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Cover Photograph: Female Western Black widow spider (*Latrodectus hesperus*). Photo by Marissa France.

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A Not-So-Deserted Microbial World: Microbiome Differences Between Urban, Desert, and Laboratory-reared Black Widow Spiders (*Latrodectus hesperus*)

Hasti Asrari[1], Todd R. Sandrin[1], and J. Chadwick Johnson[1,*]

Abstract - While the eco-evolutionary dynamics of urbanization are currently receiving a great deal of attention, the effect of urban disturbance on the microbiome of urban organisms deserves more study. Including the microbiome as a component of studying natural history may illuminate the mechanisms by which some species thrive after urbanization pest implications, while other species go locally extinct. (biodiversity implications). We investigated the gut microbiome of the Western Black Widow Spider (*Latrodectus hesperus*) from rural Sonoran Desert, urban Phoenix, AZ, and laboratory-reared populations, the latter originating from females sourced from urban habitats. Latrodectus hesperus is an ideal model system as they are a pest species of medical importance in urban ecosystems, often forming dense infestations relative to the sparse populations found in their native Sonoran Desert. High-throughput sequencing of the 16S rRNA V4 region was used to investigate the diversity of abdominal microbiota. The relative abundance of taxonomic orders Rhizobiales, Acidobacteriales, and RBC074 (class Blastocatellia) detected in desert spiders was significantly higher than those in urban and laboratory-reared spiders. However, urban spiders had a higher relative abundance of only the taxonomic order Bacillales. Intriguingly, each spider harbored a unique abdominal microbiome with considerable individual variation, irrespective of habitat. Documenting the microbiome of urban pest species and searching for individual and population differences in dominant bacteria may represent a novel approach to identify the mechanisms that underlie shifts in urban biodiversity.

Introduction

Historically, studies of urban ecology have emphasized abiotic effects such as habitat loss, and urban heat islands (Green et al. 2011, Lehner et al. 2005, Wuebbles and Hayhoe 2002). For example, impervious surfaces like buildings and roads are fragmenting natural habitats and presenting roadblocks for migratory pathways (Trombulak and Frissell 2000). However, more recently urban ecological research has begun to emphasize biotic effects (Brans et al. 2020, Cheptou and Lambrecht 2020, Diamond and Martin 2020, Ouyang et al. 2018, Pickett et al. 2016). Indeed, urbanization is an ecological disturbance that presents organisms with novel ecological and evolutionary challenges.

Studying the mechanisms by which organisms respond to urbanization has been described as a "grand challenge' for the Anthropocene, as it allows us to understand how urbanization may have a negative impact on biodiversity (Sih et al. 2010). Recent surveys of global terrestrial vertebrate diversity reveal a 52% loss in total species diversity by 2100 under modeled future urban expansion scenarios (Li et al. 2022). Despite these projected native biodiversity losses, urban environments can promote the population growth of a subset of taxa adapted to urban conditions (McKinney and Lockwood, 1999). These urban exploiters, native and sometimes non-native, are often a threat to native species diversity (Glon et al. 2018, Molnar et al. 2008, Pintor and Sih 2009). Therefore, not only is habitat

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alteration through urbanization a direct threat to native species, but also an indirect threat, as urban disturbance may favor a small set of species that can exclude other native species (McKinney and Lockwood 1999, Olden et al. 2004). For instance, in a study carried out in a residential development site, ant (Formicidae) abundance and diversity were examined during a period of construction (Buczkowski and Richmond 2012). This disturbance had a positive effect on the abundance of ant species that could tolerate the disturbance specialists), but a negative effect on the overall ant species diversity.

If urbanization affects community composition, what are the unique urban selection pressures these species are facing? For example, artificial light at night (ALAN) may impose unique urban selection pressures. ALAN disrupts the circadian rhythms of Australian Garden Orb-weaver Spiders (*Hortophora biapicata*), influencing their reproductive success and population viability (Willmott et al. 2018). ALAN affected this spider's juvenile development, reducing the number of juvenile instars, leading to earlier maturation and lower reproductive output. Similarly, work done on the Bridge Spider (*Larinioides sclopetarius*) showed spiders exposed to ALAN experienced an increase in mortality (Heiling and Herberstein 1999).

While anthropogenic activity may play a critical role in the decreased biodiversity, fitness, and altered phenotypes of organisms, its effects on the microbiome within urban organisms have only recently been addressed. (The microbiome is the collection of microorganisms living and interacting in a particular habitat host, and its composition and function could be an important mechanism influencing urban success.) Microorganisms play important roles in contributing to the basic and complex properties that build up life, with recent studies showing links between the microbiome and chemical communication (Theis et al. 2013), protection against parasites (Koch and Schmid-Hempel 2011), and predicting social networks (Tung et al. 2015). Microbes communicate with the central nervous system (specifically the brain gut-brain axis), influencing motor control and anxiety-like behavior in germ-free mice (Heijtz et al. 2011). Additionally, microbes play an essential role in internal metabolic pathways and in synthesizing vitamins essential for survival (Salem et al. 2014).

An organism's microbial community is obtained and shaped both through vertical transmission (mother-offspring) and horizontal transmission (environment-host). The association between the animal host and its microbiome is affected by various abiotic and biotic factors including host immune system (Enaud et al. 2020), nutrition (Merra et al. 2023), reproduction (Jašarević et al. 2015, Shao et al. 2019), communication (Archie and Tung 2015, Rose et al. 2023), diet (Jiang et al. 2020), life stage and geographic location (Hird et al. 2014, Gani et al. 2024), laboratory rearing (Kong et al. 2014), among others (Bahrndorff et al. 2016). As such, studying the microbiome is a relevant component of studying an organism's "natural history".

