“New” UROP Application

Title of Proposal:	Unraveling the Identities of Associated Insects in Ant-Plant Symbioses with Nanopore DNA Sequencing

PROPOSAL: STATE THE PROBLEM/TOPIC

Ant-plant mutualisms have been evolving for over 100 million years, representing ubiquitous interactions (Nelsen et al., 2018). Extensive studies have been done on conspicuous systems, where the plant provides nutritional resources to ants in the form of extrafloral nectaries or food bodies (Grasso et al., 2015), an example could be Pseudomyrmex-Acacia associations (González-Teuber & Heil, 2010). However, in cryptic ant-plant symbiotic systems, host plants do not readily provide obvious nutritional rewards, raising questions about the nature of the associations and the benefits exchanged between plants and ants. The Myrmelachista-Lauraceae mutualism falls in this category where resources are not provided by the host plants (Longino, 2006; Probst et al., 2024); these are the interactions that this project revolves around. In these instances, associated insects such as mealybugs and soft scales, both in the superfamily Coccoidea, act as intermediaries by providing enhanced plant sap known as “honeydew.” That processed sap is enriched with nitrogen, amino acids, proteins, sugars, and water thanks to Coccoidea microbiome, serves as a primary food source to their host ants (Fang et al., 2020b). However, the identities of the Coccoidea, their microbiome, and co-evolutionary history with ants are poorly understood and often overlooked.

This noticeable scarcity of in-depth studies focused on Coccoidea systematics and phylogenetics hampers our understanding of how ant-plant mutualisms might have evolved in tropical ecosystems. Most studies on Coccoidea have focused solely on their role as agricultural pests (Abd-Rabou et al., 2012; Amouroux et al., 2017; Deng et al., 2014; Pacheco da Silva et al., 2014) by focusing on species that feed on cultivars, potentially transmitting viruses and/or diseases that can lead to crop degradation (Selvarajan et al., 2016). Consequently, their ecological importance beyond agriculture has been largely underestimated. Furthermore, elucidating the microbiome of Coccoidea through DNA sequencing techniques is imperative. The microbiome plays a pivotal role as the key facilitator of these tritrophic interactions, since it is what allows Coccoidea process and enhance plant sap, yet its detailed composition and evolutionary history remain largely unexplored. Traditional alpha taxonomy struggles with the challenging morphological uniformity of Coccoidea, necessitating the integration of newer technologies such as molecular tools. DNA barcoding in Next Generation Sequencing, is a tool that allows rapid species identification by analyzing gene sequences (Vacher et al., 2016). This combined with nanopore sequencing, known for generating cost-effective high-quality data and being the only one to offer real-time analysis (Advantages of Nanopore Sequencing, n.d.), is the first step to illuminating evolutionary relationships and facilitating species identification and description.

The study of Coccoidea involved in ant-plant mutualisms represents a critical frontier in ecological research, addressing substantial gaps in our understanding of these intricate interactions. Despite their ecological significance as intermediaries in certain ant-plant symbiotic systems, Coccoidea remain inadequately studied outside of agricultural contexts. The integration of advanced molecular tools, such as nanopore DNA sequencing, offers a promising approach to unravel their taxonomic complexities and evolutionary histories. By combining modern techniques with traditional methods, this research will intend to solve some of the ecological puzzle in the inconspicuous Myrmelachista-Lauraceae mutualism.

RELEVANT BACKGROUND/LITERATURE REVIEW

The lack of distinct morphological traits to help define species boundaries has rendered the taxonomy of Coccoidea difficult to navigate. To uncover their morphology, complex techniques such as KOH cleaning are used to highlight characters (e.g., genitalia) of the insect that can be a great source for identification (Martinelli et al., 2017). Thus, molecular tools may offer a promising alternative for species identification. However, those tools might also present shortcomings for species identification. For example, a recent study indicates that

Coccoidea exhibit intricate mitochondrial genome structures, complicating sequencing efforts (Lu et al., 2020). Additionally, research suggests that primers targeting cytochrome oxidase subunit 1 (CO1) fragments (a commonly used barcoding marker) larger than 600bp have low success rates; for instance, one study reported a 65% sequencing success rate out of 524 specimens (Park et al., 2011). I have been optimizing a protocol to deal with sequencing Coccoidea barcodes by focusing on CO1 fragments that are shorter than 450bp in length, termed minibarcodes (Leray et al., 2013). I have been targeting a 313bp segment of the CO1 marker, shown to have successfully enriched barcodes from highly degraded DNA input (Leray et al., 2013). In a trial involving 25 Coccoidea samples, I was able to successfully generate DNA barcodes for all of them.

