragers and experienced increased owl predation (Makin et al. 2020). Perhaps the diversity and abundance of mycoplasmas in shrew gastrointestinal tracts have fitness consequences.

While increased taxonomic and genomic sampling are needed to reduce phylogenetic uncertainty, the unclassified Mycoplasmataceae OTUs that made up large proportions of the gut microbial communities and contributed to structural differences between gut regions formed a clade that included *Ureaplasma* OTUs. This relationship suggests mechanisms through which these organisms could potentially have critical impacts on the physiology of their shrew hosts. Many mammals such as ruminants, rodents, and humans convert ammonia—a toxic byproduct of protein catabolism in the liver—to the more benign form urea (Stewart and Smith 2005). Some urea is filtered out of the bloodstream through the kidneys and excreted as urine, but mammals can conserve water and nitrogen by transferring urea to the gastrointestinal tract where bacteria that encode the enzyme urease can convert urea into amino acids and proteins in a process known as urea nitrogen salvage (Stewart and Smith 2005). Ruminants absorb these bacterially produced amino acids and proteins in the small intestine while rodents and lagomorphs must eat their feces (coprophagy) in order to absorb these materials when they pass through the small intestine again (Torrallardona et al. 1996). While herbivores can be nitrogen-limited, shrews have the opposite problem in that their protein intake is particularly high for their body size but their kidneys do not appear to be appropriately modified to accommodate this diet and are not excreting enough protein catabolism byproducts in their urine (Singer 2002). Therefore, shrews are likely transferring large volumes of urea to their gastrointestinal tracts and providing a nitrogen source to microbes with genes for urease such as *Ureaplasma* and *Helicobacter* (Marques et al. 2016). Both of these taxa as well as unclassified Mycoplasmataceae OTUs that form a clade that includes *Ureaplasma* were prevalent in the *Crocidura* intestinal tracts, which opens the possibility that microbes help facilitate the faunivorous lifestyle of their hosts. The ability of these unclassified Mycoplasmataceae OTUs to hydrolyze urea is speculative though, as other members of this clade such as *M. microti* and *M. muris* do not hydrolyze urea (McGarrity et al. 1983; Brown et al. 2001).

The co-occurrence of five or more unclassified Mycoplasmataceae OTUs along with *Ureaplasma* within an individual shrew gastrointestinal tract raises the question of how these bacterial organisms partition niche space. Part of the answer is evident in their differential abundance in the colon and small intestine (two are more abundant in the colon and three are more abundant in the small intestine), suggesting they are partitioned by variation in the physiochemical environment along the length of the gastrointestinal tract. Additionally, the two unclassified Mycoplasmataceae OTUs found to be more abundant in the colon by PERMANOVA are sister to each other in our Mycoplasmataceae phylogenetic tree, suggesting that they share traits that facilitate life in the colon. The relatively limited taxonomic richness of shrew gut microbial communities probably makes metagenome-assembled genomes particularly tractable and could facilitate future investigations of how co-occurring Mycoplasmataceae OTUs differ in their metabolic capacities and partition niche space.

In addition to members of Mycoplasmataceae and *Helicobacter*, other bacterial taxa in *Crocidura* intestines have metabolic attributes that are likely to aid digestion of arthropods. *Romboutsia* was a component of structural differences

between the small intestines and colons of Sulawesi *Crocidura* and is a genus in the family Peptostreptococcaceae, a taxon that has the rare capacity to ferment amino acids (Smith and MacFarlane 1998). Second, a *Hafnia–Obesumbacterium* OTU was more abundant in the colons than the small intestines of Sulawesi *Crocidura*. An isolate of this taxon from a bat gastrointestinal tract produced chitinase and was capable of using chitin as its sole energy and carbon source (Whitaker et al. 2004), suggesting *Hafnia–Obesumbacterium* could play a role in making components of chitin available for use by shrews. While we cannot say whether any of these interactions are happening, the occurrence of these particular organisms in shrew gastrointestinal tracts provides fertile ground for future work.

