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ARTHROPODS AND MEDICAL ENTOMOLOGY - ORIGINAL PAPER

Molecular analysis of the blood meals and bacterial communities of bed bugs (*Cimex lectularius* L.) to assess interactions with alternative hosts

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Abstract

Common bed bugs (*Cimex lectularius* L.) are hematophagous pests present in urban environments across the globe. It is widely established that they have a strong host preference for humans. However, there are records of *C. lectularius* feeding upon a range of mammalian and avian hosts, including rodents, in the field. There is little information available about how frequently common bed bugs feed on alternative hosts in residential settings, but understanding this phenomenon has implications for both management of infestations and public health. Here, we examined cohorts of *C. lectularius* collected from 13 different dwellings in the state of New Jersey, USA, that were known to be simultaneously infested with house mice (*Mus musculus domesticus*). Host-specific quantitative polymerase chain reaction (qPCR) was used to determine if blood meals were taken from mice, while 16S rRNA gene amplicon sequencing was used to screen the bed bugs for the presence of zoonotic bacterial pathogens. We found no evidence that any of the bed bugs we collected fed on mice. Furthermore, the insects harbored depauperate bacterial communities that did not include known human pathogens. However, host-specific qPCR detected feline DNA in a pool of bed bugs from one dwelling, suggesting that interaction with domestic pets should be further investigated. Although sampling in this study was limited, the approach described herein will be useful for additional studies of the interactions between bed bugs and alternative blood meal hosts.

Keywords *Cimex* · Bed bug · House mouse · Cat · Alternative · Blood meal · 16S rRNA · Bacteria · Pathogen

Introduction

Most members of the family Cimicidae primarily parasitize an array of bird and bat species. On the other hand, there are two lineages of the common bed bug, *Cimex lectularius* L. One lineage is primarily found in association with bats, while populations typically found in human residences are a distinct lineage that is strongly adapted to humans (Balvin et al. 2012, Roth et al. 2019). However, common bed bugs have also emerged as poultry pests that can subsist on chickens as hosts (Rosen et al. 1987, Steelman et al. 2008) and they have infrequently been found on household pets such as cats and dogs (Clark et al. 2002, Little & West 2008). Some investigators rear *C. lectularius* using live mice or rats in the laboratory, demonstrating that the insects are attracted to and can sustain on rodents (Aak & Rukke 2014, Cannet et al. 2015). Moreover, there are host records of *C. lectularius* found on pigeons, sparrows, guinea pigs, mice, rats, and rabbits, suggesting these animals may occasionally serve as alternative hosts for urban infestations (Rivnay, 1930, Robaud 1928, Haag-Wackernagel and Bircher, 2010, Balvin et al. 2012). In some instances, residential *C. lectularius* infestations have appeared to originate from populations associated with non-

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human animals (e.g., *Columba livia*) (Haag-Wackernagel and Bircher, 2010). Nonetheless, little is known about the propensity of common bed bugs to feed on non-human vertebrates in residential settings and no field studies have been carried out to explicitly examine interactions with alternative hosts.

The propensity of common bed bugs to potentially feed on alternative hosts is of interest for several reasons. This property has implications for understanding the dynamics of infestations and could potentially complicate control efforts. For example, if vertebrate pests such as rodents are present in a structure that is infested with bed bugs, the distribution of bed bugs could include not only human host sleeping and resting areas but also the location of rodent nests in structural voids, appliances, and other unpredictable locations. Additionally, the insects may be able to persist for longer than expected in the absence of human hosts, avoiding starvation by feeding on the rodents. Although bed bugs are not confirmed vectors of any known human disease agents, the ability of any hematophagous arthropod to feed on non-human hosts is also relevant to the transmission of zoonotic human pathogens. Many bacterial pathogens that are transmitted by mites, ticks, and fleas are initially acquired by the arthropods from feeding on peridomestic or sylvatic reservoirs, including rodents (Bordes et al. 2015, Morand et al. 2015, Meerburg et al. 2009). This includes multiple species of *Rickettsia*, *Borrelia*, and *Bartonella*, as well as *Yersinia pestis* and others. House mice (*Mus musculus domesticus*) in particular contribute to transmission of the mite-borne pathogen *Rickettsia akari* in the New York-New Jersey metropolitan area (Paddock et al. 2003). They are also suspected reservoirs of *Francisella tularensis* (Origgi et al. 2015, Dobay et al. 2015), *Bartonella grahamii* (Holmberg et al. 2003, Mardosaite-Busaitiene et al. 2019), and *Borrelia burgdorferi* (Gern et al. 1998). Lastly, house mice shed a plethora of bacteria in their feces that could potentially be mechanically transferred on the cuticle of bed bugs (Williams et al. 2018).

