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Microbial communities exhibit host species distinguishability and phylosymbiosis along the length of the gastrointestinal tract

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Abstract

Host-associated microbial communities consist of stable and transient members that can assemble through purely stochastic processes associated with the environment or by interactions with the host. Phylosymbiosis predicts that if host–microbiota interactions impact assembly patterns, then one conceivable outcome is concordance between host evolutionary histories (phylogeny) and the ecological similarities in microbial community structures (microbiota dendrogram). This assembly pattern has been demonstrated in several clades of animal hosts in laboratory and natural populations, but in vertebrates, it has only been investigated using samples from faeces or the distal colon. Here, we collected the contents of five gut regions from seven rodent species and inventoried the bacterial communities by sequencing the 16S rRNA gene. We investigated how community structures varied across gut regions and whether the pattern of phylosymbiosis was present along the length of the gut. Gut communities varied by host species and gut region, with *Oscillospira* and *Ruminococcus* being more abundant in the stomach and hindgut regions. Gut microbial communities were highly distinguishable by host species across all gut regions, with the strength of the discrimination increasing along the length of the gut. Last, the pattern of phylosymbiosis was found in all five gut regions, as well as faeces. Aspects of the gut environment, such as oxygen levels, production of antimicrobials or other factors, may shift microbial communities across gut regions. However, regardless of these differences, host species maintain distinguishable, phylosymbiotic assemblages of microbes that may have functional impacts for the host.

KEYWORDS
bacteria, gut microbiota, microbial biology, Peromyscus

1 | INTRODUCTION

Symbiotic interactions between microbes and hosts can have profound impacts on the ecology and evolution of animals (Kohl & Carey, 2016; McFall-Ngai et al., 2013). Given the ubiquity and importance of host–microbiota interactions, hosts can be structurally defined as holobionts that refer to individual hosts plus all of their stable and transient microbes (Bordenstein & Theis, 2015; Theis et al., 2016). Several outstanding questions remain regarding the nature of holobionts. Are microbial communities stochastically assembled from environmental influences, or might there be deterministic assembly mechanisms that predict these associations? If there are

microbiota differences between species, are they meaningful in an evolutionarily informed manner? How do different anatomical sites differentially affect assembly of microbial communities?

Recently, we established the assembly pattern of phylosymbiosis as a widespread phenomenon across several animal groups (Brooks, Kohl, Brucker, van Opstal, & Bordenstein, 2016). Phylosymbiosis hypothesizes that microbial communities should be more similar within a host species compared to across species and that increasing genetic divergence between host species will be associated with greater differences in their microbial communities (Bordenstein & Theis, 2015; Brucker & Bordenstein, 2012, 2013). As a result, one would expect concordance between the evolutionary history of host species and a dendrogram of the similarities in host-associated microbial community structures (Brooks et al., 2016). Importantly, phylosymbiosis does not assume vertical transmission, co-evolution or codiversification, given that many microbial communities may be newly assembled each generation (Brooks et al., 2016).

Moreover, this pattern has a functional component such that animal hosts subjected to interspecific microbial transplants exhibit decreased performance and survival compared to hosts receiving intraspecific microbial transplants (Brooks et al., 2016). In previous studies of vertebrate hosts, phylosymbiosis was only investigated using faecal samples (Peromyscus deer mice: (Brooks et al., 2016); apes: (Ochman et al., 2010); American pikas: (Kohl, Varner, Wilkening, & Dearing, 2017b) or samples from the distal colon (bats: (Phillips et al., 2012). Faecal samples represent the composite of several gut regions, with a bias towards the hindgut, do not necessarily reflect the hindgut community (Kohl & Dearing, 2014) and do not represent a functional community as it is not interacting extensively with the host. Further, the physiochemical conditions of digesta can vary widely over the course of the gut. For example, the gastric stomach typically has a highly acidic pH, while other gut regions maintain a circumneutral pH (Kohl, Stengel, Samuni-Blank, & Dearing, 2013). Additionally, levels of oxygen, production of antimicrobial and immune products, and gut motility all vary along the length of the gut (Donaldson, Lee, & Mazmanian, 2016). As a result, the microbial communities of various animal holobionts differ across gut regions (Kohl & Dearing, 2014; Kohl et al., 2017a; Suzuki & Nachman, 2016). To date, it is unknown whether the pattern of phylosymbiosis exhibited in faecal material exists in other gut regions. Given that phylosymbiosis has a functional component, it is important to understand whether the pattern of phylosymbiosis is present throughout the gastrointestinal tract, as the functions of gut chambers as well as the nature of host–microbe interactions may also vary across gut regions.

The gut microbiome can be impacted by many dimensions of an animal's ecology, physiology and behaviour. However, in this study, we controlled for these factors by choosing closely related species (sister taxa), using animals that had been bred in captivity for >13 generations and by maintaining them on the same diet (with the exception of Neotoma). While differences in ecology, physiology or behaviour may contribute to sculpting the microbial communities, we would not expect these factors to do so in a manner that

corresponded with the evolutionary history of the group. Using the microbial inventories from this experiment, we addressed the following questions: (i) Across host species, do gut regions harbour distinct microbial communities? (ii) Within each gut region, are the microbial communities of various host species distinguishable? and if so, (iii) Does the pattern of phylosymbiosis exist across gut regions? These results will help to reveal how microbial communities structure across gut regions and host species.

