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MICROBIOME DIFFERENCES BETWEEN HUMAN HEAD AND BODY LICE ECOTYPES REVEALED BY 16S RRNA GENE AMPLICON SEQUENCING

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ABSTRACT

Human head lice and body lice (*Pediculus humanus*) are neglected ectoparasites. Head lice continue to be prevalent in children worldwide, and insecticide resistance in these insects has complicated their treatment. Meanwhile, body lice, which are most common in the developing world, are resurging among marginalized populations in developed nations. Today, the microbiome is being increasingly recognized as a key mediator of insect physiology. However, the microbial communities that inhabit human lice have remained unknown beyond only a few species of bacteria. Knowledge of the microbiomes of head and body lice could improve our understanding of the observed physiological differences between the 2 ecotypes and potentially inform the development of novel interventions against lice infestations and louse-borne infectious diseases. Toward these goals, here we performed *16S rRNA* gene amplicon sequencing to characterize the microbiomes of both head and body lice and identify patterns of interest among these communities. Our data reveal that head and body lice harbor limited but distinct communities of bacteria that include known intracellular endosymbionts ("*Candidatus* Riesia pediculicola"), extracellular bacteria that may be horizontally acquired from the host environment, and a number of taxa of known or potential public health significance. Notably, in body lice, the relative abundance of vertically transmitted endosymbionts is lower than in head lice, which is a significant driver of greater alpha diversity. Further, several differentially abundant non-endosymbiont taxa and differences in beta diversity were observed between head lice and body lice. These findings support the hypothesis that microbiome differences could contribute to the divergence between human louse ecotypes and underscore the need for future studies to better comprehend the acquisition and physiological roles of human lice microbiomes.

The human head louse, *Pediculus humanus capitis*, and the human body louse, *Pediculus humanus humanus*, are blood-feeding insects that specifically parasitize human hosts and can cause a number of clinical manifestations (Clark et al., 2013). While the taxonomic relationship between head and body lice remains debated, a mounting body of evidence indicates that the 2 are ecotypes of the same species, occurring in 6 divergent, geographically distinct clades (Ashfaq et al., 2015; Louni et al., 2018; Amanzougaghene et al., 2019). Multiple analyses of head and body lice collected from individuals suffering from dual infestations have revealed identical genotypes (Li et al., 2010; Veracx et al., 2012). Moreover, multiple efforts over decades have failed to find physiologically meaningful morphological or genetic markers that can consistently differentiate head and body lice once removed from the host, though 14 differentially expressed transcripts, numerous alternative splicing events, and a single genomic difference in a gene encoding a hypothetical protein of 69 amino acids have been identified in select populations (Olds et al., 2012; Drali et al., 2013; Tovar-Corona et al., 2015).

Though mostly indistinguishable at the morphological and genetic levels, body lice and head lice exhibit notable behavioral and physiological differences. Primarily, head lice nest and lay eggs on the head, whereas body lice do so off the host, on the clothing. Differences in fecundity, longevity, and blood-feeding habits have also been documented (Bonilla et al., 2013). In addition, it appears that head lice immunologically respond to bacterial challenge more effectively than do body lice, a property that may explain why body lice have traditionally been associated with pathogen transmission and outbreaks of human disease while head lice have not (Bonilla et al., 2013). Specifically, body lice exhibit lower phagocytic cell activity and lower production of cytotoxic reactive oxygen species than do head lice, as well as

Table I. Summary of samples collected for sequencing.

Sample name	Head or body lice	Origin	Location
HL1	head	human host	W. Palm Beach, Florida
HL2	head	human host	White Bear Lake, Minnesota
HL3	head	human host	Edmonds, Washington
HL4	head	human host	Fargo, North Dakota
HL5	head	human host	Turnersville, New Jersey
HL6	head	human host	Bedford Hills, New York
HL7	head	human host	Westmont, Illinois
HL8	head	human host	Valparaiso, Indiana
HL9	head	human host	Dothan, Alabama
HL10	head	human host	Bedford, Virginia
HL11	head	human host	Waterville, Maine
HL12	head	human host	Dallas, Georgia
HL13	head	human host	W. Bridgewater, Massachusetts
HL14	head	human host	Overland Park, Kansas
HL15	head	human host	Enid, Oklahoma
HL16	head	human host	Sioux Falls, South Dakota
BL1	body	human host	Detroit, Michigan
BL2	body	human host	Detroit, Michigan
BL3	body	human host	San Francisco, California
BL4	body	human host	San Francisco, California
BL5	body	human host	San Francisco, California
BL6	body	human host	Minneapolis, Minnesota
BL7	body	human host	Minneapolis, Minnesota
BL8	body	lab colony	Jerusalem, Israel
BL9	body	lab colony	Jerusalem, Israel

temporal delays in the humoral immune response (i.e., transcription of immunoresponsive genes such as *defensin*) relative to head lice following bacterial infection (Kim et al., 2011, 2017; Previte et al., 2014). So far, no underlying mechanisms have been elucidated to explain the physiological differences between lice ecotypes, including differential immune responses. Intriguingly, field studies have suggested that under conditions of deteriorated hygiene, body louse variants may emerge from head lice infestations (Veracx et al., 2012). This phenomenon also remains unexplained but indicates that the traits differentiating head and body lice may be plastic and that differentiation may be triggered, at least to some degree, by environmental cues.