Like bed bugs, house mice are prevalent structural pests (Zha et al. 2018) and co-infestation with both organisms occurs in multi-family housing in urban areas. For example, in a recent unpublished pest survey of 1753 apartments in the state of New Jersey led by co-authors RC and CW, 877 were found to have pests (e.g., cockroaches, bed bugs, mice). Among those infested apartments, 2.4% had both bed bugs and house mice. In our case, the collection of bed bugs from dwellings infested with mice provided a unique opportunity to investigate the relationship between the two pests and the possible connection between bed bugs and rodent-associated pathogens in a natural setting. Specifically, as part of our ongoing efforts to comprehensively examine the post-resurgence potential for human pathogen transmission by bed bugs, insects were collected from resident-occupied apartments infested with mice in urban areas of the state of New Jersey, USA. We then used host-specific quantitative polymerase chain reaction (qPCR) and 16S rRNA gene amplicon sequencing to screen these samples for (1) the presence of mouse DNA as an indicator of a recent blood meal taken from mice, and (2) the presence of zoonotic, vector-borne bacterial pathogens that may have been acquired through stochastic contact with mice. Detection of DNA from the blood meals of arthropods to identify their vertebrate host(s) is often carried out by barcoding with vertebrate primers targeting a conserved region and sequencing the resulting amplicons to discern the species of origin (Lah et al. 2012, Kjos et al. 2013, Reeves et al. 2018). Our study differed from many blood meal identification studies in that the primary host of bed bugs (i.e., humans) is well established and we sought to determine if bed bugs fed on specific alternative hosts. Therefore, we used a simple host-specific qPCR approach to amplify and detect only DNA from hosts of interest in our samples. This study is to our knowledge the first to attempt host blood meal species discrimination for bed bugs. It is also the first to specifically investigate the interactions between bed bugs and rodents in human homes.

Materials and methods

Bed bug collections from field sites

The bed bugs in this study were collected between March and August of 2019 specifically from occupied apartment units that were infested with mice. Presence of mouse activity was confirmed by placing EVO mouse stations (Bell Laboratories, Madison, WI) that contained chocolate spread and a commercial rodent attractant within the units. Live bed bugs were collected using featherweight forceps (BioQuip Products, Rancho Dominguez, CA) from infested beds and sofas and immediately placed in vials with 95% ethanol. Bed bug samples from different apartment units were stored in separate vials. In total, 39 individual bed bugs (*Cimex lectularius* L.) from 13 apartment units in 4 different buildings located in urban areas of New Jersey were examined in the study (Table 1). The bed bug samples were stored at −20 °C prior to further processing. Only bed bugs that had visible traces of a blood meal were included in downstream analyses.