2 | METHODS

2.1 | Animals and sample collection

We obtained female individuals of Peromyscus species (P. polionotus, P. maniculatus, P. leucopus, P. eremicus, P. californicus, five individuals per species, except for P. maniculatus, where n = 3) from the Peromyscus Genetic Stock Center at the University of South Carolina. We also obtained two female individuals of Neotoma lepida (Neotoma is the sister genus of Peromyscus) captured from near White Rocks, Tooele County, Utah (40°19′N, 112°54′W). Last, we obtained four female individuals of wild, outbred Mus musculus from Dr. Wayne Potts (University of Utah). The founding animals of this M. musculus colony were collected from near Gainesville, Florida, USA. These animals have been randomly bred in captivity for roughly 13 generations and maintain an outbred condition (Gaukler et al., 2016; Meagher, Penn, & Potts, 2000). All rodent species were maintained on powdered laboratory rodent chow (Formula 8904, Harlan Teklad, Madison, WI), except for woodrats, which were fed powdered rabbit chow (Formula 2031, Harlan Teklad), given that woodrats are herbivorous and develop diabetes and metabolic syndromes when fed omnivorous diets (Post, Hawkins, & Eldridge, 2015; Schmidt-Nielsen, Haines, & Hackel, 1964). Individuals of M. musculus and of Peromyscus spp. were between 5 and 8 months old and were co-housed (within species) prior to our experiment. Animals were then housed singly in shoebox cages for a period of 5 weeks. Last, animals were placed in wire-bottom metabolic cages the evening prior to dissection to collect faecal samples. Faecal samples were frozen on dry ice and stored at −80°C.

All animals were euthanized with an overdose of isoflurane. We immediately dissected animals and collected contents of the foregut (fornix ventricularis or fundus), acidic stomach, small intestine, caecum and large intestine. All contents were frozen on dry ice and stored at −80°C. All procedures involving rodents were approved under the University of Utah Institutional Animal Care and Use Committee protocol #12-12010.

2.2 | Microbial inventories and data analysis

Total DNA was extracted from samples (~0.25 g of material) using the MoBio PowerFecal DNA isolation kit, which includes vortexing with garnet beads to facilitate cell lysis. We also conducted six "blank" extractions to correct for contaminants found in DNA extraction kits (Salter et al., 2014). Extracted DNA was sent to Argonne

National Laboratory for sequencing. The primers *515F* and *806R* were used to amplify the V4 region of the 16S rRNA gene (Caporaso et al., 2012). PCRs were conducted in triplicate, and the resulting products were pooled within a single sample. DNA was quantified using PicoGreen (Life Technologies, Carlsbad, CA) and a plate reader and cleaned using the UltraClean PCR Clean-Up Kit (MoBIO, Carlsbad, CA). Amplicons were sequenced on the Illumina MiSeq platform using previously described techniques (Caporaso et al., 2012).

Microbial sequences were analysed using QIIME version 1.9.1 (Caporaso et al., 2010). We applied standard quality control settings and split sequences into libraries using default parameters in QIIME. Sequences were grouped into de novo operational taxonomic units (OTUs) using a minimum sequence identity of 99%. The most abundant sequences within each OTU were designated as a "representative sequence" and aligned against the Greengenes core set (DeSantis et al., 2006) using PYNAST (Caporaso et al., 2009) with default parameters set by QIIME. FASTTREE (Price, Dehal, & Arkin, 2009) was used to generate a phylogenetic tree of representative sequences. Taxonomic classification of OTUs was performed using UCLUST (Edgar, 2010). Singleton OTUs and sequences identified as chloroplasts or mitochondria were removed from the analysis. Any OTUs present in the "blank samples" were considered contaminants and were removed from all other samples (Salter et al., 2014).

We first tested the effects of gut region and host species on microbial community membership and diversity. We calculated Faith's phylogenetic diversity (Faith, 1992). We calculated the mean of 20 iterations for a subsampling of 280 sequences per sample, a number that was determined as the lowest sequence return for any given sample. Such sequence numbers are sufficient for differentiating microbial communities. Three studies on various sample types (soil, human- and animal- associated microbiota, etc.) have demonstrated that ~100 sequences per sample are sufficient to detect differences in community structure and membership and that sequence depth beyond that provides little utility in detecting changes (Caporaso et al., 2012; Kuczynski et al., 2010a, 2010b). Phylogenetic diversity was compared across samples using ANOVAs with the fixed effects of host species and gut region, an interaction term of host species × gut region and including individual as a random effect. Microbial community structures were compared by conducting principal coordinates analysis (PCoA) using Bray–Curtis distances calculated using 280 sequences per sample. We then conducted the ANOSIM test on the resulting distance matrix with host species and gut region as independent variables, using 999 permutations. We also investigated which microbial genera differed in relative abundance across gut regions. Relative abundances of 142 bacterial genera were compared using LEFSE (Segata et al., 2011), with a logarithmic LDA score threshold of 4.0, using gut regions as the "class" and host species as the "subclass." We applied the false discovery rate correction to *p*-values to correct for multiple tests (Benjamini & Hochberg, 1995).

