CHESTER COUNTY community FOUNDATION

REQUEST FOR PROPOSALS

Date:

Contact Information:

Organization Name: Immaculata University
Address: 1145 King Road
Immaculata, PA 19345
Phone: 484-643-8271
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Contact Name: Angela Tekely
Contact Title: VP Academic Affairs
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Year Incorporated: 1920

Have you previously received the Miller Grant Award: Yes _ No X

Organizational Information:

Geographic Area Served ***(If not all of Chester County, specify primary Chester County municipalities served):*** Immaculata University is situated in Chester country and serve a significant population within the county. The University also enrolls students from throughout the state and nationally.

Annual # of Clients & Description of Population Served:
Immaculata University's study body includes over 2,400 undergraduate and graduate students.

Mission Statement:
Immaculata University, a Catholic academic community, founded and sponsored by the Sisters, Servants of the Immaculate Heart of Mary, is committed to scholarship, formation of the whole person for leadership and service, and empowerment of all to seek truth, promote justice and engage in dialogue between faith and culture.

Organization Description:
Immaculata University (IU) is a Catholic, comprehensive, coeducational institution founded in 1920 as a women's undergraduate college in Chester County, PA. Accredited by the Middle States Association on Higher Education (MSCHE), the University serves a student population of approximately 2,500 men and women of all ages, offering 4 doctoral programs, 12 master's degree programs, and more than 60 undergraduate majors, minors, certificates, and pre-professional programs in on-campus, off-site, and/or online modalities.

Annual Budget: $42.8 M (including depreciation, but excluding scholarships)
249 # of Full-Time Paid Staff/ 215 # PT Staff
___56____ % of budget for program expenses
___42___ % of budget for administrative expenses
____2____ % of budget for fundraising expenses

23 # of Board Volunteers
Numerous Active Non-Board Volunteers
Countless Volunteer Hours

Top 3-5 funding sources:
Top three funders for Fiscal Year 2024:

Sandy Hill Foundation	$331,000
Anonymous	$109,000
Henry A. Quinn Foundation	$100,000

Proposal Information:

Grant Amount Requested: $2,500

Description of Grant Purpose: The purpose of the grant will be to identify species within Chester County, to "protect and conserve open space and the environment". It is essential there be a better documented understanding of local biodiversity, particularly with insect species. This project aimed to leverage the expertise of faculty within Immaculata University's Natural Sciences Department in conjunction with two students to conduct DNA Barcoding and Sequencing to identify, curate and house insect specimens.

MARJORIE L. AND ARTHUR P. MILLER FUND
REQUEST FOR PROPOSALS
GRANT PROPOSAL NARRATIVE

1. Organization's history, goals, key achievements and distinctiveness

Immaculata University (IU) is a Catholic, comprehensive, coeducational institution founded in 1920 as a women's undergraduate college in Chester County, PA. Accredited by the Middle States Association on Higher Education (MSCHE), the University serves a student population of approximately 2,500 men and women of all ages, offering 3 doctoral programs, 12 master's degree programs, and more than 60 undergraduate majors, minors, certificates, and pre-professional programs in on-campus, off-site, and/or online modalities.

Our goals include, (1) Create a Distinct Brand Identity within a Culture of Inclusion, (2) Achieve Enrollment Goal of 3,000 Students and a Retention Rate Above the National Average, and (3) Enhance Academic Excellence through Teaching and Learning. Some key achievements with these goals include: the increase of minority degree-seeking students to 24% of total students, new support for first-generation students, who represent 25% of the undergraduate enrollments, traditional undergraduate (first to second year) retention steady at 80% (or higher over last five years), adult student (spring – fall 23) retention rate at 84%, recent MSCHE re-accreditation on July 2024, and hiring of 12 new full time faculty in 2024.

Relating to "open space and the environment," Immaculata University is committed to "Laudato Si." Laudato Si is a 2015 cyclical letter by Pope Francis to address the urgent need to care for the environment. Immaculata has since made moves to become more sustainable. Examples of this include forming a Sustainability Committee, planting native plants in our restored water retention ponds to make them functional rain-gardens, and significantly reducing foam containers in our cafeteria. Additionally, Immaculata started an Environmental Science and Ecology minor in 2022 that is open for all majors across the university. Lastly, we frequently partner with local and national conservation organizations which include, Valley Forge National Historical Park, National Audubon Society, and New Jersey Conservation Foundation to achieve various environmental and conservation management goals.

2. Funding request:

Project Description / "Funding Request": Biodiversity loss is one of the leading threats to human society due to the "free" ecosystem services that biodiversity provides, *e.g.*, fresh air and clean water (Diaz *et al.* 2006). Insects are the most biodiverse animal group in the world; all terrestrial animals (including humans) and most plants are dependent on insects due to their contribution to pollination, nutrient cycling, decomposition, and pest control (Campbell *et al.* 2012; Rosenberg *et al.* 2023). However, due to the "insect apocalypse," insects are declining precipitously in abundance and biomass which threatens both global and local ecosystem health (van Klink *et al.*, 2020; Wagner *et al.* 2021). Some of the leading drivers of biodiversity loss and the insect apocalypse are urbanization and fragmentation (Sánchez-Bayo and

Wyckhuys, 2019; Wagner *et al.* 2021). Therefore, in order to "protect and conserve open space and the environment," it is vital to identify the species that occur there first.