Today, the microbiome is being increasingly recognized as a key mediator of insect physiology (Douglas, 2015), but the microbiomes of human lice remain largely uncharacterized. Both head and body lice have been found to harbor a number of human pathogens. The most prominent of these are the agents of trench fever (*Bartonella quintana*), louse-borne typhus (*Rickettsia prowazekii*), and louse-borne relapsing fever (*Borrelia recurrentis*) (Fournier et al., 2002; Veracx and Raoult, 2012). Additional pathogenic bacteria such as *Yersinia pestis*, *Coxiella* spp., *Ehrlichia* spp., *Anaplasma* spp., *Serratia* spp., and *Acinetobacter* spp. have also been detected in lice from some environments (Piarroux et al., 2013; Amanzougaghene et al., 2017). Beyond these pathogens, human lice harbor an intracellular endosymbiont, "*Candidatus* Riesia pediculicola" (Sasaki-Fukatsu et al., 2006). This bacterium undergoes a complex vertical transmission cycle involving multiple mycetomes and provides essential B vitamins to its hosts (Perotti et al., 2007).

Studies of louse–microbe associations have largely involved the use of low-throughput techniques such as culturing or PCR to test for the presence of specific pathogens of interest or have focused on the characterization of endosymbionts. Thus, the broader spectrum of bacteria that could be associated with lice has not been fully appreciated. In particular, whether lice horizontally acquire cuticle or gut microbiomes from their environment has remained unknown. Body lice primarily infest individuals experiencing poor living conditions (Bonilla et al., 2009; Brouqui, 2011; Ly et al., 2017), while head lice are common worldwide in children (Falagas et al., 2008). Prior work has suggested that individuals who are homeless or have previously experienced homelessness, as well as individuals living in environments of urban decay can harbor unique microbiomes and may be abnormally susceptible to certain bacterial infections (Rzotkiewicz, 2016; Leibler et al., 2017; Adebanjo et al., 2018; Brenner et al., 2018). Microbiome differences among head and body lice, therefore, seem plausible given their distinct epidemiology. In turn, we hypothesize that microbiome differences could contribute to the physiological differences between the 2 ecotypes of the same species

Here, we sought to determine whether head lice and body lice harbor distinct bacterial microbiomes in order to test the first component of this hypothesis. We also sought to determine whether lice might acquire or maintain human pathogens that have not been detected by low-coverage methods. To do so, we characterized the bacterial communities associated with different head and body lice populations collected from individuals across the United States using high-throughput *16S rRNA* gene amplicon sequencing.

MATERIALS AND METHODS

Lice samples

In total, 16 collections of head lice and 9 collections of body lice were analyzed in our study. Information on the origin of each collection is provided (Table I). In brief, 16 independent groups of head lice and 7 independent groups of body lice were collected by volunteer clinicians directly from 23 individual persons who provided consent. Lice were first identified morphologically as *P. humanus* by visual inspection and then classified as head lice or body lice based on the location on the body where they were found (i.e., head lice if from the head and body lice if from the body or clothing). No patient data or identifiers were associated with the samples to facilitate the logistics of the study. Collected lice were then shipped to the laboratory and stored under equal conditions (dry at a temperature of –20 C) prior to processing. While samples collected from each patient typically contained numerous life stages of lice, only adult female lice were used for consistency in our study. Five adult female lice from each patient were pooled in sterile PBS (GE, Chicago, Illinois) for DNA extraction. Thus, 80 head lice and 35 body lice derived from patients were included in the study. We also analyzed 2 pools of 10 adult female body lice derived from a laboratory colony that was reared on live rabbits.

DNA extractions

Microbial DNA extraction from whole insects was carried out using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Venlo,

Netherlands) with a modified protocol that included beating with glass and zirconium oxide beads and extended lysis incubation to enhance yields. Bead beating was performed using lysis matrix H tubes (MP Biomedicals, Santa Ana, California) on a BeadBug homogenizer (Benchmark Scientific, Sayreville, New Jersey). Two mock extractions using the same reagents and protocol were also conducted, pooled, and subjected to PCR and sequencing as a negative control. Owing to previously published studies indicating that surface washing of insects prior to DNA extraction does not improve sequencing-based analyses of insect microbiomes, no such steps were performed (Hammer et al., 2015).

16S rRNA gene amplicon sequencing

Library preparation and sequencing were performed at the South Dakota State University Genomics Sequencing Facility in Brookings, South Dakota. Extracted DNA samples were analyzed using a Qubit Fluorometer (ThermoFisher, Waltham, Massachusetts) to ensure sufficient quality and concentration for downstream applications. A 30-cycle PCR was performed using barcoded primers for the V4 hypervariable region of bacterial *16S rRNA* gene (F515/R806), and samples were subsequently run on a bioanalyzer (Agilent Technologies, Santa Clara, California) to verify successful amplification of experimental samples and lack of amplification in the negative control. Amplification was carried out using KAPA HiFi HotStart Ready Mix (KAPA Biosystems Inc., Wilmington, Massachusetts) using the following polymerase chain reaction (PCR) thermal profile: 3 min at 95 C, 25 cycles (30 sec at 95 C, 30 sec at 55 C, 30 sec at 72 C), 5 min at 72 C. PCR clean-up was done with a SMARTer Apollo system (Takara Inc., Mountain View, California) using AMPure XP beads (Beckman Coulter, Indianapolis, Indiana). A second round of PCR amplification was carried out to introduce individual Nextera XT (Illumina Inc., San Diego, California) indices in each library. This second amplification was carried out using KAPA HiFi HotStart Ready Mix (KAPABiosystems) using the following PCR thermal profile: 3 min at 95 C, 8 cycles (30 sec at 95 C, 30 sec at 55 C, 30 sec at 72 C), 5 min at 72 C; PCR clean-up was done the same way as described for the first PCR round. Samples were pooled at equal concentrations, and sequencing was carried out in an Illumina MiSeq (Illumina Inc.) using v 2 nano run (2×500 bp) on a single lane. Statistics and quality control metrics for the resulting reads are provided in the supplemental material (Suppl. Data, Table S1; Suppl. Data, Fig. S1). All raw sequences will be publicly available in the NCBI Sequence Read Archive upon publication of this article (PRJNA548959).