Next, we tested whether host species were distinguishable across all gut regions and whether this distinguishability varied across gut regions. For these following analyses, each gut region was tested separately, though the sample sizes and rarefied sequence numbers were identical for all gut regions. First, we conducted PCoA using Bray–Curtis distances calculated using 280 sequences per sample. We conducted 20 iterations of this analysis and compiled the resulting ANOSIM R-statistics for each gut region. Each ANOSIM still used 999 permutations; we conducted this test 20 times to obtain a range of ANOSIM *R*-values, which range from 0 to 1. An ANOSIM *R*-value of 0 means that all communities would be identical, while *R*-value of 1 signifies that communities are highly distinguishable across groups (Clarke, 1993). We qualitatively evaluated whether the ANOSIM *R*-statistic changed across the gut. We also investigated which microbial taxa distinguished host species across gut regions. Relative abundances of 142 bacterial genera were compared using LEFSE (Segata et al., 2011), with a logarithmic LDA score threshold of 4.0.

Last, we tested whether microbial signatures exhibited phylosymbiotic patterns across gut regions. We conducted the following analysis separately for each gut region. First, sequences were collapsed by host species. Then, we used the command jackknifed_beta_diversity.py within QIIME to generate UPGMA trees of the microbial communities, using 970 sequences per host species and 20 iterations. This command also produced a consensus tree. We compared topologies of microbiome dendrograms to previously published rodent host phylogenies (Platt, Amman, Keith, Thompson, & Bradley, 2015; Stepphan, Adkins, & Anderson, 2004) by calculating the Robinson–Foulds and Matching Cluster congruency scores as described previously (Brooks et al., 2016). Matching Cluster and Robinson ± Foulds *p*-values were determined by the probability of 100,000 randomized bifurcating dendrogram topologies yielding equivalent or more congruent phylosymbiotic patterns than the microbiota dendrograms (Brooks et al., 2016).

3 | RESULTS

Our sequencing effort resulted in over 419,000 sequences. Sequence return varied by host species, gut region and with a significant species × gut region interaction (*p* < .001 for all effects). Foregut and stomach samples exhibited the lowest sequence returns, while large intestine and faecal samples had the highest. Average numbers of sequences per sample type can be found in Table S1.

