Ginger Invasion in Hawaii: Sampling Arthropod Assemblages through Metagenomics of Spider Gut Contents

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ABSTRACT

The Hawaiian Islands are home for many endemic flora and fauna cannot be found elsewhere. However, in recent years, ecosystems in the Hawaiian Islands are threatened by biological invasions. *Hedychium gardnerianum*, a ginger plant native to the India's Himalaya regions, whose presence was first noted in Hawaii in 1954, and has modified the local ecosystems considerably since then. Arthropod communities are often affected by local vegetation, yet comparing the arthropod assemblages are difficult. In this study, I aim to investigate the impact of invasive ginger on native arthropod communities in Maui, HI, using gut content metagenomics with CO1 primer and a combination of 16S, 18S, 28S rDNA primers. CO1 primer successfully amplifies prey DNA from Tetragnathidae gut content, but for Philodromidae and Theridiidae, instead overwhelmingly amplifying predator DNA. I compared the gut content composition differences between spider of different sexes and collected from native versus invaded site. I detected overall gut content composition difference between Philodromidae collected from native and invaded sites; and in invaded site, more Philodromidae and Tetragnathidae preyed upon Pscoptera and more Philodromidae preyed upon Entomobrymorpha, and in native site, more Philodromidae preyed upon Hemiptera.

KEYWORDS

Invasive species, gut content metagenomics, spider diet composition, parasite, tropical conservation biology

INTRODUCTION

The Hawaiian Islands are comprised of ecosystems with unique flora and fauna that are highly susceptible to biological invasions. The Hawaiian Islands' biodiversity arises from a limited number of introductions influenced by their remoteness to the mainland, leading to species compositions of great research interests (Vitousek et al. 1987a). Invasive species, which cause extinction and ecosystem transformation, are identified as the second greatest threat to biodiversity (Park et al. 2004). Humans introduced a variety of species to Hawaii to since the Polynesian discovery 1500 years ago. In the last 200 years, over 4600 non-native plant species have been introduced, of which 800 are able to spread independently, and 86 negatively impact native species severely (Stone 1992). Invasive species alters Hawaiian ecosystems in various ways. Many invasive plant species, such as *Myrica faya*, absorb nitrogen more effectively than native species, thereby outcompeting native species and increasing nitrogen availability for the whole ecosystem (Vitousek et al. 1987, Baruch and Goldstein 1999). Invasive plant species also negatively impact recruitment of native vegetation, leading to changes in fire regimes (D'Antonio et al. 1998). Invasion of non-native arthropods, for example, the Argentine ant (*Iridomyrmex humilis*), threatens the survival of endemic species, including predators and pollinators of crucial ecological importance (Cole et al. 1992). The impacts of invasive species on Hawaiian Islands occur in multiple taxonomic groups and trophic levels, and therefore demanding further researches on the community effects of an invasive species.

Hedychium gardnerianum, a ginger plant native to the India's Himalaya regions, has invaded the Hawaiian Islands and modified the local ecosystems considerably (Naik and Panigrahi 1961). Its presence was first noted at Hawaii Volcanos National Park in 1954 and then spread to the uninvaded islands shortly after (Wester 1992). *Hedychium gardnerianum* forms dense colony in native forests and replaces native vegetations. It also favors the growth of *Psidium cattleianum*, another nonnative plant species that establishes readily at low light conditions (Minden 2009, Huenneke and Vitousek 1990). The fast litter decomposition rate of *H. gardnerianum* leads to increased soil nitrogen and phosphorous content below the plant, thus further facilitating its growth and reproduction (Allison and Vitousek 2004). In Hawaiian forests, where poor litter quality and slow litter decomposition maintains low soil nitrogen content, the positive feedback of litters of invasive species facilitating further invasion lead to significant changes in vegetation structure (Funk 2005). Despite invasive plant species usually

impacts activities of animals, there is little researches on the impacts of *H. gardnerianum* on local fauna.

Arthropod assemblages are intimately associated with the flora, and therefore greatly impacted by changes in vegetation (Gratton 2005). Changes in structural characteristics of vegetation, such as cover and height, affect arthropod movements and the reproductive success of certain taxa, resulting in a shift in arthropod community composition (Litt et al. 2014). The sensitivity of arthropod assemblages toward vegetation make them good indicators for studying the cascading effects of invasive plant species. In most cases, invasive plant species lead to reduced arthropod abundance and richness (Litt et al. 2014). Arthropod communities fill various niches and provide diverse ecological services, serving as predators, detritovores, and pollinators. To decipher the resulting changes in arthropod assemblages and to assist further analyses in altered ecosystem services, how *H. gardnerianum* affects Hawaiian arthropod communities requires study.