Bioinformatic analyses

Raw reads were processed for taxonomic assignment and bioinformatic analyses as follows. Microbiome analyses were performed using QIIME 2 2019 4.0 (Bolyen et al., 2019) on high-performance computing clusters (Lawrence-HPC, University of South Dakota, Vermillion, South Dakota). In short, raw demultiplexed sequences were quality filtered using the q2-demux plugin and denoised with DADA2 (Callahan et al., 2016) using q2-dada2. MAFFT (Katoh et al., 2002) was used to align all amplicon sequence variants (ASVs) via q2-alignment. The aligned data were used to construct phylogeny with fasttree2 (Price et al., 2010) via q2-phylogeny. Alpha-diversity metrics; Shannon and Simpson index, and beta diversity metrics; Bray–Curtis dissimilarity, distance to centroid and principal coordinates analysis (PCoA) were estimated using q2-diversity on unrarefied samples (McMurdie and Holmes, 2014). Taxonomy was assigned to ASVs using the q2-feature-classifier (Bokulich et al., 2018) against the Greengenes 13_8 reference (99% OTU threshold) (McDonald et al., 2012). Data visualization and statistics were carried out with QIIME 2 viewer as well as other tools, namely, Microbiome Analyst.ca (Dhariwal et al., 2017), Mian (Boyang, 2018), and Circos (Krzywinski et al., 2009). QIIME 2 output feature-abundance and taxonomy tables were fed to these tools to analyze taxon abundance, alpha diversity, beta diversity, phylogeny, correlation, and differential relationships among abundant taxa. Statistical analyses of differential abundance were performed in MicrobeAnalyst.ca (Dhariwal et al., 2017). The Bray–Curtis metric (Bray and Curtis, 1957) was used to assess community variability and principal coordinates analysis with louse ecotype as the experimental variable (body vs. head) and was conducted in MicrobiomeAnalyst.ca. (Dhariwal et al., 2017). Statistical analyses of alpha and beta diversity and construction of a weighted co-correlation network based on Pearson product-moment correlation coefficients were done using Mian (Boyang, 2018). Raw data were visually summarized using Circos (Krzywinski et al., 2009). Analyses were conducted in parallel with and without filtering highly abundant reads derived from *Candidatus* Riesia pediculicola to better discriminate the effects of the endosymbiont on diversity metrics and clustering. A good's coverage index of >0.999 for all samples and lack of correlation between library size and species richness indicated adequate read depth to sample existing diversity (Fig. S1)

RESULTS

As expected, the louse endosymbiont, *Candidatus* Riesia pediculicola, was the most abundant bacterium detected in all samples (Fig. 1; Table II). Statistical analysis determined that the relative abundance of endosymbionts was different between the 2 ecotypes (Mann–Whitney test, $P = 0.009$), accounting for an average of 97% of reads in head lice samples, and an average of 84% of reads in body lice samples. In addition to the louse endosymbiont, several other microbes were prevalent across our samples and were detected at appreciable relative abundances. The most abundant taxa were the following: *Staphylococcus*, *Acinetobacter*, *Propionibacterium*, *Enterobactericeae*, *Corynebacterium*, *Pseudomonas*, and *Flavobacterium* (Fig. 1; Table II). Notably, these taxa are all known components of the human skin microbiome (van Rensburg et al., 2015; Dreno et al., 2016). Of these taxa, *Acinetobacter*, *Propionibacterium*, and *Enterobacteriaceae* appeared to be differentially abundant (Table II). The relative abundance of *Acinetobacter* was higher in body lice than in head lice when considered in the context of all bacteria (Mann–Whitney test, $P = 0.004$) as well as in the context on non-endosymbiont bacteria only (Mann–Whitney test, $P = 0.0123$). These results were consistent with prior work that employed PCR to examine the prevalence of *Acinetobacter* infection in lice from Ethiopia and found this to be higher in the body ecotype (Kempf et al., 2012). While the relative abundance of *Propionibacterium* was not different when considering endosymbiont infection, within the non-endosymbiont community its relative abundance was greater in head lice (Mann–Whitney test, $P = 0.0221$). Conversely, the overall relative abundance of Enterobacteriaceae