In the case of horizontal transmission, soils act as a natural supplier. Soil microorganisms contribute to microbial diversity in terrestrial organisms; however, anthropogenic activities may impact soil microbiomes due to land-use changes which, in turn, affects terrestrial life forms which would acquire such soil microbes (Barnes et al. 2021). Nevertheless, the interaction between organisms and their environment varies amongst species, differentially shaping their microbiomes. For instance, urban habitats affect the gut microbiome of White-crowned Sparrows (*Zonotrichia leucophrys*; Berlow et al. 2021). This study found that if sparrows had more tree cover, as was the case in urban sites, they had more diverse gut microbiomes due to horizontal transmission. Alternatively, endangered Udzungwa Red Colobus Monkeys (*Procolobus gordonorum*) had higher gut microbial

diversity in undisturbed forests relative to disturbed habitats (Barelli et al. 2015). Specifically, urban colobus monkeys were found to be missing a few microbial taxa essential for the degradation of complex plant material, directly impacting their health and conservation (Barelli et al. 2015). In contrast, Coyotes (*Canis latrans*) are successful in urban environments due to their generalist and flexible diets and generally high levels of behavioral plasticity (Sugden et al. 2020). While rural coyotes harbor microbiomes containing protein-rich diet bacteria and have better overall health and fitness, urban coyotes have higher microbial diversity from anthropogenic food consumption, promoting their dependence on urban resources for survival (Sugden et al. 2020). In sum, the extent to which the internal microbiome responds to urbanization is species-specific, depending on host-environment interaction. Furthermore, microbial networks are sensitive to both natural and anthropogenic disturbances, resulting in responses that depend on variables like community composition, species richness, and species evenness.

In spiders, bacterial symbionts play an important role by helping with digestion and providing nutrients that may be limited in the diet (Zhang et al. 2018). Furthermore, such symbionts can enhance pathogen resistance and mediate the thermal tolerance of their hosts, which can be vital to their survival in hotter habitats (Feldhaar 2011). As a result, microbes can be beneficial partners to their host in maintaining metabolic homeostasis outside of other cellular processes. It is vital to understand the way an organism's microbial community is obtained and shaped because the diversity of a species' internal microbiome in a given location is a potential indicator for the overall health of the larger community. Higher internal microbial diversity may be an indicator of good health for the host (Fijan 2014, Hills et al. 2019, Panthee et al. 2022). By having a diversity of internal microbes, the host would therefore have a healthy collection of internal helpers for proper digestion, processing nutrients, and protecting against disease (Huttenhower et al. 2012). However, it is important to note that microbial diversity can have positive or negative consequences for organism health depending on microbe function (Golombos et al. 2018, Shao et al. 2017).

In Phoenix, Arizona, USA, there are dramatic differences between the city and the surrounding Sonoran Desert. Compared to urban Phoenix, the desert is less affected by human activities and inhabited by a high diversity of native plants and animals (Hope et al. 2005, Hostetler and McIntyre 2001, Walker et al. 2009). Urbanization in Phoenix involves the use of supplemental water and heightened primary productivity, resulting in the decline of native biodiversity and a dramatic population growth for taxa well-adapted to anthropogenic disturbance (Shochat et al. 2006). For example, some urban spider taxa are more densely populated in the city compared to the undisturbed Sonoran desert e.g., Lycosidae (wolf spiders; Shochat et al. 2004) and Theridiidae (cobweb spiders; Johnson et al. 2012).

This is likely due to a multitude of factors e.g., urban water and prey abundance and a higher abundance of urban web-building substrates like concrete block walls (Johnson et al. 2012). Specifically, *Latrodectus hesperus* thrives in urban ecosystems, forming dense urban infestations in Phoenix, AZ compared to the surrounding Sonoran Desert (Johnson et al. 2012, Trubl et al. 2012). The black widow's role as a medically important species leads to an oversized fear of their increased urban prevalence. *Latrodectus* venom contains neurotoxic latrotoxin proteins, making black widow spider venom hazardous to humans. As a result, widespread pesticide applications are used to combat their infestations in urban Phoenix (Johnson, pers. obs.), further differentiating the urban ecosystem.

Investigations into the microbiome of spiders are relatively scarce. In *Latrodectus hesperus* and other *Latrodectus* species, *Gilliamella*, *Wolbachia*, and *Spiroplasma* are specific bacterial symbionts shown to dominate the widow microbiome (Dunaj et al. 2020). These

symbionts are known to have an impact on arthropod fitness and reproductive behavior. While many studies on *L. hesperus* focus on their venom for medicinal applications (Chen et al. 2021) and silk for industrial usage (Parent et al. 2018), Dunaj et al. (2020) expand on this arthropod genera, by examining the internal microbiome and the connection between the microbial communities and their host species' evolutionary relationships. In particular, they found that microbial communities in multiple *Latrodectus* species are shaped by ecological and evolutionary factors, with similar microbes found amongst common ancestors. This indicates the microbes found in widow spiders have changed over time due to both their surroundings and their genetic lineage, emphasizing the combined impact of black widow ecology and evolution on these microbial populations (Dunaj et al. 2020). Notably, their work utilized only laboratory-reared spiders housed in identical conditions and fed a single-species cricket diet.