Microbial phylogenetic diversity varied significantly across species (Figure 1a; $F_{6,132} = 40.04$, *p* < .0001), such that *Mus musculus* exhibited the lowest diversity, while *Peromyscus californicus*, *Peromyscus maniculatus*, *Peromyscus leucopus* and *Peromyscus polionotus* all had the highest diversity. Additionally, phylogenetic diversity differed significantly by gut region (Figure 1a; $F_{5,132} = 77.86$, *p* < .0001), with the foregut and small intestine harbouring the lowest diversity, while the highest diversity was found in the caecum, large intestine and faecal communities. There was no significant species × gut region interaction effect for the measurement of phylogenetic diversity ($F_{30,132} = 1.18$, *p* = .25). Principal coordinate analysis of microbial inventories also demonstrated that beta diversity of microbial
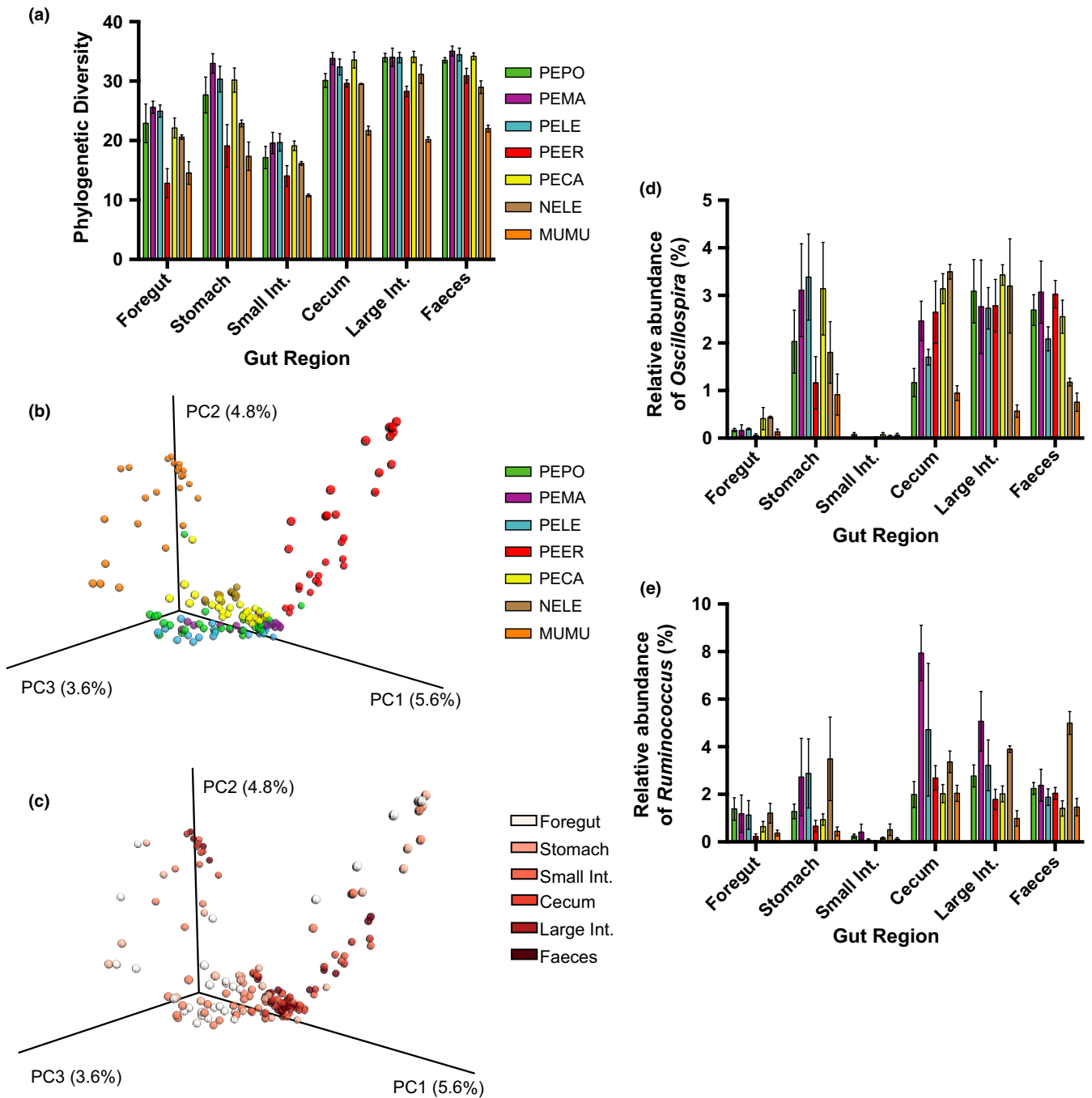
FIGURE 1 Variation in microbial communities across gut regions. (a) Faith's phylogenetic diversity of various rodent species across gut regions. (b) Principal coordinate plot of Bray–Curtis distances with samples coloured by host species. (c) Principal coordinate plot of Bray–Curtis distances with samples coloured by gut region. (d) Relative abundances of the genus *Oscillospira* across gut regions. (e) Relative abundances of the genus *Ruminococcus* across gut regions. Bars represent means ± *SEM*. PEPO: *Peromyscus polionotus*; PEMA: *P. maniculatus*; PELE: *P. leucopus*; PEER: *P. eremicus*; PECA: *P. californicus*; NELE: *Neotoma lepida*; MUMU: *Mus musculus*

communities was structured by both species and gut region. When investigating all samples, both host species (Figure 1b, ANOSIM test: $R = .76$, $p < .001$) and gut region (Figure 1c, ANOSIM test: $R = .26$, $p < .001$) had significant effects on microbial community structure. When using all samples, two microbial genera varied in abundance across gut regions: *Oscillospira* (Figure 1d; logarithmic LDA score: 5.01; FDR-corrected $p < 0.0001$) and *Ruminococcus* (Figure 1e;

logarithmic LDA score: 4.81; FDR-corrected $p < 0.0001$). These genera were most abundant in the stomach and hindgut regions.

Next, we investigated whether microbial communities were distinguishable across all gut regions. We divided samples by gut region and performed 20 iterations of the ANOSIM test in each gut region. In all cases, p-values were less than .001, demonstrating that host species are distinguishable across all gut regions. We also

qualitatively compared the ANOSIM test statistic across gut regions. Again, an ANOSIM R-value of 0 means that all communities would be identical, while an R-value of 1 signifies that communities are highly distinguishable across groups. From our data, the ANOSIM R-values increase along the length of the gut, suggesting that communities become more differentiated across host species in the hindgut (Figure 2).

We also investigated the genera that differentiate host species along the length of the gut. The relative abundances of *Lactobacillus* and *Allobaculum* differed significantly across host species in almost all gut regions (Figure 3). Additionally, many other genera differed across hosts, with the number of significantly different genera being higher in the hindgut (Figure 3).

Last, we investigated whether the distinguishable communities also followed patterns of phylosymbiosis. Indeed, using both the Robinson Foulds and Matching Cluster methods, there were significant patterns of phylosymbiosis across all gut regions (Table 1; Figure 4).
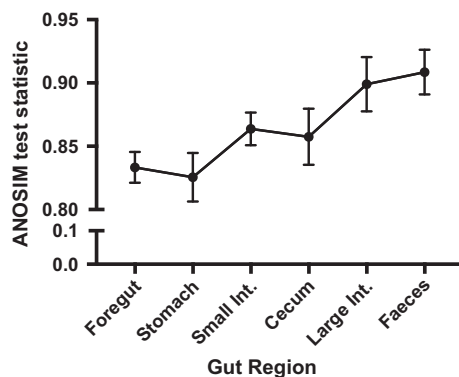
4 | DISCUSSION

Previous studies investigating assembly of gut microbial communities have demonstrated phylosymbiosis in several vertebrate holobionts. However, these studies were all conducted using faeces or samples from the distal colon (Brooks et al., 2016; Kohl et al., 2017b; Ochman et al., 2010; Phillips et al., 2012). Given that the nature of host–microbe interactions can vary across gut regions (Donaldson et al., 2016), we tested whether phylosymbiotic trends exist along the gut. We found that microbial communities vary along the length of the gastrointestinal tract, but that host species are still distinguishable in each gut region, and that phylosymbiosis also is detectable across the various microbial communities present in the different regions of the gut.