Bed bug controls

Bed bugs from a laboratory colony (Cincinnati strain, Sierra Research Laboratories Inc., established 2007) reared on commercially obtained rabbit blood (Hemostat Laboratories, Dixon, CA) using a membrane feeding system (Hemotek Ltd. Blackburn, UK) were examined as negative controls. Also included was a set of positive controls consisting of bed bugs fed blood from wild-type C57BL/6 laboratory mice. This blood was freshly extracted from necropsied mice and

Table 1 Bed bug samples included in the study. *N* nymph, *M* male, *F* female

Sample ID	City, state	Building	Unit	#insects and developmental stage
BB1	Trenton, NJ	A	1	2N
BB2	Trenton, NJ	A	2	1M,2F
BB3	Trenton, NJ	A	3	1N
BB4	Trenton, NJ	A	4	1F
BB5	Irvington, NJ	B	5	4N, 1M, 1F
BB6	Irvington, NJ	B	6	1N, 1M, 1F
BB7	Irvington, NJ	B	7	2N
BB8	Irvington, NJ	B	8	1N, 1F
BB9	Irvington, NJ	B	9	1N, 1M, 1F
BB10	Jersey City, NJ	C	10	2N, 2F
BB11	Linden, NJ	D	11	1M, 3F
BB12	Linden, NJ	D	12	1N, 1M, 1F
BB13	Linden, NJ	D	13	2N, 1M, 2F
BB14	CIN lab colony	N/A	N/A	2M, 2F

immediately administered using the membrane feeding system (Hemotek Ltd.). Both groups of control bed bugs were maintained in an incubator at 28 ± 1 °C and 60–70% relative humidity on a 12:12-h photoperiod.

DNA isolation

Bed bugs collected from the same apartment units were pooled together to increase yields for DNA extraction and molecular analyses. The insects were not subjected to additional washing once stored in ethanol in order to capture the presence of bacteria on the cuticle that could possibly be mechanically transmitted. Total DNA was isolated from pools of bed bugs using the Qiagen DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and the concentration of double stranded DNA was determined on a Qubit fluorometer (ThermoFisher, Waltham, MA).

Quantitative polymerase chain reaction

Quantitative polymerase chain reaction (qPCR) was carried out to detect the presence of mouse DNA in bed bug pools using rodent-specific primers (Order: Rodentia) designed to target a SINE (short-interspaced nuclear element) and produce an amplicon of 118 bp (Walker et al. 2004). Reactions were also carried out using primers targeting a feline-specific SINE and producing an amplicon of 98 bp (Walker et al. 2004) to examine possible interactions with a second alternative host that is commonly present in many homes. Both of these primer sets were previously validated for specificity and have been employed in blood meal host identification studies of *Triatoma infestans* (Pizarro & Stevens 2008). Ten nanograms of total DNA was used per reaction and two positive controls were run for rodent-specific primers. First, the primers were tested on genomic DNA extracted from wild-type C57BL/6 mouse cells. Second, to determine sensitivity, the primers were tested on DNA extracted from laboratory-reared bed bugs that were fed mouse blood 1, 4, and 7 days prior. In addition, to confirm that DNA extracted from field-collected bed bugs was of suitable quality, conventional PCR was run for each sample using primers that target the RPL18 gene of *C. lectularius* and produce an amplicon of 137 bp, as previously described (Fisher et al. 2018). Cycle conditions were as follows: for rodent and feline-specific primers: 1 min at 95 °C followed by 40 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s (Walker et al. 2004, Pizarro & Stevens 2008). For bed bug-specific primers, 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min were run (Fisher et al. 2018). All qPCR reactions were run in duplicate using PowerUp SYBR Green universal qPCR mastermix (ThermoFisher) on an Applied Biosystems QuantStudio Flex Real-Time PCR system (Applied Biosystems, Waltham, MA).

16S rRNA gene amplicon sequencing

Isolated DNA was subjected to 16S rRNA gene amplicon sequencing to identify the bacterial communities present in bed bug pools. A negative control sample consisting of a mock DNA extraction was also sequenced. In brief, primers for the V4 hypervariable region of the bacterial 16S rRNA gene (515F/806R) were used to conduct PCR using the HotStarTaq Plus Master Mix Kit (Qiagen). Cycle conditions were as follows: 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 53 °C for 40 s, and 72 °C for 1 min, after which

a final elongation step at 72 °C for 10 min was performed. Following PCR, products were run on agarose gels to verify successful amplification. The multiplexed amplicons were then pooled and purified with calibrated Ampure XP beads (Beckman Coulter, Brea, CA). Purified amplicons were subsequently used for DNA library preparation according to the Illumina Truseq protocol. Sequencing was carried out on an Illumina MiSeq system (Illumina, San Diego, CA) according to the manufacturer's guidelines. All raw sequences were deposited into the NCBI Sequence Read Archive and will be publicly available upon publication of this article (PRJNA641337).