In contrast, a recent study examining native and invasive populations of the Brown Widow Spider (*Latrodectus geometricus*) found no significant differences in microbiome composition across field sites, with communities largely dominated by *Rhabdochlamydia*, a putatively maternally inherited endosymbiont (Mowery et al. 2024). This pattern differs from the more diverse and variable microbial communities reported in black widows (Dunaj et al. 2020), suggesting that microbiome structure within the genus *Latrodectus* may vary substantially across species and ecological contexts. Here, we explore the microbiome of *L. hesperus* collected from Sonoran Desert and urban Phoenix habitats and compare this with the microbiome of urban spiders reared from egg to adulthood in the laboratory. Examining spiders collected from their natural habitats allows us to directly observe how urbanization and environmental factors shape internal microbial communities. It is crucial to understand this, as the diversity of their internal microbiome in different habitats can serve as a key indicator of the overall health and stability of populations and the surrounding ecological community.

In this study differences between the abdominal bacterial community of *L. hesperus* from urban Phoenix, rural Sonoran Desert populations in Arizona, and laboratory-reared populations were investigated. We asked the following questions: 1) Are there habitat differences in the diversity of the *L. hesperus* microbiome urban versus desert versus laboratory? 2) Are specific microbes more dominant than others in these different habitats? We predicted, compared to desert spiders, that urban (and laboratory-reared) widows would have a lower abdominal microbial diversity due to limited prey diversity in urban and controlled laboratory environments. Moreover, we expected desert spiders to host bacterial taxonomic groups primarily associated with local soils, because of the environmental characteristics specific to their web-building location.

Methods

Sample collection and preparation

Western Black Widow Spiders (*Latrodectus hesperus*) were collected across metropolitan Phoenix, AZ in early May 2021. The desert spiders utilized for this study were sourced from 4 populations in the Sonoran Desert, situated to the north of all urban infrastructure, and 4 urban populations within Glendale, a city in the Phoenix metropolitan area near Arizona State University, West Valley Campus. One individual spider was sampled from each site (n = 10 total; n = 4 urban, n = 4 desert, n = 2 laboratory). Figure 1 showcases a map of the collection sites. Notably, each population had to be a minimum of 3.5 km apart from one another. We additionally selected 2 second filial generation (F1)

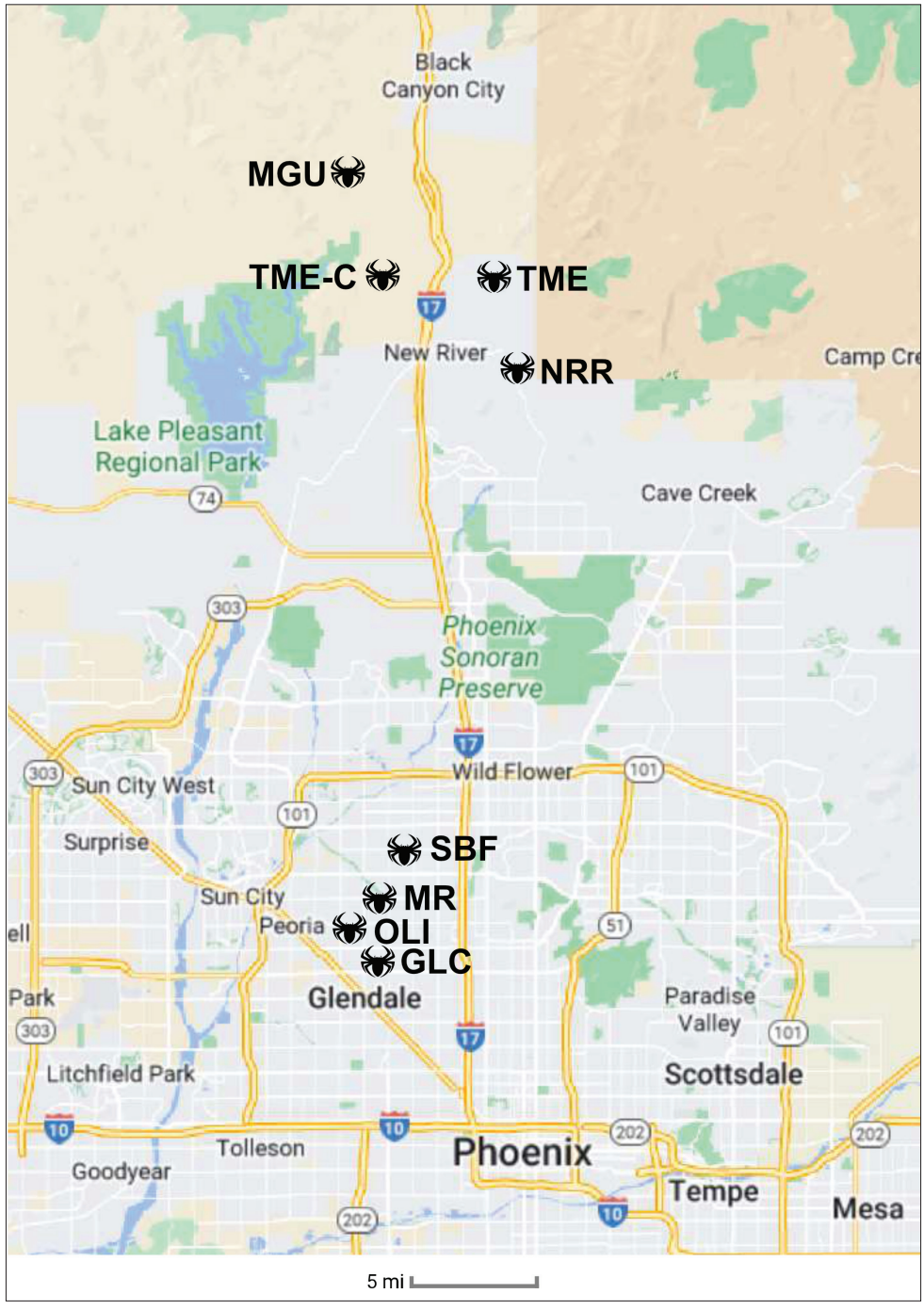

Figure 1. Four desert Table Mesa East (TME), Table Mesa East-Central (TME-C), New River Road (NRR), and Moores Gulch (MGU), and 4 urban Olive Street (OLI), Grace Lutheran Church (GLC), Marshall Ranch Elementary (MR), and Sunburst Farms (SBF) widow spider collection sites. One spider from each site is represented in the study, in addition to 2 urban-collected MR F1 sub-adult female spiders from the Johnson black widow laboratory at Arizona State University, West Valley Campus.