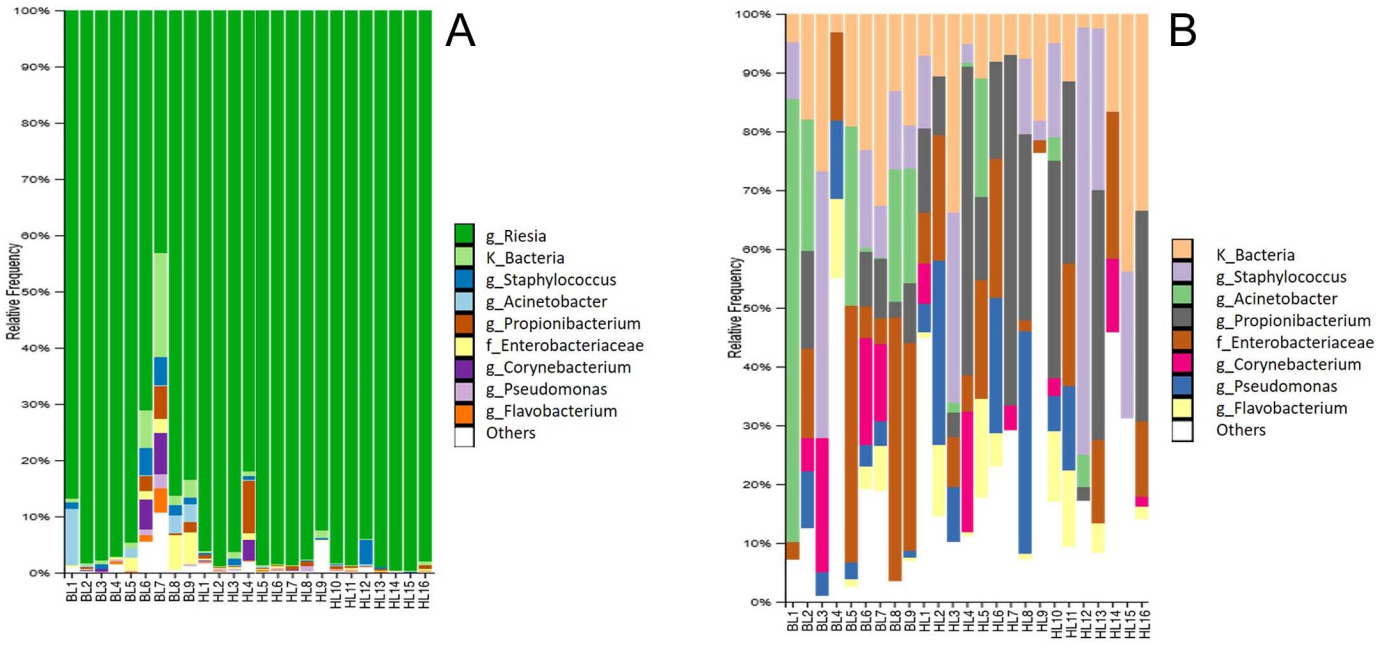

Figure 1. Relative abundances of bacterial taxa present in head and body lice samples. Each bar corresponds to an individual pool of head (HL) or body (BL) lice. (**A**) Data include reads from the highly abundant louse endosymbiont *Candidatus* Riesia pediculicola, and taxa are shown to the lowest taxonomic level called. (**B**) Data excluding reads from *Candidatus* Riesia pediculicola. n = 9 for body lice, n = 16 for head lice.

was greater in body lice than in head lice (Mann–Whitney test, $P = 0.0053$), but its relative abundance within the non-endosymbiont communities was not significantly different.

A number of other bacteria were found at lower relative abundances than the aforementioned taxa in our samples (Fig. 1). Among these were genera that contain several known or possible pathogens, demonstrating that high-depth sequencing can be a useful tool for pathogen discovery in lice. For instance, *Bartonella* was detected in body lice (sample BL4). Additional genera that contain pathogenic species that have not been previously described in lice included *Neisseria* (sample BL6; Liu et al., 2015), *Mycobacterium* (samples BL7, BL8, HL3; Cosma et al., 2003), *Anaerococcus* (samples BL5, BL6, BL7; Murphy and Frick, 2013), and *Peptoniphilus* (samples BL5, BL6, BL7; Murphy and Frick, 2013). We also detected *Burkholderia* (sample HL4; Eberl and Vandamme, 2016).

When we performed diversity analyses of the communities associated with lice, several intriguing patterns emerged. First, alpha diversity was significantly greater in body lice than in head lice (Fig. 2), as indicated by the Shannon diversity measure (Wilcoxon rank-sum, $P = 0.007$). This difference was largely driven by the relative abundance of endosymbionts in body lice, since analysis after filtering to remove these reads revealed no significant difference (Wilcoxon rank-sum, $P = 0.86$). The same trend was observed when analyzing the Simpson diversity measure. In addition, while hierarchical clustering showed many mixed clades, beta diversity was also greater in body lice (Fig. 3A, C). Principal coordinates analysis (Fig. 4A) to determine the similarity of the microbial communities further revealed that the microbiomes of lice group by ecotype when endosymbiont-derived reads are included in the analyses. Three principal coordinates accounted for 99.1% of the variation among head and body lice samples. Meanwhile, abundance-driven interconnectivity analysis (Fig. 4B) qualitatively showed homogenous connectivity and stronger correlations within samples from the same ecotype. In other words, the relative abundances of microbial taxa were generally more strongly correlated between samples of body lice than between samples of head lice and body

Table II. Statistical analyses (Mann–Whitney test) of the most abundant bacterial taxa at the genus (g) or family (f) level detected in sequenced samples. Bold values indicate statistically significant differences.

Taxon	Head abundance (of total reads)	Body abundance (of total reads)	*P* value (+Riesia)	*P* value (−Riesia)
g_Acinetobacter	0.0005	0.0206	**0.004**	**0.0123**
g_Corynebacterium	0.0026	0.0148	0.371	0.4820
g_Flavobacterium	0.0007	0.0067	0.598	0.6822
g_Propionibacterium	0.0091	0.0119	0.082	**0.0221**
g_Pseudomonas	0.0015	0.0048	0.139	0.7226
g_Staphylococcus	0.0048	0.0168	0.084	0.8837
f_Enterobacteriaceae	0.0022	0.0215	**0.0053**	0.2112
g_Riesia	0.9666	0.8434	**0.009**	not calculated