Gut content metagenomics provides a convenient tool for studying arthropod assemblage. Despite the importance of studying impact of invasive vegetation on local arthropod assemblages, collecting and identifying arthropod samples are very time-consuming and error-prone. The data required for inferring such impacts are lacking (Hart et al., 2017). With the popularity of high-throughput sequencing, arthropod assemblage shifts can be inferred through a shift in gut contents of predators. However, CO1, the traditional primer used for such analyses, tends to over-amplify predator sequences in Philodromidae and Theridiidae gut content analysis due to homoplasy (Krehenwinkel et al., 2017). Therefore, rDNA primers are designed for selectively blocking predator DNA during amplification the process (Krenhenwinkel et al., 2019).

In this study, I aim to analyze the impact of invasive ginger on Hawaiian arthropod community. Specifically, I investigate in 1) the performance of CO1 and rDNAs, the primers used for amplification; 2) How the gut content composition differs between native and invaded sites. I hypothesize that CO1 primers over-amplify predator DNAs for non-Tetragnathidae, while the rDNAs successfully amplify other clades. I also hypothesize that there will be gut content composition differences between native and invaded sites.

METHODS

Sample collection and DNA extraction

The Gillespie group collected over five hundred spiders by beating from Maui, HI. Lab members removed all legs from each spider, and then separate the prosoma and opisthosoma. They then put the opisthoma into lysis buffer and crushed it with a pestle. They extracted DNA using the Qiagen Puregene Tissue kit based on the manufacturer's protocol. They performed PCR amplification with CO1 primer and rDNA primers using the Qiagen Multiplex PCR kit. They then constructed dual-indexed Illumina libraries. These samples and sequences are used for a larger project studying the prey composition of Hawaiian spiders. I obtained reads for my study from their sequencing results.

Sequence analysis

To assign taxonomic information of sequences recovered from each sample, I cleaned and analyzed the reads. I assembled the sequences with PEAR (Zhang et al. 2013) with a minimum overlap of 50bp and quality threshold of 20. I quality-filtered the merged reads for >90% of the sequence having quality >30 with Fastx-toolkit (Gordon & Hannon 2010). I then trimmed off the primer sequences with Unix command awk. I performed OTU clustering with 97% identity using the UPARSE algorithm for sequences amplified with both primers, and ZOTU clustering with Unoise3 algorithm for sequences amplified with rDNA primers (Edgar 2013). I then blasted all the OTUs against the NCBI database to obtain the best 10 taxonomy assignment for the OTUs (e-value < 10^-3) (Krenhenwinkel et al. 2019). I constructed the OTU table with USEARCH and combined OTUs with same species assignment with Python3 (Edgar 2013).

Primer performance analysis

To compare the performance of the CO1 and rDNAs primers, I computed the percentage of number of non-spider and non-contamination reads in total reads. I treated reads belong to mammals and plants as contamination, and defined gut reads as non-spider, non-contamination reads. For each primer – spider family combination, I counted the total number of reads and the total number of gut reads, as well as the number of unique orders among all

non-contamination, non-spider sequences. I calculated percentage of prey reads as #gut reads / #total reads.

Gut content composition analysis

To compare the gut content composition of spiders of each family and sex group, I calculated abundance of each order of gut reads and performed statistical tests. I first rarefied the OTU table with the GUniFrac Rarefy function in R (Chen 2018). To examine the overall gut content composition, I used NMDS to visualize and ANOSIM to compare the clustering. (Oksanen et al. 2019) For each order identified, the occurrence and abundance of reads were summed up with Python3 from all samples with a threshold of >10 reads recovered (Python Software Foundation 2018). Then, I conducted z-tests for sample proportion between native and invaded samples for each order of content and spider group with R (R Core Team, 2016).

RESULTS

Sequencing results

Both rounds of sequencing produced high-quality reads of a wide variety of arthropods. For CO1 primer, from the gut content metagenomic sequences extracted from spiders of three families (Tetragnathidae, Philodromidae, and Theridiidae) collected in non-invaded sites (168) and spiders of three families collected in ginger-invaded sites (167), I recovered 7219725 arthropod sequences (minimum e-value 10^-3). For rDNA primer, from the Philodromidae samples (83), I recovered 73332 reads. Of the 48 orders were recovered, 16 OTUs were from prey.