sub-adult female spiders born from different urban females, originally collected from the Marshall Ranch Elementary (MR) site, and laboratory reared to provide insight into how a controlled diet and habitat influence the abdominal microbial framework of those raised in the laboratory (Johnson et al. 2019).

Immediately after field collection spiders were individually placed in pre-weighed sterilized collection tubes and weighed on a portable microbalance. Whole spiders were stored at −80 °C within 2 hours after field collection. One to three days later, depending on the collection date, 1× saline-sodium citrate (SSC) dissection buffer was added to each vial for both spider preservation and their associated microbial communities. Samples were stored in −80 °C after the addition of the SSC dissection buffer for later preparation.

All samples went through an aseptic surface wash protocol to remove microbes from their body surface, which involved room temperature thawing, submersion in 1× SSC buffer, light and brief vortexing, and transfer into a new SSC buffer for repeated surface washing a total of three times. After three washes whole spiders were weighed on a microbalance to determine the final weight of the spider after −80 °C storage post-collection and surface washing. Urban and desert spiders did not differ significantly in this weight. All surface-washed samples were shipped overnight in dry ice to the University of Arizona's Microbiome Core in Tucson, AZ for downstream processing, DNA extraction, PCR, and 16S rRNA library construction.

DNA extraction preparation and processing

Spider abdomens were processed for DNA extraction using Qiagen's QIAamp PowerFecal Pro DNA Kit. To homogenize tissues, frozen spiders were briefly dipped in liquid nitrogen and mechanically disrupted on aluminum foil before transfer into bead tubes from the kit. For larger specimens, abdomens were divided into 2 parts and processed separately to avoid clogging the DNA columns. Throughout the procedure liquid nitrogen was used to keep samples frozen and minimize DNA degradation. Two extraction controls containing only lysis buffer were included. No individual internal structures (e.g., gut, silk glands) were dissected; instead, the entire abdominal contents were processed to capture the whole abdominal microbiome.

PCR and 16S rRNA library construction

Polymerase chain reaction (PCR) was performed to target the V4 bacterial 16S rRNA gene region with 515F/806R primers. PCR settings were based on MyFi recommendations with times increased to improve yield: pre-incubation (1 cycle) at 95 °C for 120 sec; three step incubation (35 cycles) at 95 °C for 30 sec, 50 °C for 30 sec, and 72 °C for 30 sec; pre-incubation (1 cycle) at 72 °C for 60 sec; and cooling (1 cycle) at 37 °C for 30 sec. Invitrogen's Quant-iT PicoGreen dsDNA Assay Kit was used to quantify DNA amplification following the manufacturer's protocol. Samples were normalized based on the calculations from PicoGreen. 16S rRNA gene sequencing on an Illumina MiSeq instrument was done alongside extraction and PCR negative controls to identify, classify, and quantify the microbes within the spider samples. Those spiders with split abdomens (left and right sides) were sequenced separately and combined at the individual level for downstream analyses. No samples from different individuals were pooled. For field-collected habitats, 1 spider was sequenced per site, resulting in 4 spiders sequenced per habitat (urban and desert), and 2 laboratory-reared spiders were sequenced, for a total of 10 spiders.

Sequence processing

Raw sequence data were processed in QIIME2 amplicon version 2023.9 pipeline for microbial community analyses (Bolyen et al. 2019). QIIME2 was used to add barcodes to the read files and demultiplex the joined paired-end reads. The Divisive Amplicon Denoising Algorithm (DADA2) package was used for quality control to remove sequence errors, trim primers, and assign the sequences to operational taxonomic units (OTU; Callahan et al. 2016). Spiders with left and right abdomens sequenced separately had their OTU data and sequences merged appropriately so they could be analyzed alongside the spiders whose abdomens were kept whole. These OTUs were aligned to the GTDB v207 database and assigned taxonomy (Parks et al. 2022). Taxa bar plots were generated and sequences that were classified as chloroplasts and mitochondria were filtered out.

Diversity and statistical analysis

The Shannon Diversity metric was used to analyze alpha diversity which regards OTU richness and evenness (Shannon 1948). The Kruskal-Wallis test was utilized for pairwise and group comparison between sourced habitats (Kruskal and Wallis 1952). The Bray-Curtis dissimilarity beta diversity metric was used to measure individual variation of microbial community structure between spider samples (Bray and Curtis 1957). A dissimilarity distance metric for Bray-Curtis was constructed in QIIME2 and visualized with a principal coordinate analysis (PCoA) plot. Furthermore, linear discriminant analysis effect size (LEfSe; Segata et al. 2011) was used to compare bacterial orders and family differentially abundant in urban and desert widow microbiomes. PCoA and LEfSe, along with other statistical analyses and figure generation, were performed in RStudio (Posit team 2023). Packages utilized in RStudio for analysis included qiime2R (Bisanz 2018), tidyverse (Wickham et al. 2019), gplots (Warnes et al. 2022), vegan (Oksanen et al. 2022), dplyr (Wickham et al. 2023), DESeq2 (Love et al. 2014), kableExtra (Zhu 2024), and ANCOMBC (Lin and Peddada 2020, Lin et al. 2022). Lastly, phyla abundance was calculated as percent relative abundance by normalizing each taxon's abundance to the total abdominal microbiota of each spider and averaging across individuals within each habitat.