First, we compared diversity and microbial community composition across gut regions. Measurements of phylogenetic diversity

FIGURE 2 Values of the ANOSIM test statistic across gut regions, using host species as the explanatory variable. An ANOSIM R-value of 0 means that all communities would be identical, while R-value of 1 signifies that communities are highly distinguishable across groups. Points represent mean ± *SEM* for 20 iterations of the analysis
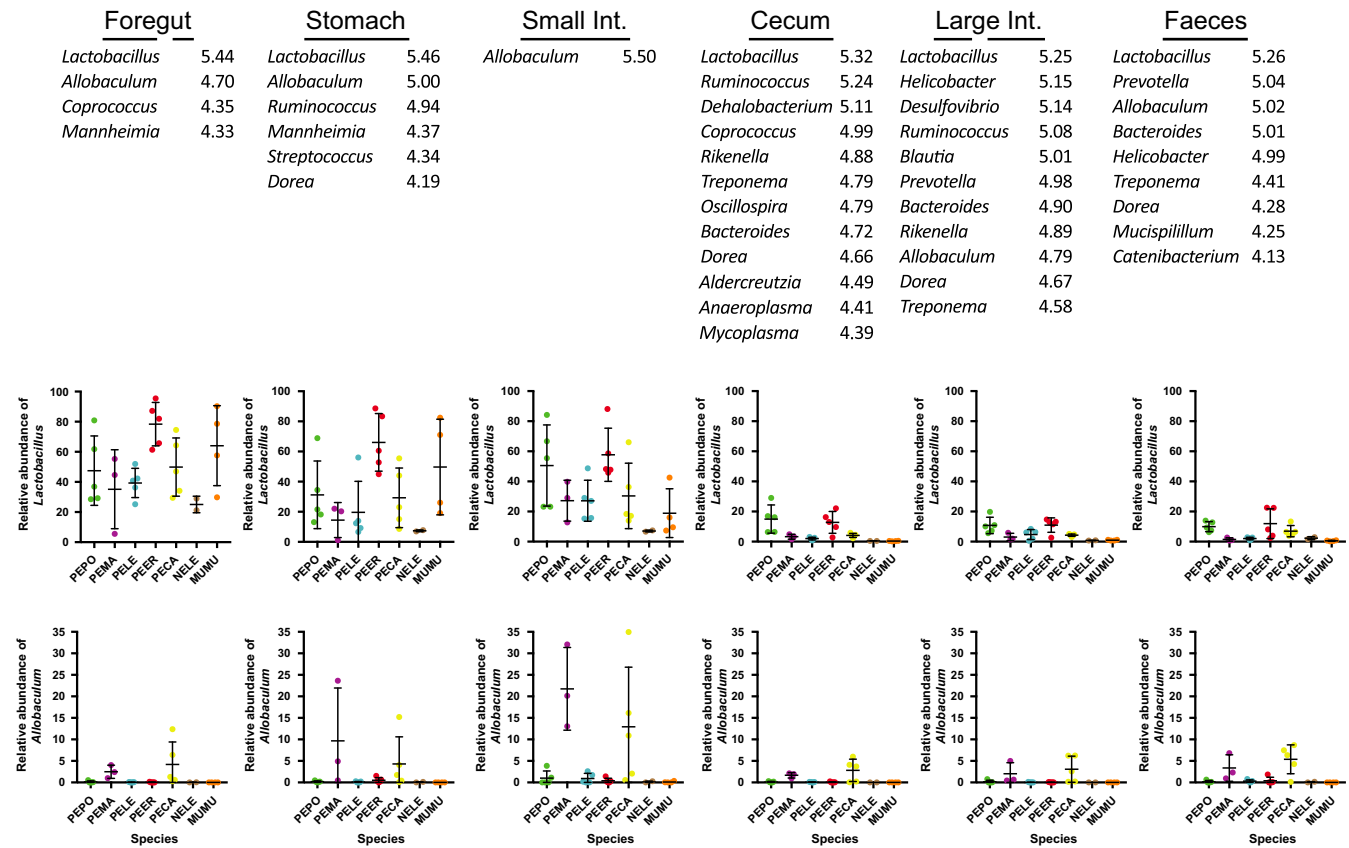
varied significantly, such that the hindgut regions (caecum, large intestine, faeces) had the highest diversity. These results are consistent with other studies that found highest diversity in the hindgut regions of rodents (Kohl & Dearing, 2014; Suzuki & Nachman, 2016) and reptiles (Colston, Noonan, & Jackson, 2015; Kohl et al., 2017a). In general, the hindgut is more supportive for microbial growth, as it has lower oxygen concentrations, neutral pH and reduced production of antimicrobial compounds (Donaldson et al., 2016), which may favour the growth of a more diverse microbial community. For example, the caecum is a region known for high microbial activity as evidenced by high levels of VFA production, which the host can use an energy source (Stevens & Hume, 2004).