Primer performance

For the CO1 primer, although 84.6% of the reads recovered from Tetragnathidae samples were non-spider sequences, only 0.63%, and 0.25% of the reads recovered from Philodromidae and Theridiidae were non-spider sequences (Figure 1). The spider sequences were excluded from down-stream analyses. The remaining sequences contained 283 OTUs belong to 228 species of 10 orders (Figure 2). The order Diptera (47%), Lepidoptera (29%), and Hemiptera (12%) had the most sequences. For rDNA primer, 71.3% of the reads were non-

spider and non-contamination, and I excluded the rest for downstream analysis. With 97% identity OTU clustering, I identified 77 OTUs belong to 64 species of 16 orders. With ZOTU clustering, I identified 135 OTUs belong to 57 species of 16 orders (Figure 2). The Hemiptera (45%), Pscoptera (19%) and Entomobrymorpha (13%) had the most sequences. I used ZOTU clustering results for downstream analysis.

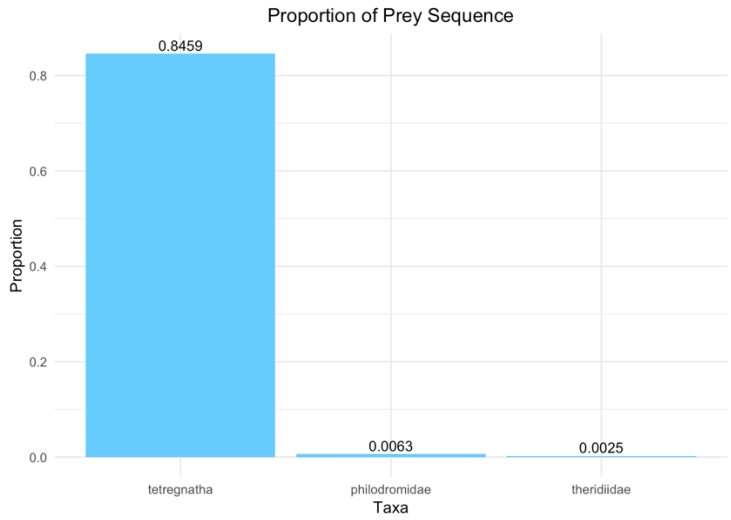

Figure 1. Proportion of gut content sequences. The proportion of non-spider sequences recovered from Tetragnathidae (total reads = 2308032), Philodromidae (total reads = 2573104), and Theridiidae (total reads = 2338589) samples.

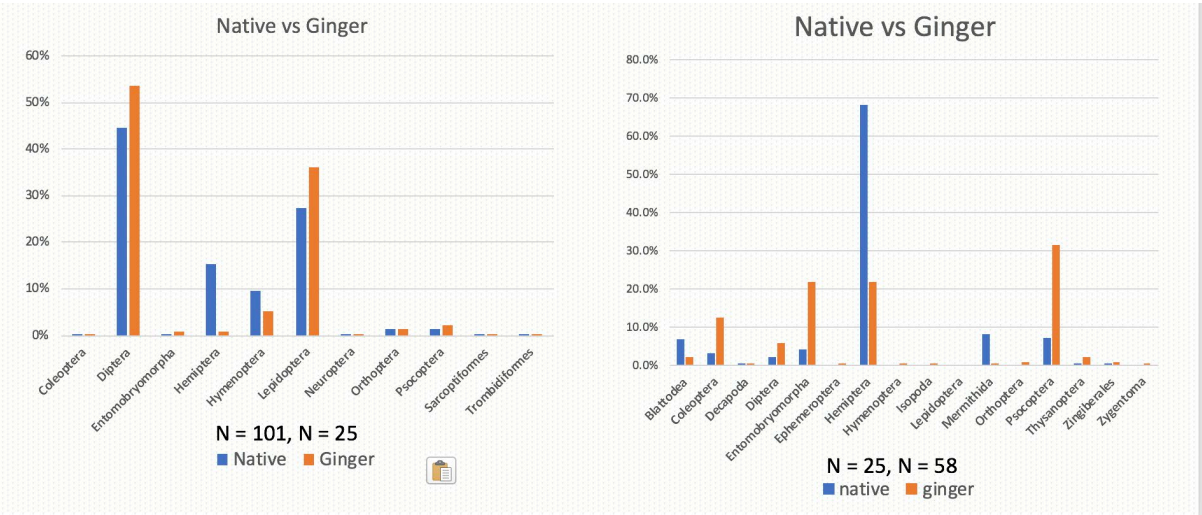

Figure 2. Distribution of content reads. The percentage of reads recovered for each insect order from spiders of each family from native and ginger sites. Left: CO1 primer. Right: rDNAs primer.