Results

Sequencing depth

After DADA2 processing and the removal of chloroplast and mitochondrial sequences, there were 7,263,815 16S rRNA sequences retained (mean = 726,361.5, SD = 502,906.13; see Supplemental File 1, available online at https://eaglehill.us/urnaonline/suppl-files/urna-250-Johnson-s1.pdf for sequence and OTU counts per sample). There were also 4739 unique prokaryotic (archaeal and bacterial) OTUs obtained.

Composition of bacterial taxa

We found high variation in microbial relative abundance among 10 spiders across habitats, with some microorganisms being more abundant than others, but similarly present amongst all samples. The dominant bacterial phyla across all samples were Proteobacteria (23% desert, 18% urban, and 14% laboratory relative abundance) and Actinobacteria (11% desert, 20% urban, and 10% laboratory relative abundance; see Supplemental File 2, available online at https://eaglehill.us/urnaonline/suppl-files/urna-250-Johnson-s2.pdf). Bacteria identified as phylum Firmicutes, class Bacilli, with unknown lower levels of taxonomic classification, were especially dominant in 1 spider from each of the three habitat

sources (Fig. 2). Despite their high abundance in a few samples, Firmicutes, as well as Proteobacteria and Actinobacteria, were not present in all spiders, preventing them from being considered a uniformly dominant group.

These prominent bacterial groups are included with the 10 taxa with the highest relative abundance across all spider samples, and are represented by the colored bars in Fig. 2. Across all individuals, a total of 339 taxa were identified to at least the family level. All remaining taxa were consolidated into the "Remainder" category for visualization, representing a diverse assemblage of lower-abundance taxa. The relative size of this category varied among spiders, reflecting substantial inter-individual variation in low-abundance community members and underscoring the extensive microbial diversity harbored by each individual.

To identify bacterial taxa that differed significantly in relative abundance between source habitats, linear discriminant analysis (LDA) was utilized following Kruskal-Wallis tests with FDR correction. There were 9 bacterial orders that were differentially abundant when comparing desert versus urban spiders, each with an FDR-adjusted q-value of 0.047 (Kruskal-Wallis test). Desert spiders had significantly higher abundances of Rhizobiales, Acidobacteriales, and an unidentified order RBC074 (class Blastocatellia), whereas urban spiders showed significantly higher relative abundances of Bacillales (see Supplemental File 3, available online at https://eaglehill.us/urnaonline/suppl-files/urna-250-Johnson-s3.pdf). LDA scores were used to estimate the effect size of these differentially abundant taxa, indicating the strength of association between specific bacterial orders and source habitat. Several bacterial orders showed strong positive associations with desert spider abdominal microbiomes, suggesting habitat-specific enrichment patterns. This indicates that although overall community composition was variable among individuals, certain bacterial groups were consistently enriched in desert relative to urban spider populations, illuminating the unique microbial landscape characterizing the widow communities in arid environments.

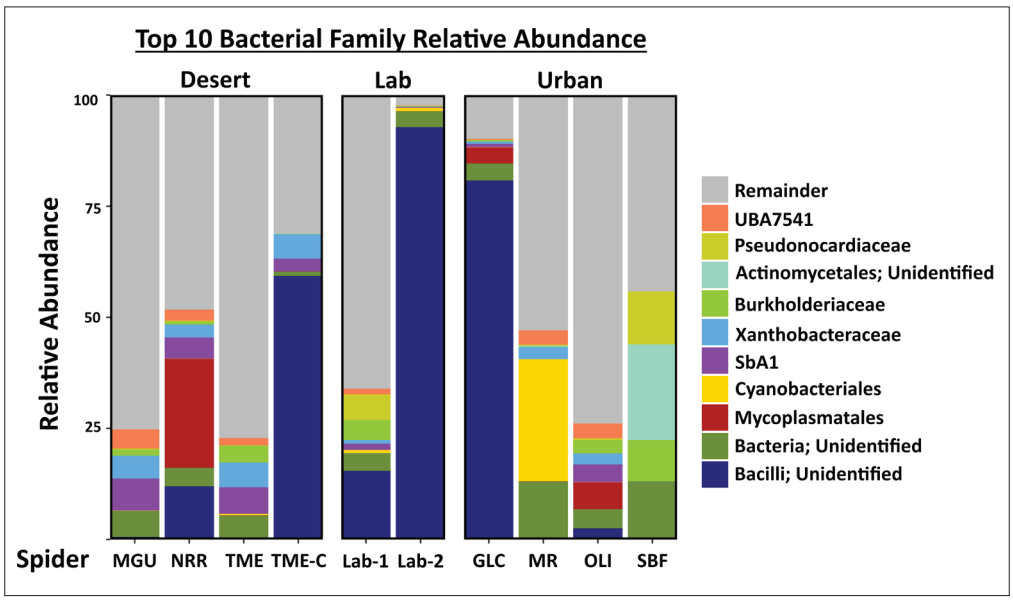

Figure 2. Bar plot of the relative abundance of specific bacterial taxa in 4 desert, 4 urban, and 2 laboratory-reared black widow spiders, filtered for those minimally identified at the family level. The bars are colored according to those who have the top 10 highest relative abundance across the samples and the remaining taxa are bundled together as a single gray color in the "Remainder" category.