Sequencing data processing and analysis

Raw reads were processed using FastQC and MultiQC quality assessment tools (Andrews 2010, Ewels et al. 2016). The mean quality value across each base position in the read was checked and the average quality score was registered (Phred Score of 36). Per base, per tile, overrepresentation, and per base sequence content qualities were also assessed. Furthermore, the sequence length, duplicate level, and GC and N nucleotide content qualities were checked. We also checked for successful removal of adapters in the raw reads by checking the adapters per sequence content among the reads. Out of the 14 samples sequenced, 13 passed QC assessment. One sample (BB2) failed slightly but was not removed from the analysis as it still met our quality threshold (Phred score of 25).

After quality checking with FastQC, sequence data were first processed using the R-Studio 1.3 (Ihaka and Gentleman, 1996) package DADA2 1.16.0 (Callahan et al. 2016a) built upon the Bioconductor 3.11 (Huber et al. 2015) framework on a personal computer with 16 GB of RAM and a hexa-core processor. A guide pipeline from (Callahan et al. 2016b) was followed to aid in the initial processing of the raw reads. Reads were filtered at a maxN (ambiguous nucleotides) = 0, maxEE (expected error) = 2, and truncated at 240 nt for forward and 150 nt for reverse reads, after which reads were dereplicated to reduce redundant comparisons. Sequencing error rates were also estimated using unsupervised learning by observing sample inference and parameter estimation of the model of substitution errors until both values were consistent. Sample composition and amplicon sequence variants (ASVs) were processed and reads were then concatenated to form contiguous sequences which were assigned taxonomic rankings based on the Silva Reference Database 138 SSU Ref NR 99 (Quast et al. 2012). Chimeric sequences were removed and phylogenetic trees were created to be used for downstream data processing and visualization in the R package phyloseq 3.11 (McMurdie & Holmes 2013). Uncharacterized and other ambiguous sequences were filtered out and a sample count table was produced. Abundance counts and compositional amounts were recorded and transformed with phyloseq and visualized with ggplot2 (Wickham 2009). Beta-diversity was calculated using the Bray-Curtis dissimilarity index and MDS/PCoA. Figures were all generated with a combination of phyloseq extended and core functions and visualized with ggplot2.

Results

We used a simple host-specific qPCR approach to amplify and detect DNA from hosts of interest in our samples. In these experiments, no rodent DNA was detected in any of the field-collected bed bug pools (BB1-BB13) nor in laboratory-reared bed bugs that fed on rabbit blood (BB14). Meanwhile, positive controls consisting of bed bugs that fed on mouse blood in the laboratory 1, 4, and 7 days prior to testing showed amplification with rodent-specific primers. The average cycle threshold (CT) values for these controls were 25.5, 33.2, and 37.4, respectively. Curiously, one sample of field-collected bed bugs (BB1) showed amplification with feline-specific primers while all others were negative. The average CT value for this positive sample was 32.7 and amplification of the expected product was further confirmed by gel electrophoresis (Fig. 1). Reactions on each field-collected sample using bed