Across all samples, we found that both gut region and host species determined microbial community structure, with host species having a greater effect. The relative abundances of two microbial genera, *Ruminococcus* and *Oscillospira*, exhibited differential abundances across gut regions. Notably, their abundances were higher in hindgut regions and the gastric stomach. The genus *Ruminococcus* is known to degrade fibre (Leatherwood, 1965) and dominates the hindgut communities of other species, such as koalas (Barker, Gillett, Polkinghorne, & Timms, 2013) and lizards (Kohl et al., 2017a). The specific functions of the genus *Oscillospira* are unclear due to the fact that it has not been cultured (Konikoff & Gophna, 2016). In humans, relative abundances of this genus increase when individuals are feeding on animal-based diets (David et al., 2014), and genome reconstruction from metagenomic analysis of the human gut microbiota suggests that human-associated species of *Oscillospira* utilize host-produced sugars (Gophna, Konikoff, & Nielsen, 2017). However, in other animals, the genus *Oscillospira* has been hypothesized to play roles in fibre fermentation, given its presence in the guts of many ruminants and other herbivores (Kohl & Dearing, 2014; Mackie et al., 2003). For example, ruminants fed fresh forage exhibit higher abundances of *Oscillospira* compared to those fed low-fibre grain, and microscopy reveals that these bacteria associate with the surfaces of plant material in the gut (Mackie et al., 2003). Further, high-fibre diets increase the abundance of *Oscillospira* in the guts of lizards (Kohl et al., 2016a). The rodents in our study may maintain higher abundance of *Ruminococcus* and *Oscillospira* in the hindgut to aid in the digestion of dietary fibre.

It is unclear why the relative abundances of *Ruminococcus* and *Oscillospira* are also high in the gastric stomach. Isolated species of *Ruminococcus* cannot grow below a pH of ~6 (Russell & Dombrowski, 1980), yet the pH of the rodent gastric stomach is between 1.5 and 4 (Kohl et al., 2013). A recent study that inventoried of the gut microbiota of Plateau pikas (*Ochotona curzoniae*) and Daurian pikas (*Ochotona daurica*) also found that *Oscillospira* and *Ruminoccocus* dominate the stomach communities of these mammals (Li, Li, Tu, Kou, & Li, 2017), and similar results have been found in another woodrat species, *Neotoma albigula* (Kohl & Dearing, 2014). However, it should be noted that relative abundances of microbes based on 16S rRNA sequences cannot discriminate between live and dead bacteria. Future studies could probe the microbial function of the gastric stomach through metatranscriptomic approaches.

Foregut		Stomach		Small Int.		Cecum		Large Int.		Faeces	
Lactobacillus	5.44	*Lactobacillus*	5.46	*Allobaculum*	5.50	*Lactobacillus*	5.32	*Lactobacillus*	5.25	*Lactobacillus*	5.26
Allobaculum	4.70	*Allobaculum*	5.00			*Ruminococcus*	5.24	*Helicobacter*	5.15	*Prevotella*	5.04
Coprococcus	4.35	*Ruminococcus*	4.94			*Dehalobacterium*	5.11	*Desulfovibrio*	5.14	*Allobaculum*	5.02
Mannheimia	4.33	*Mannheimia*	4.37			*Coprococcus*	4.99	*Ruminococcus*	5.08	*Bacteroides*	5.01
		Streptococcus	4.34			*Rikenella*	4.88	*Blautia*	5.01	*Helicobacter*	4.99
		Dorea	4.19			*Treponema*	4.79	*Prevotella*	4.98	*Treponema*	4.41
						Oscillospira	4.79	*Bacteroides*	4.90	*Dorea*	4.28
						Bacteroides	4.72	*Rikenella*	4.89	*Mucispilillum*	4.25
						Dorea	4.66	*Allobaculum*	4.79	*Catenibacterium*	4.13
						Aldercreutzia	4.49	*Dorea*	4.67		
						Anaeroplasma	4.41	*Treponema*	4.58		
						Mycoplasma	4.39				

FIGURE 3 Microbial genera that were significantly different across host species in each gut region. LEFSE analysis was conducted to determine genera that were differentially abundant in each gut section. Values next to genus names are the logarithmic LDA scores. Graphs depict mean ± *SEM* abundances of *Lactobacillus* and *Allobaculum*, as these genera often were differentially abundance across host species. PEPO: *Peromyscus polionotus*. PEMA: *P. maniculatus*; PELE: *P. leucopus*; PEER: *P. eremicus*; PECA: *P. californicus*; NELE: *Neotoma lepida*; MUMU: *Mus musculus* [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Results from analyses for phylosymbiosis across gut regions

|	Robinson–Foulds		Matching cluster	
	nRF	***p***	**nMC**	***p***
Foregut	0.42	.005	0.34	.005
Stomach	0.58	.005	0.32	<.001
Small Int.	0.60	.04	0.33	.005
Caecum	0.52	.005	0.28	<.001
Large Int.	0.56	.005	0.33	.005
Faeces	0.44	.005	0.33	<.001

Normalized Robinson–Foulds (RF) and normalized Matching Cluster (MC) metrics were determined following the methods by Brooks et al. (2016). Normalized metrics (nRF and nMC) scale from 0.0 (complete congruence) to 1.0 (complete incongruence).