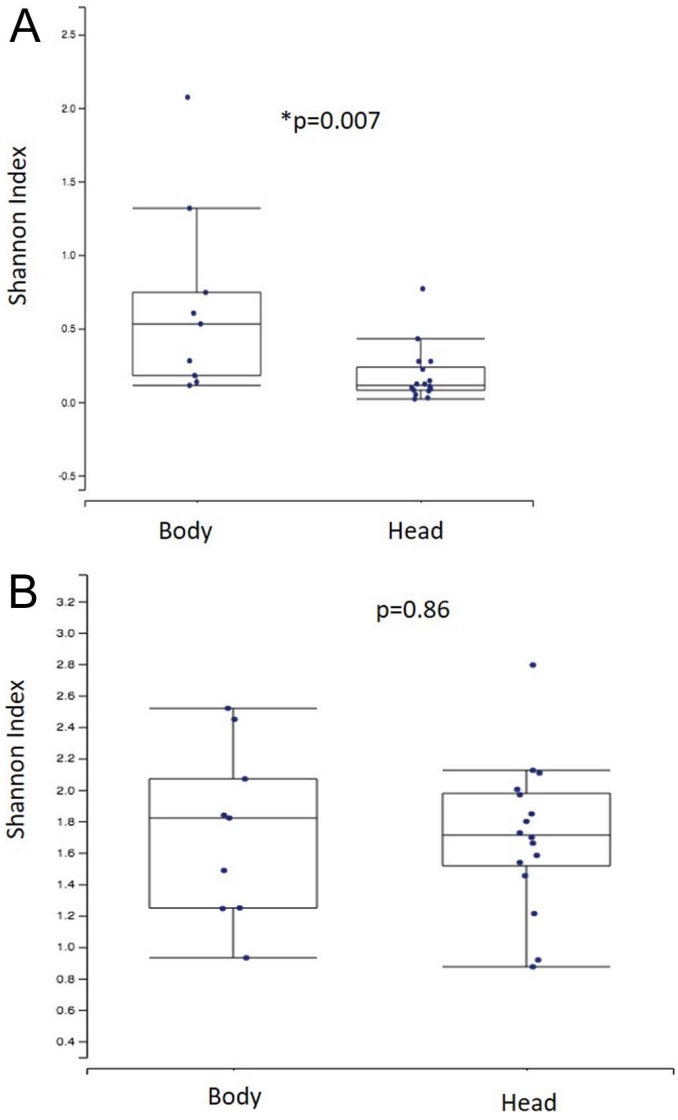

Figure 2. Alpha diversity of head and body lice microbiomes. (**A**) Including reads from *Candidatus* Riesia pediculicola. (**B**) Excluding reads from *Candidatus* Riesia pediculicola. Dots shown are individual Shannon alpha diversity measures. Box plots indicate median, Q1, and Q3 for head and body lice groups. n = 9 for body lice, n = 16 for head lice. Data were analyzed using the Wilcoxon rank-sum test.

lice. When endosymbiont-derived reads were removed, some discrimination between ecotypes was lost in principal coordinates analysis (Fig. 4C), but beta diversity remained higher in body lice (Fig. 3B, D). These results indicated that microbiome differences between body lice and head lice are driven not only by endosymbionts but also to a lesser extent by other taxa.

Lastly, both positive and negative correlations between the relative abundances of certain taxa were observed across all samples (Fig. 4D). In particular, the relative abundances of *Corynebacterium* and *Propionibacterium* were strongly correlated ($r = 0.959$), as were the relative abundances of *Corynebacterium* and *Flavobacterium* ($r = 0.6244$), *Acinetobacter* and *Staphylococcus* ($r = 0.7859$), *Staphyloccus* and *Flavobacterium* ($r = 0.598$), and *Staphylococcus* and *Pseudomonas* ($r = 0.568$). *Riesia* was negatively correlated with all other taxa. These relationships are depicted in (Fig. 5). In summation, body lice harbor a microbial community with higher alpha diversity and a lower relative abundance of endosymbionts relative to horizontally acquired bacteria than do head lice. In addition, several differentially abundant non-endosymbiont taxa and differences in beta diversity were observed between the 2 louse ecotypes.

DISCUSSION

Herein, we report the first broad characterization of the bacterial communities associated with head and body lice from different host individuals using high-depth sequencing. In addition to detecting endosymbionts and pathogens previously known to be associated with lice, we also detected a moderate relative abundance of bacterial taxa that are known to colonize human skin as well as several potential pathogens that have not been previously described in lice. While the communities of bacteria associated with lice appear depauperate, patterns that distinguish the microbiomes of head and body lice were apparent. Together, these results provide support for the hypothesis that the microbiome may contribute to the differences between head and body ecotypes.

A study published in 2007 showed that the dynamics of *Candidatus* Riesia pediculicola endosymbiosis in the bodies of head and body lice are distinct (Perotti et al., 2007), suggesting that these microbes are perhaps involved in regulating differential aspects of louse physiology. However, others found few transcriptomic differences between the endosymbionts of head and body lice, arguing against this hypothesis (Olds et al., 2012). While our work found that the relative abundance of endosymbionts is significantly higher in head lice than in body lice, this could be due to dilution from a greater amount of environmentally acquired bacteria in body lice samples and may not necessarily reflect a biologically relevant difference in endosymbiont titer between the 2 ecotypes. Environmental acquisition of microbiome components from the environment is known to occur in blood-feeding insects such as mosquitoes (Zouache et al., 2011; Muturi et al., 2018). Further, a recent analysis of *Acinetobacter baumanii*, a well-documented associate of lice, from the skin of homeless individuals supports this route of acquisition for the bacterium (LaScola and Raoult, 2004; Ly et al., 2019). Our work suggests this finding may extend to several other important bacteria present in lice, but additional field studies are needed to determine whether there is a significant correlation between the microbiomes of individual lice and those of their hosts.