Beyond species identification of Coccoidea, to further understand their natural history it is crucial that we sequence their microbiomes using 16S barcoding region. Without their microbiome, Coccoidea wouldn't be able to facilitate ant-plant interactions because their bacterial symbionts provide Coccoidea with essential nutrients by metabolizing them (Szabó et al., 2016). By generating DNA barcodes of the Coccoidea along with information on their microbiomes we will be able to uncover and dive deep into the co-evolution patterns with their Myrmelachista hosts.

SPECIFIC ACTIVITIES AND TIMELINE

For this project, Coccoidea specimens obtained from colonies of 11 Myrmelachista species will be sequenced along with their microbiomes with the purpose of understanding their identities, diversities, and natural histories. Throughout the timeline of this project, I will be in close contact with my mentor, Dr. XXX, with a weekly meeting where I will be updating him with the status of the project, addressing progress and shortcomings in the project.

So far:

- I optimized a pipeline using primers for DNA minibarcodes targeting a fragment of 313bp of the CO1 marker.
- Conducted pilot study on 25 samples recovering DNA minibarcodes for all samples.
- Confirmed the identity of the Coccoidea (at least to family level) using the BLAST algorithm (McGinnis & Madden, 2004).
- Have been running phylogenetic analyses incorporating additional Coccoidea BOLD barcodes (Ratnasingham & Herbert, 2007).

August:

- Coccoidea specimens from already collected Myrmelachista colonies (n=200) will be sorted and processed.
- I will run DNA extractions on selected specimens.
- I will conduct PCR and obtain sequenced amplicons for the new specimens.
- New minibarcodes will be added to the current dataset, expanding our sequence matrix. Phylogenetic analyses will be conducted with that updated dataset.

September:

- I will compare the phylogeny obtained from Coccoidea minibarcodes with the already existing phylogenetic tree of Myrmelachista ants (Probst et al., 2024) to test for co-evolutionary hypotheses.
- I will discuss with Dr. XXX a pipeline to sequence the microbiomes of the Coccoidea and associated Myrmelachista.

October:

- I will run DNA extractions on the microbiomes of Coccoidea and Myrmelachista.

- I will amplify and sequence the 16S barcoding region for both groups' microbiomes.
- I will run phylogenetic analyses to compare the composition of microbiomes for Coccoidea and ants.
- I will organize and present my findings at the 2024 National Diversity in STEM (NDiSTEM) SACNAS conference to which I have been selected to present.

November and December:

- I will start drafting a manuscript with the supervision of Dr. XXX
- I will focus on writing, editing and finalizing that manuscript.

RELATIONSHIP OF WORK TO THE EXPERTISE OF THE MENTOR

My faculty mentor Dr. XXX is a postdoctoral fellow at the Science Research Initiative (SRI) program at the University of Utah. Dr. XXX is an expert in molecular biology, phylogenetics, and entomology. He has developed a DNA barcoding pipeline focused on nanopore sequencing, being selected by Oxford Nanopore Technologies (ONT) to be part of their Education Beta program. Additionally, he has been working with ant-plant symbioses, specifically the MyrmelachistaLauraceae system. I have been working under his mentorship since Spring of 2023, and thanks to this I have learned data processing and logging, different methods for DNA extraction, and library preparation focused on CO1 markers, DNA sequencing using nanopore technology, and phylogenetic analyses; all of which are crucial for my research. Aside from the skills I have acquired, Dr. XXX has also been supportive, encouraging, and has been giving active feedback which has allowed me to grow confidence and feel comfortable in navigating and exploring research topics, methods, and network outreach.

RELATIONSHIP OF THE WORK TO YOUR FUTURE GOALS

Once I obtain my bachelor's degree in biology, my trajectory extends to a Ph.D. where I aim to delve deeper into research in biotechnological and/or pharmaceutical fields. Working on this project has not only given me a set of skills that I can apply towards a Ph.D., but it was also what solidified my decision to continue to pursue research in the future. Participating in UROP will provide me with valuable research experience, impacting my performance in a lab setting by exposing me to new tools, analyses, and the peer-review method. It will also allow me to grow as a science communicator which is indispensable for aspiring researchers like myself.

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