We observed that microbial communities were distinguishable across host species for all gut regions. Additionally, the distinguishability of host-associated microbiotas seemed to increase along the length of the gut. Similarly, the effect of "individual" is a strong determinant of the hindgut microbial community membership and structure in mice, but less so in the upper gastrointestinal tract (oesophagus, stomach, small intestine; Suzuki & Nachman, 2016). Thus, there seems to be higher selectivity for individual- or species-specific microbes towards the end of the gut. This result could be due to the digestion and removal of transient microbes, which are often present in food material (Lang, Eisen, & Zivkovic, 2014), yet make up a small proportion of the faecal microbiome (Kohl et al., 2017a). Additionally, there may be variation in the gut environment, such as lower oxygen concentrations and differential production of immune compounds that select for a specific microbiome in the hindgut. In rodents, the hindgut, particularly the caecum, is critical in the generation of energy for the host through the fermentation of fibre (Stevens & Hume, 2004). It is possible that this critical function of the hindgut drives the strong relationship between host evolutionary history and microbial communities. This notion is also consistent with the prediction of phylosymbiosis that function ultimately governs the underlying community structure.

Last, we observed that the pattern of phylosymbiosis existed across all gut regions. Thus, there is significant ecological structuring of gut microbial communities in concordance with host phylogeny. We have previously discussed mechanisms which may underlie patterns of phylosymbiosis (Brooks et al., 2016), such as control by the host immune system (Franzenburg et al., 2013), vertical transmission
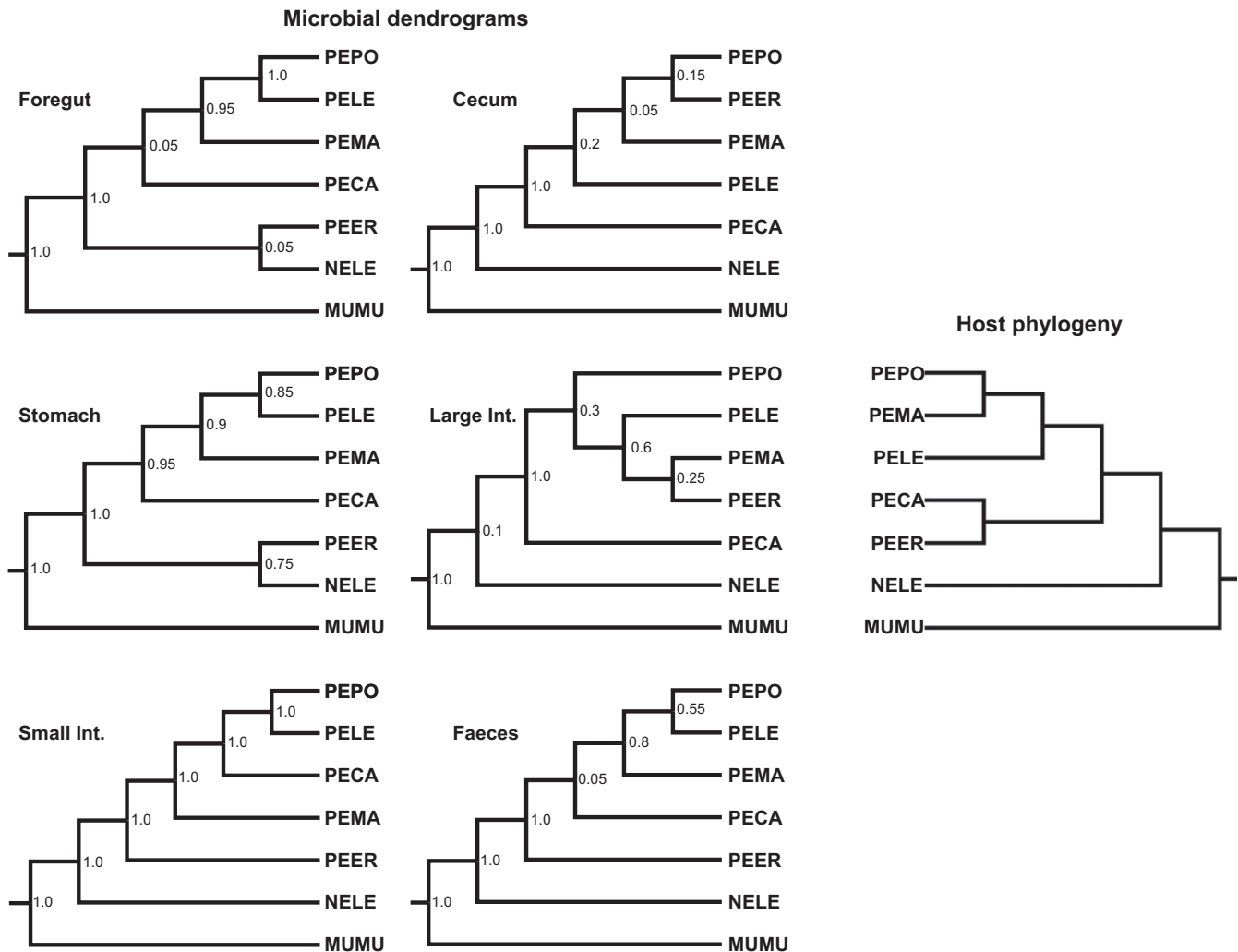
FIGURE 4 Comparison of dendrograms of microbial community structures and host phylogeny. Microbial dendrograms are consensus trees from 20 iterations of a subsampling of 970 sequences. Values at nodes are jackknife support values. Even though these values are low at some nodes, it should be noted that this only represents a consensus tree, and statistics were conducting using the full set of iterations. Host phylogeny is from Platt et al. (2015) and Stepphan et al. (2004). PEPO: *Peromyscus polionotus*; PEMA: *P. maniculatus*; PELE: *P. leucopus*; PEER: *P. eremicus*; PECA: *P. californicus*; NELE: *Neotoma lepida*; MUMU: *Mus musculus*