Although the detection of *Bartonella* in a body lice sample was not an unexpected finding, since *B. quintana* is known to be transmitted by body lice (Fournier et al., 2002), previous high-depth sequencing studies to detect pathogens had not been carried out in human lice, and it was unknown whether low-abundance pathogens could be detected by this method in the context of highly abundant endosymbiont infection. Our work shows that metabarcoding is a promising avenue for the detection of low-abundance pathogens in lice, but future work is needed to test the sensitivity of these methods against targeted amplification. Since our methods did not discriminate species, it could not be confirmed if the *Neisseria, Mycobacterium, Anaerococcus, Peptinophilus*, and *Burkholderia* we detected are pathogenic or non-pathogenic members of their respective genera. Nonetheless, the

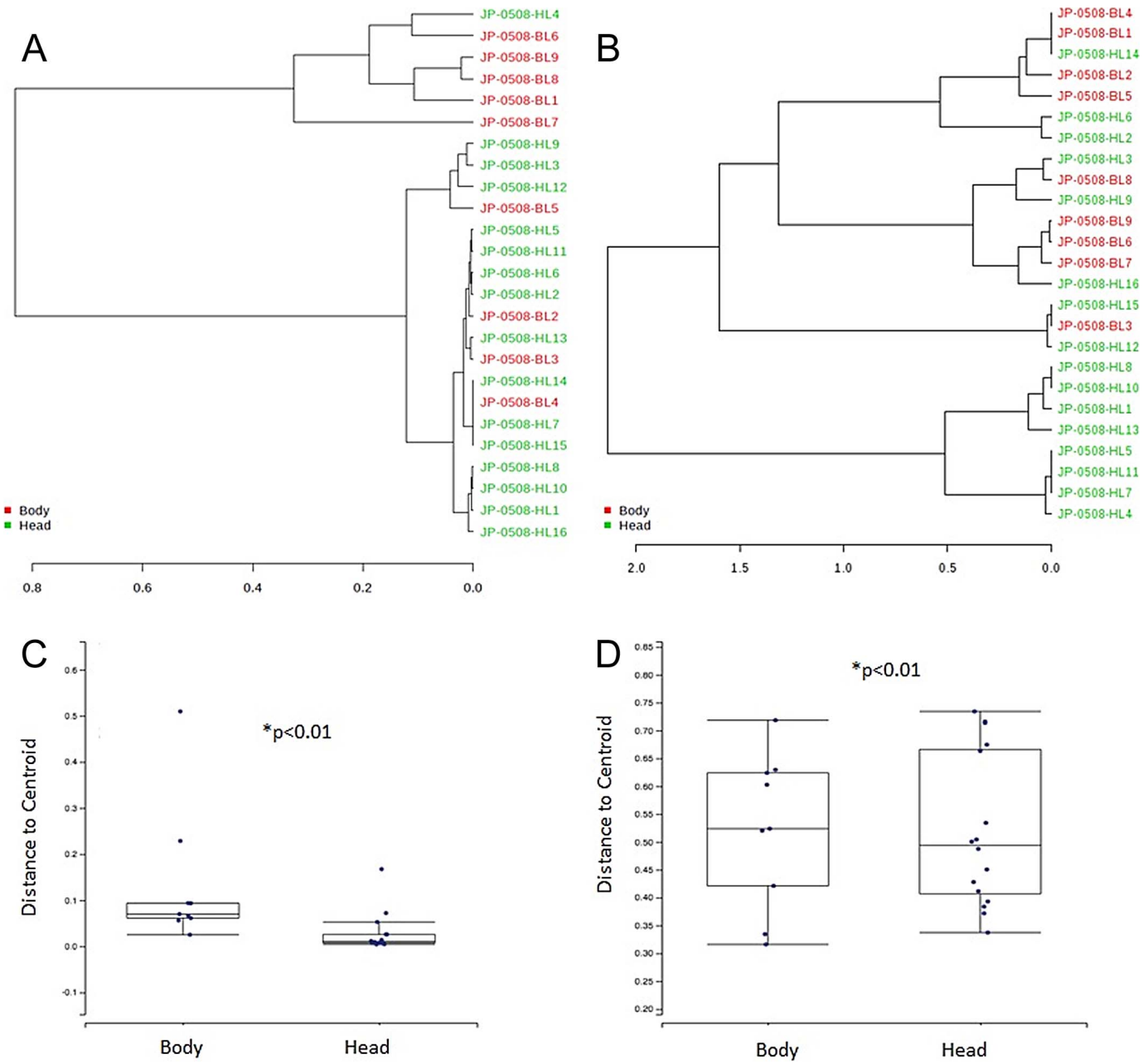

Figure 3. Hierarchical clustering of head and body lice microbiomes. Head lice (HL) samples are shown in green, and body lice samples (BL) are shown in red. Bray–Curtis dissimilarity is used as the distance metric for the dendrograms. (**A**) Including reads from *Candidatus* Riesia pediculicola. (**B**) Excluding reads from *Candidatus* Riesia pediculicola. (**C**) Distance to centroid with reads from *Candidatus* Riesia pediculicola analyzed by PERMANOVA. (**D**) Distance to centroid without reads from *Candidatus* Riesia pediculicola analyzed by PERMANOVA.

detection of *Burkholderia* was particularly intriguing because a recent survey of bed bugs in the United States detected the human pathogen *Burkholderia multivorans*, indicating it could possibly be vector-borne (Saenz et al., 2013). It is also not known at this time whether lice are capable of biologically or mechanically vectoring any of the above microbes between human hosts, but species identification and closer examination of their interactions with lice are merited given the importance of some pathogens in these genera. Our data further suggest that the extent to which lice contribute to skin or opportunistic infections via transmission of pathogenic *Streptococcus* or *Staphylococcus* strains should be examined more deeply. The ability to transmit these agents has been speculated but not proven for lice as well as bed bugs, which often overlap with lice infestations under deteriorated living conditions (Lowe and Romney, 2011; Barbarin et al., 2014).