Diversity metrics

Figure 3 and Supplementary File 4 (see Supplemental File 4, available online at https://eaglehill.us/urnaonline/suppl-files/urna-250-Johnson-s4.pdf) show the results of our analysis using QIIME2 to assess alpha microbiome diversity within black widow spiders residing in different habitats (desert, urban, and laboratory). The Shannon group significance boxplots show a non-significant trend for desert spiders to have a higher mean microbial diversity (Figure 3; Kruskal-Wallis $P_{desert\text{-}urban} = 0.386$, $P_{desert\text{-}laboratory} = 0.355$, $P_{urban\text{-}laboratory} = 0.643$, and $P_{overall} = 0.527$). While the comparison of Shannon alpha diversity across habitats does not yield statistical significance, notable individual variation is evident. Indeed, the Shannon diversity index rarefaction curve shows alpha diversity differences between all spider samples, but an absence of habitat differences (Supp. File 4). Furthermore, these patterns can be visualized in the Bray-Curtis dissimilarity principal coordinate analysis (PCoA; Fig. 4). The Bray-Curtis PCoA plot demonstrates such high levels of individual variation that spiders from different habitats have more similar microbiome diversity than those from the same habitat (Fig. 4). For instance, it is worth noting the three segregated points on the far left of the PCoA are from the same three black widow spider samples, from different source habitats, dominated by the bacterial phylum Firmicutes, class Bacilli (Fig. 4).

Discussion

Dominant and differentially abundant bacterial taxa

Dominant bacterial phyla across all samples were Proteobacteria and Actinobacteria; both known to play an important role in nutrient and energy metabolism in spiders (Hu et al. 2019). As these phyla were dominant across widow samples regardless of habitat, they may harbor essential microbial groups comprising the widow "core microbiome" (Risely 2020).

The phylum Firmicutes, specifically class Bacilli, showed individual variation in abun-

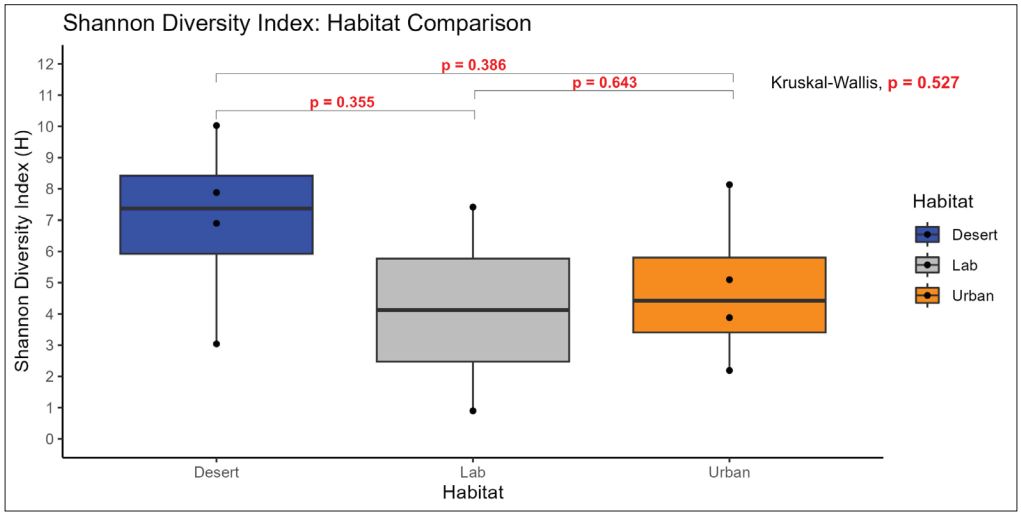

Figure 3. Alpha diversity analysis using the Shannon metric, comparing source habitats (desert n = 4, urban n = 4, and laboratory n = 2). Each depicted as a box and whisker plot colored according to habitat with individual spider samples plotted as solid black dots. P-values for Kruskal-Wallis pairwise comparisons between habitats are bolded in red.

dance among spiders even from the same habitat (Fig. 2). Firmicutes is known to play a similar metabolic role in spiders similar to roles Proteobacteria and Actinobacteria play (Hu et al. 2019). Prior work has further revealed different features about this taxonomic group, such as its presence being linked to recent high-fat intake (Hildebrandt et al. 2009) or increased energy demand, which Firmicutes might support (Gao et al. 2022). However, it is unclear why this group is dominant in only some spiders and nearly absent in others. The assumption of a uniform microbiome observed in laboratory-reared organisms (Tinker and Ottensen 2021, Cheng et al. 2015, Kong et al. 2014) is especially challenged by our dataset, given that 2 black widow spiders reared from egg to adulthood under identical laboratory conditions (e.g., diet, photoperiod, temperature) varied dramatically in their composition of Firmicutes.

Variation in time since last feeding may also contribute to the observed inter-individual differences in bacterial community composition, particularly for taxa such as Firmicutes that have been linked to dietary intake and metabolic state. For laboratory-reared spiders, feeding schedules were standardized, and individuals were sampled at comparable times relative to their most recent feeding event. The differences observed between laboratory-reared spiders therefore suggest that factors other than short-term feeding history (such as vertical microbial transmission from maternal lineages or stochastic acquisition from prey-associated microbiota) may play a role in shaping individual microbiomes. In contrast, time since last feeding was not controlled for wild-caught spiders, as individuals were sampled directly from natural environments with no prior monitoring. Desert spiders were collected on the same night, while urban spiders were collected on a different night, but feeding history in the field likely varied among individuals due to differences in prey availability, web