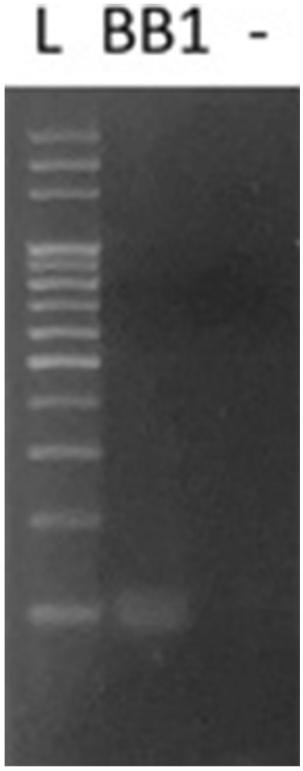

Fig. 1 Detection of feline DNA in a bed bug sample (BB1). Primers specific for a feline SINE were used in qPCR (Walker et al. 2004). To confirm amplification detected by qPCR, the resulting amplicons were visualized by ultraviolet illumination after electrophoresis on agarose containing SYBR safe dye (ThermoFisher). "L" indicates 100-bp DNA ladder. "-"indicates no template control

bug-specific primers were all positive in conventional assays, indicating that DNA extracted from these was of suitable quality for PCR (Fig. 2).

We next analyzed the bacterial communities present in the bed bug samples. Consistent with the lack of detection of mouse DNA, these bacterial communities did not include common zoonotic pathogens such as those in the following genera: *Bartonella*, *Borrelia*, *Francisella*, *Leptospira*, *Salmonella*, *Streptobacillus*, *Rickettsia*, or *Yersinia* (Bordes et al. 2015, Morand et al. 2015, Meerburg et al. 2009). Instead, the communities of field-collected samples were mostly dominated by two symbionts, *Wolbachia* and a member of the Pectobacteriacea family (genus *Brenneria*) (Table 2). These two bacteria typically comprised >98% of all classified reads. In the field-collected samples, several additional genera were occasionally present, but at very low abundances. These included *Acinetobacter*, *Corynebacterium*, *Lawsonella*, *Ornithonicoccus*, and *Pseudomonas* (Table 2). The composition of the bacterial communities was surprisingly similar across all infestations and did not cluster by location of collection (Fig. 3). However, one sample (BB7) did harbor a notably higher diversity of bacteria than the rest (Table 2). Some differences relative to individuals from the laboratory colony were observed, but even these were minimal. The most notable variation between field and lab samples was the lack of *Pectobacteriacea* and abundance of another member of the Enterobacteriaceae family (genus *Lelliottia*) in the latter.

Discussion

Prior studies have shown that PCR-suitable DNA from a human blood meal is detectable in bed bugs for 4.5–60 days post-feeding, depending on the methods and primers used (Szalanski et al. 2006, Schal et al. 2018). We confirmed that our methods could detect mouse DNA in bed bugs for up to 7 days after a meal of mouse blood. Therefore, our inability to detect rodent DNA in a representative sample of field-collected bed bugs with visible traces of a blood meal is strong evidence against recent feeding on mice. Although previous host records indicate that bed bugs may feed on both rats and mice in the field (Rivnay 1930, Robaud 1928), our data support anecdotal accounts that this probably occurs only infrequently. Perhaps, spatial separation between typical bed bug harborage sites (e.g., the bed and furniture) and the nests of rodent pests (e.g., structural and non-structural voids) is a key ecological factor that may prevents frequent interaction between the two. Additional possibilities are that the phenomenon only occurs in vacant units, or that bed bugs that feed on mice establish separate harborage sites closer to these alternative hosts, as indicated by a previous report of bed bugs specifically infesting a mouse colony located in the attic of a home (Robaud 1928). Such populations would not have been captured by our sampling, which was a limitation of our work. It is also possible that interactions between bed bugs and mice may be affected by the severity of infestation of either or both which could bring the two pests into closer proximity to one another and increase the possibility of opportunistic feeding. Future studies should include apartments varying in degree of bed bug and rodent infestations as well as rodent-infested apartments that have been vacated.