Gut content composition

The prey species composition and abundance from the invaded and non-invaded sites were different by prey species. Because of the poor CO1 amplification result for Philodromidae

and Theridiidae, those results are excluded from analyses. NMDS results indicate no statistically significant overall gut content composition difference between Tetragnathidae from native and ginger sites (p = 0.668) or between sexes (p = 0.018). From the rDNA primer sequencing result of Philodromidae, I identified different overall gut content composition difference between sites (p = 0.001), but not sexes (p = 0.053) (Figure 3).

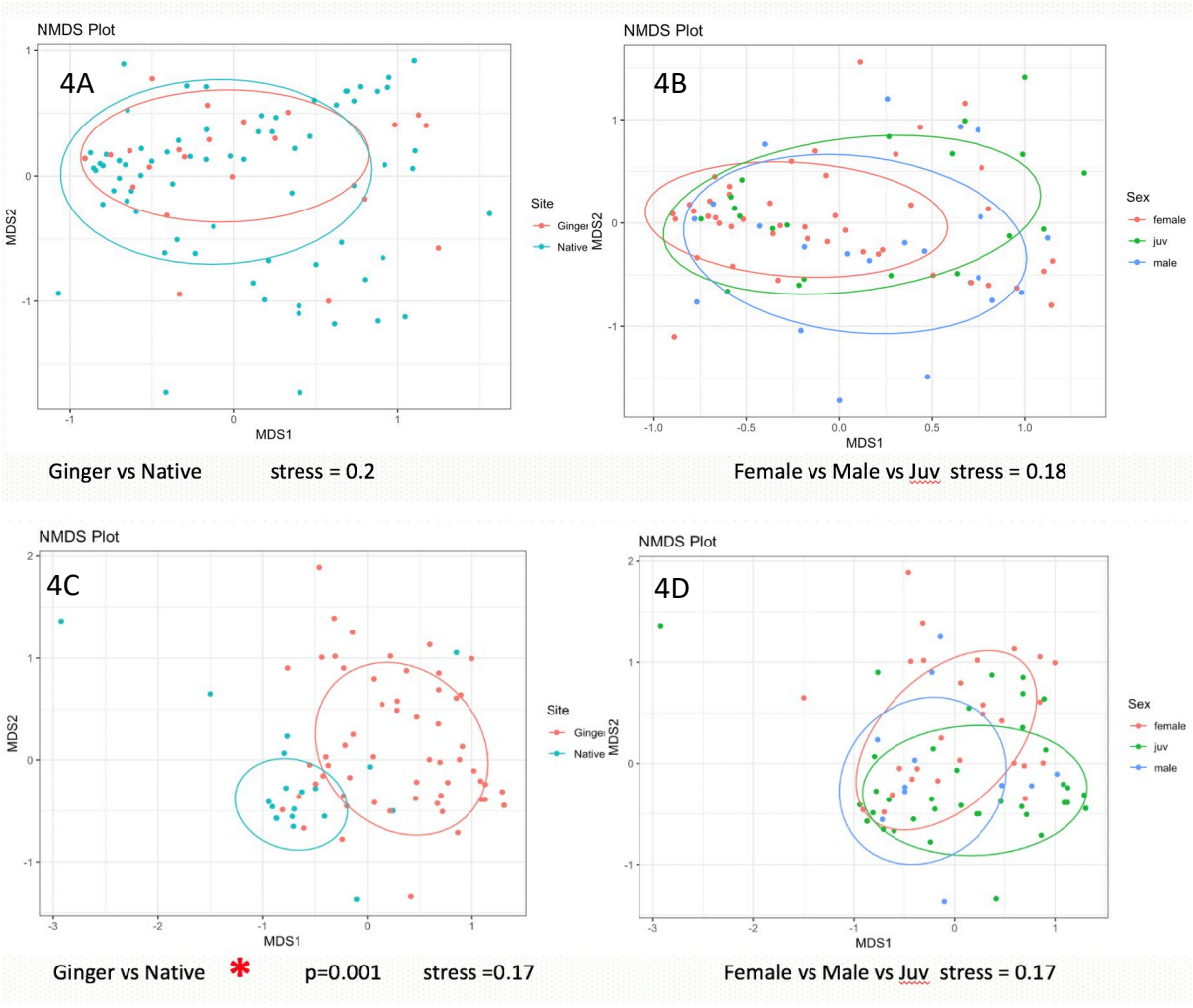

Figure 3. NMDS results for gut content difference. 4A: Tetragnathidae, native vs. ginger. n_native = 101, n_ginger = 25. 4B: Tetragnathidae, female vs. male vs. juvenile. 4C: Philodromidae, native vs. ginger. n_native = 25, n_ginger = 58. 4D: Philodromidae, female vs. male vs. juvenile. "*" indicates $p < 0.05$.