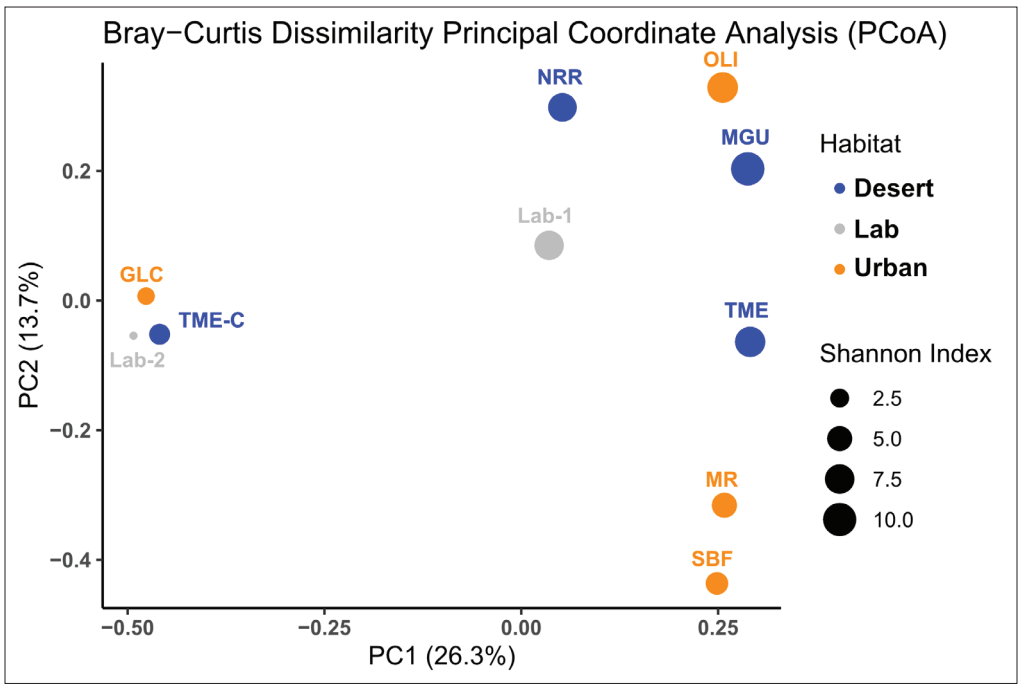

Figure 4. Principal coordinate analysis PCoA beta diversity plot of all spider samples, using the Bray-Curtis dissimilarity metric. Points on the plot are colored according to source habitat desert, urban, and laboratory, labeled with the associated spider sample, and sized according to their Shannon index. In this analysis, the closer data points are to each other, the more similar they are in microbiome composition.

success, and local ecological conditions. Such variation is difficult to control in natural populations and may contribute to the high individual-level microbiome diversity observed within habitats. Future studies incorporating controlled feeding experiments or finer-scale sampling within sites would help disentangle the relative roles of diet, feeding history, and environmental exposure in shaping spider microbiomes.

Besides dominant taxa, some microbes were more abundant than others depending on the source/habitat of the spider. Desert spiders had higher abundances of taxonomic orders Rhizobiales, Acidobacteriales, and RBC074 (class Blastocatellia) compared to urban and laboratory-reared widows. Both Acidobacteriales and RBC074 are a part of the Acidobacteria phylum which is ubiquitous in many types of soils and sediments (Janssen 2006, Lee et al. 2008, Foesel et al. 2014, Ivanova et al. 2020, Ji et al. 2016). A study of Arizona soil microbial communities found Acidobacteria to be the most abundant group (Dunbar et al. 2002). The order Rhizobiales is a part of the phylum Proteobacteria, which is also known to have members that dominate soil microbial communities, including Arizona soils (Dunbar et al. 2002). Desert widows anchor their webs to the soil to intercept prey, which may explain why their microbiome contains soil microbes like these groups (Banerjee and van der Heijden 2023). In contrast, urban widows collected for this study were found extending their web from residential walls or bushes to cement sidewalks. A similar shift in web-building behavior has been observed in Urban Golden Silk Spiders (*Nephilia clavipes*), which build webs more elevated from the ground and exploit man-made structures for predation advantages (Ripp et al. 2018). Therefore, habitats where widows build webs may play a vital role in the kinds of microbes that colonize their abdominal microbiome. Considering how an organism's microbial community is acquired and shaped, soil microorganisms are significant contributors to terrestrial microbiomes (Banerjee and van der Heijden 2023). However, the loss of natural soils in urban habitats through concrete sidewalks and other infrastructure hinders acquisition of these microbial groups by city-dwelling organisms (Barnes et al. 2021). Understanding these dynamics can reveal how different habitats support or limit certain microorganisms and how these microbes, in turn, influence the larger ecosystem.

As mentioned earlier, we found a decreased prevalence of Acidobacterales and Rhizobiales in the abdominal microbiome of urban widows compared to desert spiders. Simonin et al. (2019) showed Acidobacterales and Rhizobiales to substantially decline in streams along an increasing urbanization gradient. The authors suggest the observed sensitivity of these taxonomic groups to urbanization stems from heightened nutrient input into streams. This phenomenon is particularly pertinent given the oligotrophic nature of microorganisms belonging to these taxonomic groups, as they exhibit a preference for environments characterized by low nutrient levels conducive to their growth. The features of urbanization, like heightened nutrient input, may also be exerting an influence on black widows and their internal microbial communities. The significantly lower abundances of these indicator taxa in urban black widow spiders serve as preliminary evidence of the direct impact of urbanization on natural microbial groups and how this, in turn, influences the microbiomes of urban spiders.