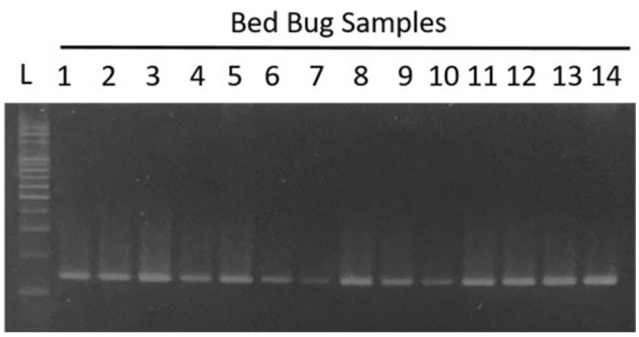

Fig. 2 Detection of bed bug DNA in study samples by conventional PCR. Primers specific for the RPL18 gene of *C. lectularius* were used in conventional PCR (Fisher et al. 2018). The resulting amplicons were visualized by ultraviolet illumination after electrophoresis on agarose containing SYBR safe dye (ThermoFisher). "L" indicates 100-bp DNA ladder

On the other hand, the detection of feline DNA in bed bugs from one dwelling suggests that opportunistic feeding on domestic cats may be more common than is currently appreciated based on case reports alone. To date, evidence for bed bugs feeding on pets has been limited to isolated instances in which the insects were physically found on these alternative hosts (Clark et al. 2002, Pinto et al. 2007, Little & West 2008). Although cat activity was not specifically recorded during our survey and we could not confirm how many of the infested dwellings housed cats, our result supports the idea that bed bugs occasionally parasitize domestic pets. This phenomenon could facilitate the transmission of some zoonotic pathogens such as *Bartonella henselae* or *Rickettsia felis*. In the future, it would be relevant to conduct additional sampling in homes specifically known to have pets to more accurately determine the frequency with which bed bugs may feed upon non-human animals. The success of positive controls in the present study signals that our approach will be useful for additional molecular studies to better understand bed bug interactions with alternative hosts. Importantly, taxon-specific qPCR primers have been designed for an array of vertebrates (e.g., dogs, birds) (Walker et al. 2004). Many of these have been validated in blood meal identification studies of other insects (Pizarro & Stevens 2008) but were not pursued in our work as limited quantities of DNA were available.

The results of our bacterial community analyses were consistent with the few existing studies of the microbiota of laboratory strains and field-collected bed bugs from locations not known to be infested with mice (Meriweather et al. 2013,

Table 2 Read counts of bacterial genera detected in bed bugs. Only genera for which more than 10 reads were present in at least one bed bug sample and no reads were present in the negative control are shown