of microbial communities or keystone taxa (Funkhouser & Bordenstein, 2013) or microbial selection of appropriate environments (Costello, Stagaman, Dethlefsen, Bohannan, & Relman, 2012). Our results notably suggest that these mechanisms apply to communities along the entire length of the gut, even early on in the foregut. It would be interesting to investigate these trends in other body sites, such as inventorying the oral, respiratory or skin microbiome, which may help to illuminate how widespread this phenomenon is across the bodies of vertebrates. Interestingly, phylosymbiosis has been observed in the host-associated microbiota of sponges (Easson & Thacker, 2014) and hydra (Fraune & Bosch, 2007), which are largely exposed to the external environment, suggesting strong host control of these communities.

Our study aimed to minimize the effects of environmental variability using animals that have been bred in captivity for >13 generations (with the exception of *Neotoma*). In nature, animals are exposed to the microbiota of soil, their food sources, conspecifics and other microbial sources (Kohl & Dearing, 2014; Kohl et al., 2016b; Smith, Snowberg, Caporaso, Knight, & Bolnick, 2015). Moreover, ecological differences such as social interactions (Tung et al., 2015), diet (Bolnick et al., 2014) or rates of coprophagy (Fitzgerald, Gustafsson, & McDaniel, 1964) can influence gut microbial communities. These exposures and ecological differences may enhance or overwhelm the ability to detect phylosymbiotic signatures. Indeed, when comparing the microbiota of syntopic individuals of *P. maniculatus* and *P. leucopus*, these species were indistinguishable (Baxter et al., 2015). It is possible that some ecological differences from nature persist in captivity, as wild-caught rodents retain a majority of their wild microbiome (Kohl & Dearing, 2014), and some of the gut microbiota is transmitted from generation to generation (Funkhouser & Bordenstein, 2013; Kohl, Skopec, & Dearing, 2014).

Additionally, physiological differences between species may persist in captivity, and obscure the ability to detect phylosymbiosis.

In our study and a previous investigation into phylosymbiosis (Brooks et al., 2016), the samples from *P. eremicus* exhibit strong differences from other species of *Peromyscus* and do not follow the patterns of phylosymbiosis. For example, in the current study, *P. eremicus* exhibited much lower microbial diversity compared to the other *Peromsycus* species. The colony of *P. eremicus* has been bred in captivity since 1993 and was being fed the same diet as all other species. However, *P. eremicus*, also known as the cactus mouse, is a desert-adapted species and exhibits much lower rates of voluntary drinking compared to other *Peromyscus* species (Glenn, 1970). This difference, or other physiological adaptations in this species, may affect the gut microbial community structure, even in captivity.

Overall, we found that the gut microbiota varies across gut regions of closely related rodent hosts and that this variance is consistent with host evolutionary history, resulting in a pattern of phylosymbiosis. We have previously demonstrated that phylosymbiosis among species of *Permoyscus* has a functional component such that individuals of *P. polionotus* inoculated with the microbial communities from more distantly related host species exhibited a reduced ability to digest food material (Brooks et al., 2016). Future studies could investigate how these interspecific inoculations impact phylosymbiosis across gut regions to determine where recipient animals are most affected and how these effects might contribute to the overall decrease in digestibility. Together, investigations into phylosymbiosis across body sites as well as further studies focused on underlying mechanisms and functional effects will enhance our understanding of how natural selection may act on the collective of the host and microbiome known as the holobiont.

DATA ACCESSIBILITY

All 16S rRNA sequences have been deposited in the Sequence Read Archive (SRA) under Accession no. PRJNA412990.

AUTHOR CONTRIBUTION

K.D.K. Carried out the experiment, analyzed the data, and wrote the manuscript. M.D.D and S.R.B Oversaw the study, provided valuable feedback, and helped to review the manuscript.

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