Habitat differences in microbiome diversity

While we observed differences in the relative abundance of microbial groups in the widow abdominal microbiome, habitat did not appear to affect diversity. This finding is surprising considering previous studies that have documented significant habitat-related microbiome variations based on dietary changes (see Introduction). In particular, desert black widows have access to approximately 31 different ground-based arthropod prey families, while urban widows prey on only 18 arthropod families (Bang and Faeth 2011).

The microbial diversity differences in our study suggest that despite these dietary differences, other factors must be obscuring habitat-level differences in microbiome diversity.

Additionally, while black widow spiders thrive in urban landscapes, warming from the urban heat island (UHI) may increase variability in their microbiomes and obscure differences between habitats. Because UHI effects are spatially patchy, urban spider webs experience site- and web-specific temperature fluctuations during the day that could temper consistent urban-desert contrasts (Clark and Johnson 2024). Similar temperature-driven effects on microbiome diversity have been observed in the Common, or Viviparous lizard (*Zootoca vivipara*) and tadpoles of the Northern leopard frog (Lithobates pipiens), where warmer climates reduced bacterial diversity and increased variability in host microbial communities (Bestion et al. 2017, Kohl and Yahn 2016). Like lizards and tadpoles, black widows are ectothermic and, therefore, likely experience comparable body temperature fluctuations; suggesting the UHI, rather than urbanization alone, may shape their microbiomes. Future work could simulate the UHI effect on laboratory-reared spiders to see how it affects their internal microbiome diversity; testing to see if warmer conditions increase microbial community variability or reduce diversity as seen with lizards (Bestion et al. 2017) and salamanders (Fontaine et al. 2018).

The large proportion of bacterial groups falling within the "Remainder" category further highlights the substantial diversity of low-abundance taxa identified within individual spiders. Although the 10 most abundant taxa were broadly shared across habitats, many lower-abundance taxa varied considerably among individuals, contributing to differences in overall diversity metrics. For example, individuals with smaller "Remainder" fractions tended to exhibit lower Shannon diversity, suggesting that variation in these low-abundance taxa meaningfully contributes to overall community diversity. Because 16S rRNA amplicon sequencing targets a relatively conserved gene region, strain-level or functional differences among bacteria may not be fully resolved. Future studies using deeper sequencing approaches, such as shotgun metagenomics, would enable improved taxonomic and functional resolution and offer greater insight into the ecological roles of both dominant and low-abundance taxa.

Individual variation in microbiome composition and diversity

The variation in microbial diversity among spiders from the same habitat was unexpected at the start of this study. In wild-caught widows, we observed differences in the abundance of certain microbial taxa. However, these differences were not consistent enough within spiders from the same habitat to yield the habitat-level differences we expected. Individual widows in the wild have varying chances of successfully capturing different prey types, depending on relatively random prey encounters in their webs. The 4 sampled sites within each habitat also have unique characteristics, such as available web-building sites, types of prey, widow spider populations, and levels of disturbance (e.g., pesticide applications; Trubl et al. 2012). This spatial patchiness within each habitat, similar to that suggested for levels of the UHI (see text above), may explain the absence of habitat differences in microbiome diversity found in this study. Although previous studies have shown that diet and temperature differences across habitats can influence microbiome composition and diversity, individual black widows appear to experience unique variations within habitats, based on specific environmental factors. Lastly, even 2 laboratory-reared spiders, despite being reared under identical conditions from egg to adulthood, showed significant microbiome variation. We hesitate to speculate on this finding, however, as the result stems from such a small sample of laboratory-reared spiders.

Indeed, a primary limitation of this study is the sample size (4 desert, 4 urban, and 2 laboratory-reared spiders), yet this limitation is informative in itself. If strong habitat-level differences in microbiome composition were present, we would expect at least preliminary evidence of such patterns to emerge even with the limited sampling. Instead, the pronounced variability observed among individuals within the same habitat, including among laboratory-reared spiders maintained under identical conditions, suggests individual-level factors may outweigh broader habitat effects in shaping widow spider microbiomes. In this context, the absence of clear habitat-level differentiation may not solely reflect insufficient sampling, but rather may highlight microbiome heterogeneity among individual spiders.

Future studies incorporating larger sample sizes will be critical for disentangling this individual-level variation from finer-scale spatial or ecological effects. In particular, sampling multiple spiders from the same site would allow evaluation of whether microbiome similarity emerges at the site level, potentially revealing micro-environmental influences not captured by broader habitat classifications. Additional metadata, such as inter-web distance, web height, prey availability, and localized disturbance regimes, could further clarify the drivers of microbiome variation. Together, such approaches would enable a more precise understanding of how environmental context and individual ecology interact to shape microbial communities in widow spiders.

Conclusions

We report here variation in the microbial composition of a common urban pest. While we found habitat differences in the relative abundance of different microbial taxa, we found little evidence for the simplistic hypothesis that microbiome diversity is based on habitat differences. Our investigation into black widows reveals unique individual variations possibly attributable to site-specific conditions, diet, and external factors, including temperature and prey availability. These results suggest complex interactions between habitat, microbial diversity, and host species exist, highlighting the need for further exploration into how environmental variables specifically shape the widow microbiome. By considering the microbiome's potential as a critical trait for understanding urban adaptation (its role in this species' natural history), our study advocates for its integration into future ecological and evolutionary research. Ultimately, our work provides a foundation for understanding potential impacts of urbanization on microbiomes and the ecological success of black widows, offering broader insights into the role of microbial community dynamics in shaping environmental and population-level processes.

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Statement of Data Availability

Raw sequences and corresponding metadata will be archived within the Central Arizona-Phoenix Long-Term Ecological Research Program (CAP LTER) database. Additional data and scripts for analysis will be made available in a public GitHub repository: https://github.com/Hasrari/WMP-Manuscript.

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