Genus	BB1	BB2	BB3	BB4	BB5	BB6	BB7	BB8	BB9	BB10	BB11	BB12	BB13	BB14
Acinetobacter	4	0	0	0	0	0	41	0	8	0	0	0	31	0
Aerococcus	0	0	0	0	0	0	16	0	0	0	0	0	0	0
Allorhizobium	0	0	0	0	0	0	32	0	0	0	0	0	0	0
Altererythrobacter	0	0	0	0	0	0	18	0	0	0	0	0	0	0
BBMC-4	0	0	0	0	0	0	38	0	0	0	0	0	0	0
Brenneria	11762	679	17819	10524	95	24952	15808	12269	3246	21003	11350	3680	8008	0
Brevibacterium	0	0	0	0	0	0	15	0	0	0	0	0	0	0
Cellulosilyticum	0	0	0	0	0	0	24	0	0	0	0	0	0	0
Clostridium_sensu_stricto_1	0	0	0	0	0	0	74	0	0	0	0	0	0	0
Corynebacterium	0	0	0	0	0	110	19	0	0	0	0	0	0	0
Dietzia	0	0	0	0	0	0	74	0	0	0	0	0	0	0
Dyadobacter	0	0	0	0	0	5	0	0	0	0	0	0	22	0
Flavobacterium	0	0	0	0	0	0	19	0	0	0	0	0	0	0
Humibacillus	0	0	0	0	0	0	39	0	0	0	0	0	0	0
Janibacter	0	0	0	0	0	0	42	0	0	0	0	0	0	0
Lautropia	0	0	0	0	0	12	0	0	0	0	0	0	0	0
Lawsonella	0	0	57	0	0	54	0	0	0	0	0	0	14	0
Lelliottia	0	0	0	0	0	0	0	0	0	0	0	0	0	6297
Leptotrichia	0	0	0	0	0	15	0	0	0	0	0	0	0	0
Marinobacterium	0	0	0	0	0	0	10	0	0	0	0	0	0	0
Membranicola	0	0	0	0	0	0	37	0	0	0	0	0	0	0
Nocardioides	0	0	0	0	0	0	126	0	0	0	0	0	0	0
Ornithinicoccus	0	0	0	0	0	0	232	0	0	0	0	0	0	0
Ornithinimicrobium	0	0	0	0	0	0	76	0	0	0	0	0	0	0
Ornithobacterium	0	0	0	0	0	0	19	0	0	0	0	0	0	0
Pelagibacterium	0	0	0	0	0	0	21	0	0	0	0	0	0	0
Prevotellaceae_NK3B31_group	0	0	0	0	0	11	0	0	0	0	0	0	0	0
Proteiniclasticum	0	0	0	0	0	0	15	0	0	0	0	0	0	0
Proteiniphilum	0	0	0	0	0	0	45	0	0	0	0	0	0	0
Pseudomonas	0	0	24	0	0	0	0	0	46	0	0	0	0	52
Rubrivirga	0	0	0	0	0	0	49	0	0	0	0	0	0	0
Salinimicrobium	0	0	0	0	0	0	33	0	0	0	0	0	0	0
Sanguibacter	0	0	0	0	0	0	27	0	0	0	0	0	0	0
Staphylococcus	0	0	0	0	0	0	0	0	0	0	0	0	17	0
Sumerlaea	0	0	0	0	0	0	19	0	0	0	0	0	0	0
Tunicatimonas	0	0	0	0	0	0	12	0	0	0	0	0	0	0
Vibrionimonas	0	0	11	0	0	0	0	0	0	0	0	0	0	0
Wenxinia	0	0	0	0	0	0	11	0	0	0	0	0	0	0
Wolbachia	112427	13159	93170	123524	153099	147356	88166	47431	176193	69741	125264	94758	162425	68920

Potts et al. 2020, Pietri, 2020, Lim & Majid 2020, Kakumanu et al. 2020). In particular, our data provide further evidence that the core microbiota of bed bugs is lacking in diversity and minimally influenced by the environment, in contrast to another structural pest, the German cockroach (Kakumanu et al. 2018). Nonetheless, one sample in our study harbored several unique genera not present in others, suggesting that at least in some cases bed bugs may acquire environmental bacteria, though it could not be determined if these were on the cuticle or hosted in the gut. Our results also support our previous