Several bacterial taxa in our data set were probably associated with prey items and were not residents of the shrew gastrointestinal tract. *Wolbachia* in particular is a well-known parasite of arthropod reproductive organs (La Scola et al. 2015) and our recovery of *Wolbachia* amplicons in the colon suggests that prey-associated DNA can persist through the digestive process. One *C. rhoditis* specimen yielded *Hepatoplasma* amplicons, a member of Entomoplasmatales that has previously only been found in isopods (Bouchon et al. 2016). While members of Mycoplasmataceae are overwhelmingly associated with vertebrates, a handful of taxa are found in termites and consequently we cannot rule out that the unclassified Mycoplasmataceae OTUs originated in prey items (Volokhov et al. 2012). Similarly, *Amoebophilus* and *Cardinium* are the described genera of Amoebophilaceae and are reduced genome endosymbionts of amoebas and insects, respectively (Santos-Garcia et al. 2014). The unclassified Amoebophilaceae taxa we found could be endosymbionts of prey organisms or possibly parasites that compete with Mycoplasmataceae taxa for niche space.

We showed that bacterial community composition differed between the small intestine and colon in shrews, even though they have a simple gut configuration that resembles a tube without a specialized fermentation chamber. These differences between gut regions were consistent across the four species in this study and suggest that mutualisms with Bacteria contribute to digestion in mammalian faunivores. More research is needed to understand which aspects of the intestinal physiochemical environment such as epithelium histology, mucin thickness, abundance of antimicrobial peptides, and nutrient availability vary along the length of the gut and influence bacterial community composition. Genomic characterization and culturing experiments could also test whether microbial symbionts play a role in facilitating the digestion of protein-rich, faunivorous diets in shrews.

## Acknowledgments

This work was supported by National Science Foundation DEB-1441634 and -1754393 to JAE, the Alfred L. Gardner and Mark S. Hafner Mammalogy Fund, and by an American Society of Mammalogists Grant-in-Aid of Research and LSU Department of Biological Sciences Ron and Mary Neal Superior Graduate Student Fellowship to MTS. We thank Louisiana State University Museum of Natural Science and Museum Zoologicum Bogoriense for providing access to museum specimens. Portions of this research were conducted with high-performance computing resources provided by Louisiana State University. We are grateful to Reksa Robi and Pak Julius, Pak Eno, Pak Allo, and the people of Desa Insil for making fieldwork at Mount Ambang possible. We thank Kementerian Riset Teknologi dan Pendidikan Tinggi, Kementerian Lingkungan Hidup dan Kehutanan, BKSDA Sulawesi Utara for providing research permits and materials.

## Supplementary Data

Supplementary data are available at *Journal of Mammalogy* online.

**Supplementary Data SD1.**—Museum catalog numbers for specimens and NCBI BioSample Accession Numbers for all raw sequencing reads used in this study.

## Literature Cited

Albertsen M., Karst S.M., Ziegler A.S., Kirkegaard R.H., Nielsen P.H. 2015. Back to basics - the influence of DNA extraction and primer choice on phylogenetic analysis of activated sludge communities. PLoS One 10:1–15.

Anderson M.J. 2017. Permutational multivariate analysis of variance (PERMANOVA). Wiley StatsRef: Statistics Reference Online :1–15. doi:10.1002/9781118445112.stat07841

Borgstrom B., Dahlqvist A., Lundh G., Sjovall J. 1957. Studies of intestinal digestion and absorption in the human. The Journal of Clinical Investigation 36:1521–1536.

Bouchon D., Zimmer M., Dittmer J. 2016. The terrestrial isopod microbiome: an all-in-one toolbox for animal–microbe interactions of ecological relevance. Frontiers in Microbiology 7:1–19.

Brown D.R., Talkington D.F., Thacker W.L., Brown M.B., Dillehay D.L., Tully J.G. 2001. *Mycoplasma microti* sp. nov., isolated from the respiratory tract of prairie voles (*Microtus ochrogaster*). International Journal of Systematic and Evolutionary Microbiology 51:409–412.

Burgin C.J., Colella J.P., Kahn P.L., Upham N.S. 2018. How many species of mammals are there? Journal of Mammalogy 99:1–14.

Caporaso J.G., et al. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME Journal 6:1621–1624.

Churchfield S. 1990. Natural History of Shrews. Comstock Publishing/Cornell University Press, Ithaca, New York, USA.

Delsuc F., Metcalf J.L., Parfrey L.W., Song S.J., González A., Knight R. 2014. Convergence of gut microbiomes in myrmecophagous mammals. Molecular Ecology 23:1301–1317.