The overall differences are reflected by differences at Order levels. Statistical significantly more Tetragnathidae spiders in invaded sites were identified with Psocoptera (p = 0.0040) (Figure 4A). After grouping the Tetragnathidae spiders by sex, more female spiders in native sites were identified with Diptera (p = 0.0216), more in invaded site were identified with Entomobryomorpha (p = 0.0191); and more juvenile spiders in native sites were identified with Hemiptera (p=0.0249). For Philodromidae, I identified more Philodromidae preyed upon

Entomobrymorpha (p = 0.0001) and Pscoptera (p = 0.0003) in invaded site, and more preyed upon Hemiptera in natural site (p = 0.0002). After grouping by sex, I identified more female spiders in invaded site with Entomobrymorpha (p = 0.001) and Pscoptera (p = 0.02) sequences, and more juvenile spiders in invaded site with Entomobrymorpha (p = 0.04).

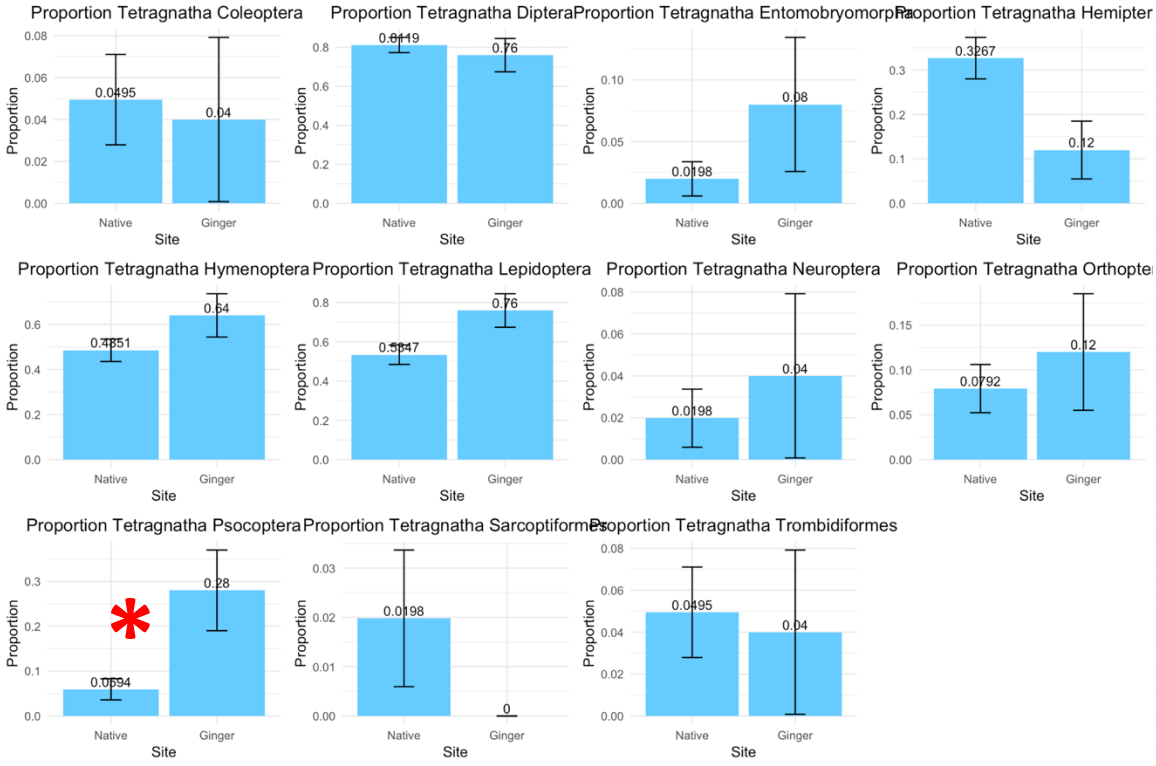

Figure 4A. Orders of Tetragnathidae Gut Content. Proportion of Tetragnathidae spiders identified with each order of arthropod sequences. "*" indicates $p < 0.05$. n_native = 101, n_ginger = 25.