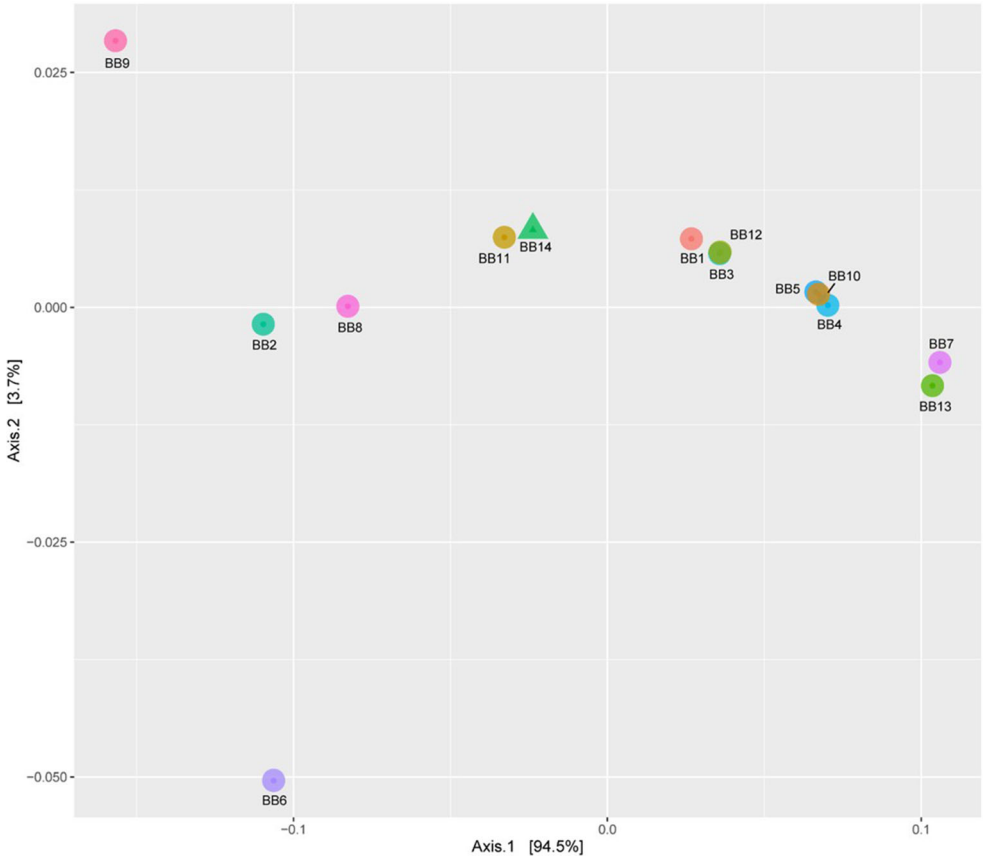

Fig. 3 Principal coordinates analysis (PCoA) of bacterial communities of bed bugs from mouse-infested dwellings. MDS/PCoA on weighted UniFrac distance

assertions that the prevalence of infection with *Rickettsia* is low in bed bugs and occurs in geographic clusters (Potts et al. 2020). Likely, the detection of *Brenneria* represents the common secondary endosymbiont of bed bugs, often referred to as BEV-like symbiont (Sakamoto & Rasgon 2006). This bacterium has been assigned to various related plant-pathogen taxa in 16S amplicon studies depending on the classifier used (Pietri, 2020, Potts et al. 2020). Conversely, *Lelliottia* may be a separate symbiont that became established in the laboratory population. This genus has been previously detected in and is a likely symbiont of mosquitoes and honeybees (Dada et al. 2018). While no human pathogens were detected here, monitoring for pathogens in bed bug populations remains inadequate (Pietri et al., 2020) and should be of continued interest.

Taken together, our findings indicate that mice are unlikely to significantly influence the dynamics of bed bug infestations and that bed bugs probably pose minimal risk as intermediate hosts or vectors of pathogens with rodent reservoirs in residential settings. That is, they do not appear to frequently feed on rodents in situations where human hosts are also readily available for feeding. This is in contrast to established vectors of zoonotic pathogens such as fleas and mites that primarily feed on non-human hosts and opportunistically feed on humans. Nonetheless, ours was a geographically restricted survey. It is possible that under different conditions or environments, such as severe infestations, temporary starvation, or in rural homes in developing countries where bed bugs experience greater exposure to sylvatic animals, acquisition of pathogens from rodents and incidental transmission to human hosts could occur sporadically. Experiments testing vector competence for any emerging zoonotic *Bartonella*, *Borrelia*, and *Rickettsia* should therefore still be encouraged, but vector-borne pathogens for which humans or domestic pets are the primary reservoir seem to be more plausible candidates for transmission by bed bugs based on previous studies (Angelakis et al. 2013, Leulmi et al. 2015, El Hamzaoui et al. 2019, Pietri, 2020, Pietri et al., 2020).

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Declarations

Conflict of interest The authors declare no competing interests.

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