Donaldson G.P., Lee S.M., Mazmanian S.K. 2015. Gut biogeography of the bacterial microbiota. Nature Reviews Microbiology 14:20–32.

Edgar R.C. 2018. Updating the 97% identity threshold for 16S ribosomal RNA OTUs. Bioinformatics 34:2371–2375.

Edgar R.C., Haas B.J., Clemente J.C., Quince C., Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200.

Esselstyn J.A., Achmadi A.S., Handika H., Giarla T.C., Rowe K.C. 2019. A new climbing shrew from Sulawesi highlights the tangled taxonomy of an endemic radiation. Journal of Mammalogy 100:1713–1725.

Esselstyn J.A., Achmadi A.S., Handika H., Swanson M.T., Giarla T.C., Rowe K.C. 2021. Fourteen new, endemic species of shrew (genus *Crocidura*) from Sulawesi reveal a spectacular island radiation. Bulletin of the American Museum of Natural History 454:1–108.

Fuller M.F., Reeds P.J. 1998. Nitrogen cycling in the gut. Annual Review of Nutrition 18:385–411.

Gillman S.J., McKenney E.A., Lafferty D.J.R. 2020. Wild black bears harbor simple gut microbial communities with little difference between the jejunum and colon. Scientific Reports 10:1–11.

Glöckner F.O., et al. 2017. 25 years of serving the community with ribosomal RNA gene reference databases and tools. Journal of Biotechnology 261:169–176.

Greiman S.E., Cook J.A., Odom T., Cranmer K., Liphardt S.W., Menning D.M., Sonsthagen S.A., Talbot S.L. 2020. Microbiomes from biorepositories? 16S rRNA bacterial amplicon sequencing of archived and contemporary intestinal samples of wild mammals (Eulipotyphla: Soricidae). Frontiers in Ecology and Evolution 8:1–15.

Grond K., Lanctot R.B., Jumpponen A., Sandercock B.K. 2017. Recruitment and establishment of the gut microbiome in arctic shorebirds. FEMS Microbiology Ecology 93:1–9.

Henson M.W., Lanclos V.C., Pitre D.M., Weckhorst J.L., Lucchesi A.M., Cheng C., Temperton B., Thrash J.C. 2020. Expanding the diversity of bacterioplankton isolates and modeling isolation efficacy with large-scale dilution-to-extinction cultivation. Applied and Environmental Microbiology 86:1–23.

Hoang D.T., Chernomor O., von Haeseler A., Minh B.Q., Vinh L.S. 2018. UFBoot2: improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35:518–522.

Hu Y., et al. 2018. Herbivorous turtle ants obtain essential nutrients from a conserved nitrogen-recycling gut microbiome. Nature Communications 9:1–14.

Ingala M.R., Simmons N.B., Wultsch C., Krampis K., Speer K.A., Perkins S.L. 2018. Comparing microbiome sampling methods in a wild mammal: fecal and intestinal samples record different signals of host ecology, evolution. Frontiers in Microbiology 9:1–13.

Kalyaanamoorthy S., Minh B.Q., Wong T.K.F., von Haeseler A., Jermiin L.S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14:587–589.

Karasov W.H., Douglas A.E. 2013. Comparative digestive physiology. Comprehensive Physiology 3:741–783.

Karasov W.H., Martinez del Rio C. 2007. Physiological ecology: how animals process energy, nutrients, and toxins. Princeton University Press, Princeton, New Jersey, USA.

Knowles S.C.L., Eccles R.M., Baltrūnaitė L. 2019. Species identity dominates over environment in shaping the microbiota of small mammals. Ecology Letters 22:826–837.

La Scola B., Bandi C., Raoult D. 2015. *Wolbachia*. In: Whitman W.B., editor. Bergey's manual of systematics of archaea and bacteria. Wiley. doi:10.1002/9781118960608.gbm00906

Langer P. 2017. Comparative anatomy of the gastrointestinal tract in Eutheria. Walter de Gruyter GmbH and Co. KG, Berlin, Germany.

Ley R.E., Hamady E., Lozupone C., Turnbaugh P.J., Ramey R.R., Bircher J.S., Schlegel M.L., Tucker T.A., Schrenzel M.D., Knight R., et al. 2008. Evolution of mammals and their gut microbes. Science 320:1647–1651. [This article has 11 authors].

