Biotic crisis: Identifying Hymenoptera decline through Microbiome inspection Using Diversity Metrics

Hymenopterans are an important order of insects consisting of bees (Apoidea), wasps (various families) and ants (Formicidae) significantly contributing to ecosystem function. Many important pollinators in this group are declining worldwide. Can sociality and disease be a driver of these declines? We documented spatial and socially influenced distributions hymenopterans inhabiting the Bighorn Basin using point-of-collection software (Anecdata.org) in 2022-2023. We investigated the microbiome of these samples expanding our collection over 2023-2024. We extracted DNA from whole-body homogenates, amplifying both 16S ribosomal DNA (V4/V5 region) and 18S COI regions following the Earth Microbiome Project. Additionally, we assessed the microbiome of various castes contained within a bald-faced hornet nest (*Dolichovespula maculata*). We characterized metabarcode data via the QIIME2 pipeline comparing patterns of microbial diversity across space and bee/ant/wasp taxa and caste.

In our recent sampling, from highest occurrence to lowest, bacterial phyla most well-represented were: Proteobacteria (67.6%), Firmicutes/Bacillota (23.7%), Cyanobacteria (3.4%; potential environmental/substrate contaminants?), Bacteroidota (2.8%), and Actinobacteriota (1.8%) following a similar pattern in our 2022 data: in our previous samples Proteobacteria (83.7%), Firmicutes/Bacillota (8.4%), Bacteroidota (4.1%), Actinobacteria (3.6%), and Cyanobacteria (0.1%).

Previously we found the common insect parasite, *Wolbachia* sp., in only 1 sample (of 33), from an overwintering European paper wasp (*Polistes dominula*) queen. However, in our recent sampling effort of 69 samples, 10 samples contained Wolbachia including 2 European Honeybees (*Apis mellifera*) and 3 bald-faced hornet samples (a larva and a nest substrate). Other Wolbachia-positive samples remain to be identified but included 1 ant. We also identified 20+ other harmful bacteria in our samples. These included *Gilliamella* and *Snodgrassella,* which both live in the gut biome of the insects, negatively affecting their immune systems.

Can non-native species like *Polistes dominula* be spreading bacterial disease among native community members? Furthermore, we assessed whether the degree of sociality can predict disease occurrence and microbial diversity for we hypothesized that asocial (solitary) hymenopterans may individually harbor less species (OTU) richness but increased ß diversity compared to more social and eusocial members of the order. After computing α and ß diversity metrics, we have finally been able to review the trends between each caste member.