Considering the epidemiology of head and body lice infestations may explain some of the microbiome patterns we discerned. Note that the confinement of sampling to the United States is 1 limitation of our study. Nonetheless, within our sampling locations, head lice primarily affect school-age children, while

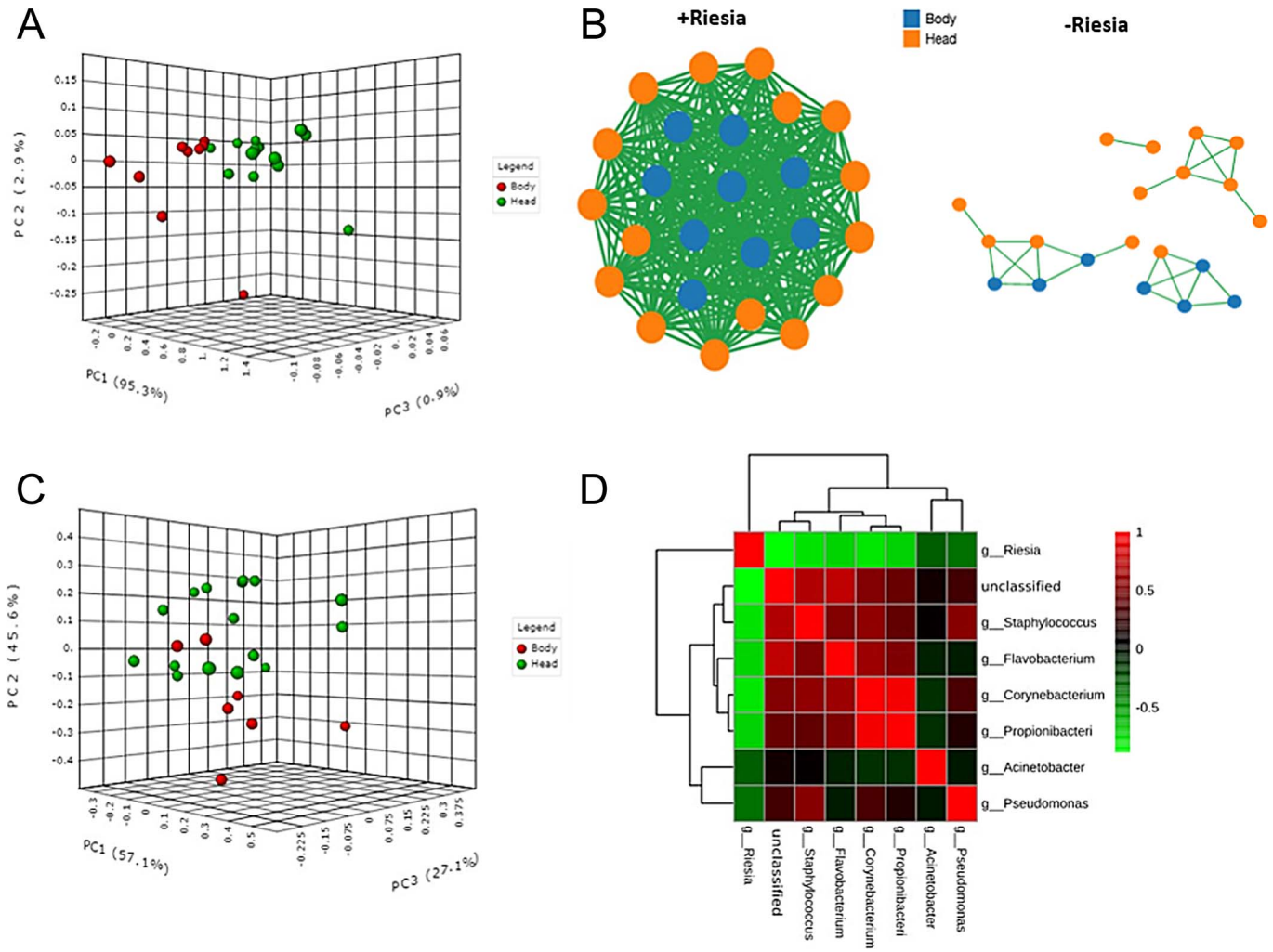

Figure 4. Pattern identification in head and body lice microbiomes. (**A**) 3D principal coordinates analysis based on Bray–Curtis distances and focusing on the 8 most abundant taxa shows strong discrimination between head and body lice. (**B**) Abundance-driven sample interconnectivity analysis including all taxa (±Riesia) shows homogenous connectivity and stronger correlation between samples from the same ecotype. Lines emanating from each node denote significant correlations to other nodes (0.75 Pearson product-moment correlation coefficient cutoff). (**C**) Excluding reads from *Candidatus* Riesia pediculicola eliminates discrimination between head and body lice in principal coordinates analysis. (**D**) Heat map of correlation between the abundances of the top 8 taxa across all samples. n = 9 for body lice, n = 16 for head lice.

body lice are seen largely in marginalized populations without adequate access to hygiene or healthcare. It is possible that the relative balance of the scalp microbiome of children in contrast to potential dysbiosis that may be present in individuals that are most susceptible to body lice is reflected in the microbiomes of the specimens we examined (Dreno et al., 2016). Whether similar patterns are conserved in additional lice samples from other geographical regions is of interest, and these data would further understanding of whether microbiomes are truly consistent distinguishers of the ecotypes, but again requires further study.