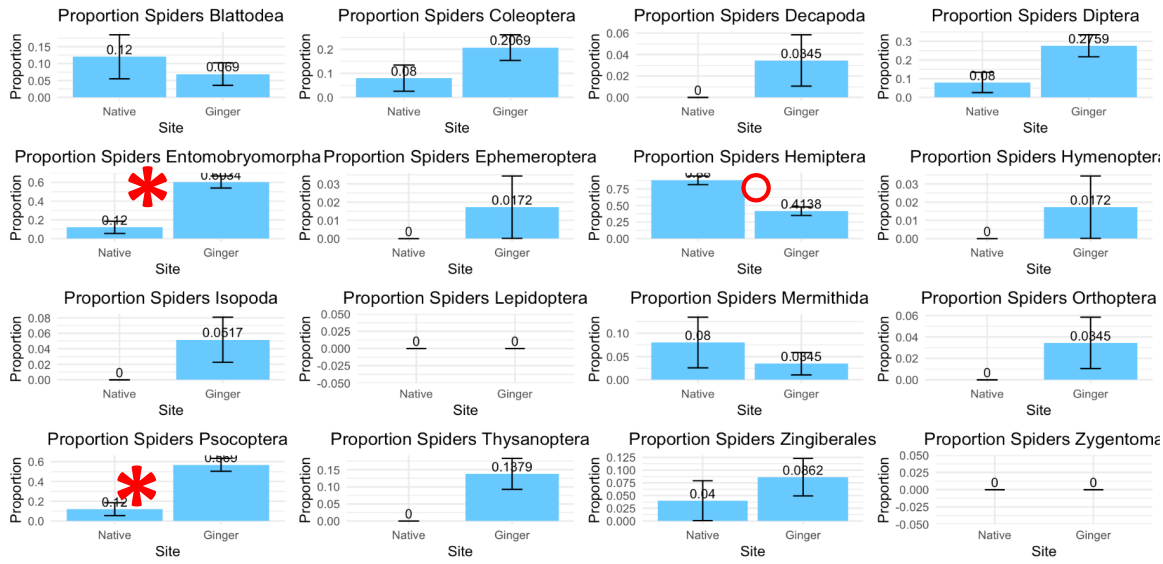

Figure 4A. Orders of Philodromidae Gut Content. Proportion of Philodromidae spiders identified with each order of arthropod sequences. "*" indicates $p < 0.05$, more in ginger; "○ " indicates $p < 0.05$, more in native. n_native = 25, n_ginger = 58.

DISCUSSION

Spider gut content metagenomics provides valuable information for inferring the arthropod community assemblage shifts influenced by invasive ginger. The sequencing results indicate that CO1 primer amplified only Tetragnathidae gut sequences sufficiently, and rDNA primers solved the problem of spider DNA over-abundance for Philodromidae. I identified the overall gut content composition difference of Philodromidae amplified with rDNA primers between native and invaded sites. I identified no overall gut composition difference between spiders of different sexes, and between Tetragnathidae collected from native and invaded site.

Primer performance

The CO1 primer effectively amplified content sequences extracted from Tetragnathidae, but not from Philodromidae or Theridiidae, while the rDNA primer effectively amplified Philodromidae sequences. The inconsistency of CO1 primer performance is probably due to homoplasy of mitochondrial DNA, which enhances primer binding of spiders and thereby the over-amplification of spider sequences (Krehenwinkel et al. 2019). Although the sequencing

result for rDNA primer is preliminary, rDNA primer amplified content DNA well. The rDNA primer resulted in fewer OTUs obtained than the CO1 primer did. Since 16S, 18S and 28S rDNAs are relatively conserved, clustering OTUs with 97% identity may fail to differentiate closely related lineages (77 OTUs identified), but clustering with ZOTU (135 OTUs) leads to comparable OTU counts to the CO1 primer (283 OTUs).