Linnenbrink M., Wang J., Hardouin E.A., Künzel S., Metzler D., Baines J.F. 2013. The role of biogeography in shaping diversity of the intestinal microbiota in house mice. Molecular Ecology 22:1904–1916.

Love M.I., Huber W., Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15:1–21.

Lundberg D.S., Yourstone S., Mieczkowski P., Jones C.D., Dangl J.L. 2013. Practical innovations for high-throughput amplicon sequencing. Nature Methods 10:999–1002.

Makin D.F., Kotler B.P., Brown J.S., Garrido M., Menezes J.F.S. 2020. The enemy within: how does a bacterium inhibit the foraging aptitude and risk management behavior of Allenby's gerbils? The American Naturalist 196:717–729.

Marques L.M., Rezende I.S., Barbosa M.S., Guimarães A.M.S., Martins H.B., Campos G.B., do Noscimento N.C., dos Santos A.P., Amorim A.T., Santos V.M., et al. 2016. *Ureaplasma diversum* genome provides new insights about the interaction of the surface molecules of this bacterium with the host. PLoS One 11:1–22. [This article has 19 authors].

Martin B.D., Witten D., Willis A.D. 2020. Modeling microbial abundances and dysbiosis with beta-binomial regression. Annals of Applied Statistics 14:94–114.

McGarrity G., Rose D., Kwiatkowski V., Dion A., Phillips D., Tully J. 1983. Characterization of *Mycoplasma muris*, a new species from urogenital tracts of laboratory mice. Yale Journal of Biology and Medicine 56:906–907.

McMurdie P.J., Holmes S. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8:1–11.

Milani C., Alessandri G., Mancabelli L., Mangifesta M., Lugli G.A., Viappiani A., Longhi G., Anzalone R., Duranti S., Turroni F., et al. 2020. Multi-omics approaches to decipher the impact of diet and host physiology on the mammalian gut microbiome. Applied and Environmental Microbiology 86:1–21. [This article has 13 authors].

Minh B.Q., Schmidt H.A., Chernomor O., Schrempf D., Woodhams M.D., von Haeseler A., Lanfear R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution 37:1530–1534.

Oksanen J., et al. 2020. vegan: community ecology package. R package version 2.5. https://CRAN.R-project.org/package=vegan. Accessed 25 January 2021.

Pacheco-Sandoval A., Schramm Y., Heckel G., Brassea-Pérez E., Martínez-Porchas M., Lago-Lestón A. 2019. The Pacific harbor seal gut microbiota in Mexico: its relationship with diet and functional inferences. PLoS One 14:1–21.

Parks D.H., Chuvochina M., Waite D.W., Rinke C., Skarshewski A., Chaumeil P.A., Hugenholtz P. 2018. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nature Biotechnology 36:1–14.

Penry D.L., Jumars P.A. 1987. Modeling animal guts as chemical reactors. The American Naturalist 129:69–96.

Perofsky A.C., Lewis R.J., Meyers L.A. 2019. Terrestriality and bacterial transfer: a comparative study of gut microbiomes in sympatric Malagasy mammals. The ISME Journal 13:50–63.

Porter E.M., Bevins C.L., Ghosh D., Ganz T. 2002. The multifaceted Paneth cell. Cellular and Molecular Life Sciences 59:156–170.

Price M.N., Dehal P.S., Arkin A.P. 2010. FastTree 2 - approximately maximum-likelihood trees for large alignments. PLoS One 5:1–10.

Quast C., Pruesse E., Yilmaz P., Gerken J., Schweer T., Yarza P., Peplies J., Glöckner F.O. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41:D590–D596.

R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed 25 January 2021.

Razin S., Yogev D., Naot Y. 1998. Molecular biology and pathogenicity of mycoplasmas. Microbiology and Molecular Biology Reviews 62:1094–1156.

Revell L.J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution 3:217–223.

Sanders J.G., Beichman A.C., Roman J., Scott J.J., Emerson D., McCarthy J.J., Girguis P.R. 2015. Baleen whales host a unique gut microbiome with similarities to both carnivores and herbivores. Nature Communications 6:1–8.