From our experiments, it also remains uncertain whether the particular bacteria we detected in our samples can transiently or stably colonize the gut of lice through ingestion during piercing of the skin, or whether they can survive on the external surface of lice for extended time periods. These questions are difficult to address using *16S rRNA* gene amplicon sequencing of field-collected lice samples because protocols such as surface sterilization may not completely eliminate contaminating reads from dead bacteria on the cuticle and may even negatively affect the quantitation of internal bacteria (Hammer et al., 2015). We posit that in nature 4 possible scenarios exist when lice are exposed to bacteria: (1) the bacteria remain on the surface of the lice without entering the gut, (2) the bacteria enter the gut during blood feeding but are rapidly killed resulting only in transient exposure, (3) the bacteria enter the gut and transiently colonize but are eventually shed, and (4) the bacteria enter and stably associate with the gut. Other studies have isolated viable bacteria, including *Serratia* spp. and *Acinetobacter* spp., from the gut of field-collected body lice, indicating that colonization by at least some species does occur (LaScola et al., 2001). The optimization of culture conditions and tightly controlled experiments using laboratory-reared lice will likely be required to investigate colonization by taxa of interest in more detail, making these experiments beyond the scope of the present work.

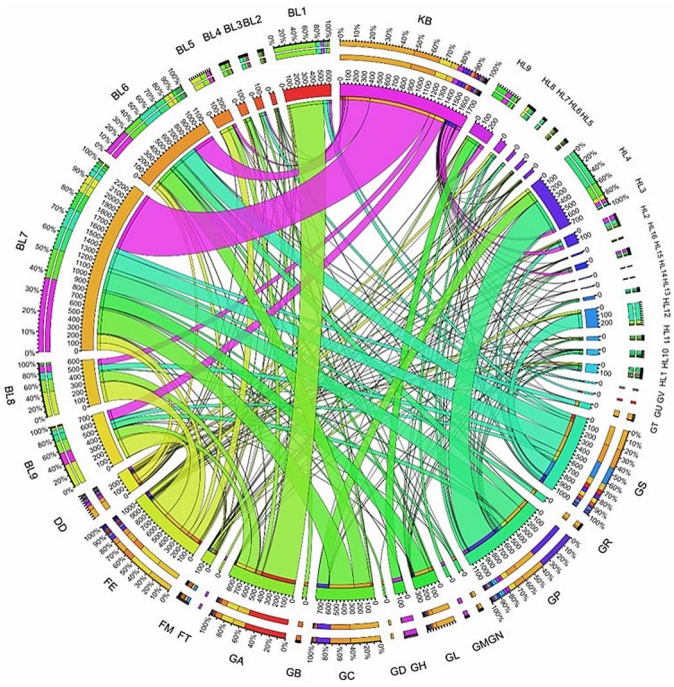

Figure 5. Summary of lice–microbiome relationships. On the circle border are individual samples of head lice (BL) or body lice (BL) with bar size indicating library size. Bacterial taxa are also shown on the border with bar size indicating the total number of reads across all libraries. Colored bands across the middle of the circle represent connections between taxa and samples, and the size of bands corresponds to the number of reads in the connection. The top 20 taxa are shown, excluding *Riesia pediculicola*. Genus *Acinetobacter* (GA), Genus *Propionibacterium* (GP), Family *Enterobacteriaceae* (FE), Genus *Staphylococcus* (GS), Kingdom bacteria (KB), Genus *Corynebacterium* (GC), Genus *Pseudomonas* (DD), mitochondria (FM), Genus *Flavobacterium* (GL), Genus *Sphingomonas* (GH), Genus *Kushneria* (GU), Genus *Bartonella* (GB), Genus *Methylobacterium* (GM), Genus *Peptinophilus* (GT), Genus *Finegoldia* (GN), Family *Metholobacteriaceas* (FT), Genus "*Candidatus* Rhodoluna" (GD), Genus *Beillonella* (GV), Genus *Anaerococcus* (GR).

Ultimately, if environmentally acquired bacteria can survive in lice even for a short period of time, then we speculate that differences in the communities they are exposed to could contribute to the physiological differences between lice species. In other arthropods, the microbiome can regulate life history (e.g., development) and behavior as well as the immune response to pathogens and vector competence (Coon et al., 2014; Dennison et al., 2014; Wada-Katsumata et al., 2015, de la Fuente et al., 2017). These parameters are among the key differentiators of head and body lice. We propose a model for future investigation in which shifts in the human microbiome could be passed on to lice and could impact their physiology, thereby facilitating ecotype shift and explaining the emergence of body lice epidemics from head lice under certain conditions. For instance, one hypothesis is that in body lice, increased exposure to environmentally acquired bacteria could result in negative feedback on the immune system in a way that delays or inhibits responses upon infection with human pathogens such as *B. quintana*, increasing vector competence.

On the clinical front, prior studies have identified the louse endosymbiont *Candidatus* Riesia pediculicola as a possible target for treating lice (Kirkness et al., 2010, Sangare et al., 2015). Because these bacteria provide critical functions such as vitamin synthesis and are essential for embryonic development, killing the bacteria using clinically obsolete antibiotics could be an efficient method to eliminate even insecticide-resistant lice infestations. Although this approach may be largely impractical for head lice, which are not considered a significant public health threat, and even for most body lice infestations, it may have some clinical applications against especially problematic (i.e., insecticide-resistant) body lice infestations or co-morbid body lice infestation and louse-borne bacterial infection. Our work similarly suggests that additional bacteria could be future therapeutic targets. That is, if any of the newly detected taxa are shown to impact relevant traits, then manipulating these may be a useful, novel strategy to mitigate lice infestation and/or louse-borne pathogen transmission.

Although our understanding of the roles that the microbiome plays in the divergence between head and body lice undoubtedly remains incomplete, the work presented here provides evidence in support of the first piece of a complex hypothesis. That is, our results demonstrate that the microbiomes of the 2 ecotypes are indeed distinct. We conclude that additional epidemiological field studies, as well as controlled laboratory experiments, are needed to fully establish the dynamics of microbiome acquisition in lice and to determine how specific bacteria may affect important aspects of louse physiology.

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