Overall gut content composition

The gut content species identified from sequences recovered from each of the three spider families (Tetragnathidae, Philodromidae, and Theridiidae) had different compositions, reflecting different spider dietary preferences and life histories. Diptera, Hemiptera, and Hymenoptera species each contributed to nearly twice as many as sequences for Tetragnathidae and Theridiidae than for Philodromidae. The clustering of Philodromidae was also separated from that of the other two families as a result of these prey taxa. Because most of the Tetragnathidae and all the Theridiidae we collected were web-building species, which prefer small flying insects, yet Philodromidae only use silk for draglines and egg sacks, we expect such differences in diet (Gillespie 1997). Because Theridiidae and most of the Tetragnathidae are web-builders, we expect them occupy similar niches. It was unexpected that we observed large contribution by Entomobryomorpha (Collembola) in Theridiidae and Philodromidae samples, yet not in Tetragnathidae samples. However, the CO1 primer tends to amplify Collembola sequences at a lower rate than many other arthropod sequences due to lower affinity (Krehenwinkel et al., 2019). Such property may contribute to fewer Collembola sequence amplified for Tetragnathidae using CO1 primer.

Difference in native and ginger - invaded sites

Gut content composition and abundance from the invaded and non-invaded sites differed, suggesting different arthropod community composition. For Tetragnathidae, I identified more spiders from invaded sites with Pscoptera sequences. For Philodromidae, more spiders from invaded sites were identified with Pscoptera and Collembola sequences, while more of those from native sites were identified with Hemiptera sequences. The observation on Collembola is as expected, because previous studies reported higher Collembola abundance is associated with invasive ginger and these organisms could serve as alternative prey for spiders in non-native ecosystems (Agusti et al. 2003, Lawrence et al. 1999). Moreover, the Collembola

identified are majorly composed of Salina and Tomoceroidea, and a lot of Pscoptera identified are Lobocaecilius, which are likewise non-native (Schaffer 1898, Lee and Thornton 1967). However, it is surprising that a lot of the Pscoptera identified are native Kilauella, leaving it uncertain whether Kilauella is positively impacted by invasive ginger (Thorton 1984). Because site does not directly affect spider diets, I can use diet difference to infer arthropod assemblage differences (Gillespie 1997). However, it is not certain whether invasive ginger reduces local Diptera and Hymenoptera abundance or their reduced observations were due to spiders relying on Collembola more for diet. Another unexpected result is that, although not statistically significant, more spiders are identified with Mermithoidea (spider parasites) and Ichneumonoidea in the native sites (George 2005). The Ichneumonidae are Lepidoptera parasites; since they were not present with their associated hosts, it is more likely that spiders directly preyed upon them (Beardsley 1960). I hypothesize that invasive ginger may reduce local assemblage complexity and interrupts the life cycle of the parasites, leading to overall less parasitism. In conclusion, I inferred arthropod community assemblage shift due to invasive ginger from spider gut content metagenomics, favoring some nonnative arthropods like Collembola and Kilauella, and modifying species interaction dynamics.

Limitations and future directions

One of the major limitations in this study is that, in non-Tetragnathidae samples, CO1 primers amplified spider DNA more readily than gut contents. As a result of under-representation of content DNA, detected sequences are more subjected to stochastic processes, leading to fewer results of statistical significance observed, especially for Theridiidae and Philodromidae spiders. The Gillespie group has developed a new protocol to block amplification of spider DNA, and future studies may yield more meaningful results (Krehenwinkel et al., 2019). Another future direction is to sample along a gradient of time of invasion, so we can study how arthropod assemblage change across time (Mogobozi et al. 2008).

Broader implications

Impacts of invasive species on local communities has been a major theme in ecology research. Because arthropod communities perform many important ecological services, and are closely related to other flora and fauna through ecological interactions, it's very important to

study the response of local arthropod assemblage to invasive species. However, as a result of sampling and identification difficulties, the corresponding food-web and parasitism dynamics and changes have rarely been studied in a high-throughput manner. This study not only examines the response of local arthropod communities to invasive ginger, but also exemplifies high-throughput study of arthropod communities using next generation sequencing and bioinformatics tools. With similar studies in the future, we may better understand the consequences of invasive species at multiple trophic levels, and thus better inform mitigation plans and prevention strategies.

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