Santos-Garcia D., Rollat-Farnier P.A., Beitia F., Zchori-Fein E., Vavre F., Mouton L., Moya A., Latorre A., Silva F.A. 2014. The genome of *Cardinium cBtQ1* provides insights into genome reduction, symbiont motility, and its settlement in *Bemisia tabaci*. Genome Biology and Evolution 6:1013–1030.

Sato J.J., Bradford T.M., Armstrong K.N., Donnellan S.C., Echenique-Diaz L.M., Begue-Quiala G., Gamez-Diez J., Yamaguchi N., Nguyen S.T., Kita M., et al. 2019. Post K-Pg diversification of the mammalian order Eulipotyphla as suggested by phylogenomic analyses of ultra-conserved elements. Molecular Phylogenetics and Evolution 141:1–14. [This article has 11 authors].

Schloss P.D. 2021. Amplicon sequence variants artificially split bacterial genomes into separate clusters. mSphere 6:e00191-21.

Schloss P.D., Westcott S.L., Ryabin T., Hall J.R., Hartmann M., Hollister E.B., Lesniewski R.A., Oakley B.B., Parks D.H., Robinson C.J., et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75:7537–7541. [This article has 15 authors].

Sikes R.S., and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy 97:663–688.

Singer M.A. 2002. Vampire bat, shrew, and bear: comparative physiology and chronic renal failure. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 282:R1583–R1592.

Smith E.A., MacFarlane G.T. 1998. Enumeration of amino acid fermenting bacteria in the human large intestine: effects of pH and starch on peptide metabolism and dissimilation of amino acids. FEMS Microbiology Ecology 25:355–368.

Song S.J., Sanders J.G., Delsuc F., Metcalf J., Amato K., Taylor M.W., Mazel F., Lutz H.L., Winker K., Graves G.R., et al. 2020. Comparative analyses of vertebrate gut microbiomes reveal convergence between birds and bats. mBio 11:e02901-19. [This Article has 26 authors].

Stewart G.S., Smith C.P. 2005. Urea nitrogen salvage mechanisms and their relevance to ruminants, non-ruminants and man. Nutrition Research Reviews 18:49–62.

Torrallardona D., Harris C.I., Fuller M.F. 1996. Microbial amino acid synthesis and utilization in rats: the role of coprophagy. British Journal of Nutrition 76:701–709.

Van Soest P.J. 1994. Nutritional ecology of the ruminant. Cornell University Press, Ithaca, New York, USA.

Vogel P. 1976. Energy consumption of European and African shrews. Acta Theriologica 21:195–206.

Volokhov D.V., Simonyan V., Davidson M.K., Chizhikov V.E. 2012. RNA polymerase beta subunit (rpoB) gene and the 16S-23S rRNA intergenic transcribed spacer region (ITS) as complementary molecular markers in addition to the 16S rRNA gene for phylogenetic analysis and identification of the species of the fam-ily Mycoplasmataceae. Molecular Phylogenetics and Evolution 62:515–528.

Westcott S.L., Schloss P.D. 2017. OptiClust, an improved method for assigning amplicon-based sequence data to operational taxonomic units. mSphere 2:e00073-17.

Whitaker J.O., Dannelly H.K., Prentice D.A. 2004. Chitinase in insectivorous bats. Journal of Mammalogy 85:15–18.

Yilmaz P., Parfrey L.W., Yarza P., Gerken J., Pruesse E., Quast C., Schweer T., Peplies J., Ludwig W., Glöckner F.O. 2014. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Research 42:D643–D648.

Youngblut N.D., Reischer G.H., Walters W., Schuster N., Walzer C., Stalder G., Ley R.E., Farnleitner A.H. 2019. Host diet and evolutionary history explain different aspects of gut microbiome diversity among vertebrate clades. Nature Communications 10:1–15.

Zhang Y., Hill G.E., Ge Z., Park N.R., Taylor H.A., Andreasen V., Tardy L., Kavazis A.N., Bonneaud C., Hood W.R. 2021. Effects of a bacterial infection on mitochondrial function and oxidative stress in a songbird. Physiological and Biochemical Zoology 94:71–82.

Zhu L., Wu Q., Deng C., Zhang M., Zhang C., Chen H., Lu G., Wei F. 2018. Adaptive evolution to a high purine and fat diet of carnivorans revealed by gut microbiomes and host genomes. Environmental Microbiology 20:1711–1722.