Chester County, PA is also rapidly becoming more developed and urbanized (USGS, 2016). The more charactmastic insect species are readily known to our area, *e.g.,* butterflies and dragonflies (GBIF, 2024), but we are likely losing thousands of other local species before we are even aware that they occur here. These insect species are often the harder to identify and more species-rich taxa such as bees and ants (Hymenoptera: Apoidea and Formicidae) and beetles (Coleoptera). To help solve this issue, we will use DNA Barcoding to identify insect specimens. DNA barcoding has been successfully used to determine insect specimens to species and is more efficient than traditional morphological taxonomy (Ashfaq *et al.*, 2022; Wührl *et al.* 2021). Essentially, DNA will be extracted from each sample (e.g., an insect leg), and PCR will be used to amplify a small sequence of DNA of the mitochondrial gene COI (Cytochrome oxidase subunit 1). The amplified DNA will be sequenced, and we can compare sequences to published sequences of known species ("barcodes") to identify the genus and species of each specimen. It is of utmost importance to learn what our local insect fauna is before they are lost via development and urbanization.

Lastly, we will collaborate with the Entomology Department at the Academy of Natural Sciences in Philadelphia (Mason *et al.* 2020). These research specimens can last hundreds of years ("lasting in nature") and be used for further research projects to broaden their individual impact (Mason *et al.* 2020). There is no doubt that the entomologists at the Academy of Natural Sciences will also be interested in the insect specimens we collect. This especially goes for specimens that are in their area of expertise, *i.e.,* moths (Lepidoptera), crane flies (Diptera: Tipulidae), and stink bugs (Hemiptera: Pentatomidae).

The goals of this project are to (1) advance our understanding of the local insect fauna for Chester County, PA, (2) learn what insect species occur in IU's forests using DNA barcoding, and (3) involve undergraduate students in local biodiversity research projects. Immaculata University's forests are genuinely "valuable resources that are worthy of being preserved." We just need to know what these resources (insects) are and how they can contribute to the surrounding Chester Country community.

Grant Criteria that will directly be satisfied:

1. learning what our "local land resources are"
2. becoming better stewards of "important parcels of land"
3. "Creative ways for education"
4. "school-based environmental education effort"
5. "Research about important an important environmental issue"
6. "Involve collaboration with environmental agencies and organizations"

Role of Funds: We are requesting $2,500 for DNA Barcoding and Sequencing material ($1,500) and Cornell Insect Drawers ($1,000) to curate and house the specimens. These materials will directly support Immaculata undergraduate biology students who will be working on this research project. The insect drawers can last decades and will specifically be used to house Immaculata (*i.e.,* Chester County) insect specimens.

3. Timetable, with anticipated outcomes and their relevance to the nonprofit's mission

Once the grant is awarded, our timeline will be to order supplies within the first month. When our Spring Semester starts (January 2025), we will recruit two students from our majors-level Genetics (BIOL 234) class. It will take one month to train students to curate insect samples and perform the DNA extraction/PCR protocol. Each student and both principal investigators of this grant will spend at least two hours a week on the project throughout the spring and following fall semesters. Once trained, the students will process at least a dozen specimens per week, or about 50 specimens per month.

The main outcome of this project will be a preliminary species list of insects that occur on Immaculata's campus. It is anticipated that this species list will continue to grow the longer this project continues throughout the years. Eventually when enough specimens and species have been identified, a data paper can be published and updated to the Global Biodiversity Information Facility (GBIF, 2024) for other scientists to use these data points for their own research projects. It is very possible to discover new county records, particularly with insect species that are not studied and documented very well.

Lastly, this project will directly contribute to Immaculata University's mission with "scholarship," "seeking truth," and "leadership." Our students will be taking the lead on this project by learning more about the natural resources on campus and discovering which local species of insects occur here. Students will advance their own education by learning skills such as DNA barcoding, taxonomy, collection management, ecology, natural history, and conservation.

4. How impact and results will be demonstrated

a. Provide Chester County Community Foundation with a species list of the insects we find on Immaculata University's campus.
b. Student-lead research poster presentations
c. Be available to present our results to the Chester County Community Foundation and/or public.

Current Progress:
There are already set and collected eight pitfall traps in Immaculata University's forests in our Ecology and Biodiversity (BIOL 307) classes. Pitfall traps are commonly and effectively used to collect surface-active insects (Mason et al. 2023). Each of these traps literally contain hundreds of insect specimens that are already prepared to be identified. Additionally, hundreds of other insect specimens have been collected using sweep-nets, which target flying insects. The insects that are relatively easy to identify, mostly the butterflies and ground beetles (Coleoptera: Carabidae), have been determined to species already. Most importantly, we have two students who recently learned the DNA barcoding technique and have used this skill to begin identifying the ants from the aforementioned pitfall traps. This led to a local poster presentation in Spring of 2024 entitled "Using DNA Barcoding to Analyze Ant (Hymenoptera: Formicidae) Biodiversity on Immaculata University's Campus (Supplemental Data 1).

Estimated Budget:

Company	Description	Cost	URL
Qiagen	QIAwave DNA Blood & Tissue Eco-friendlier Kit (250) (Cat# 69556)	$1,000	https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/dneasy-blood-and-tissue-kit?catno=69556
Insect Storage	Cornell Drawer, Museum Grade (two 6 packs)	$1,000	https://ecologysupplies.com/products/cornell-drawer/?srsltid=AfmBOoqAczu6ZbL1EM6Dnlwhv2LPiPPsVau1pzArDkGgP0BOuoU_Bn8v
Azenta	Sanger Sequencing	$500	https://clims4.genewiz.com/SangerSequencing/SimpleOrder
Estimated Total		$2,500	

Challenge Component:

Dr. Kelly Orlando and Dr. Steve Mason along with two students will dedicate a total of 240 hours of their time towards this project.

	Hourly Rate	Total Hour	Challenge Component
Faculty	$30	120	$3,600
Students	$9	120	$1,080
Total			**$4,680**

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Using DNA Barcoding to Analyze Ant (Hymenoptera: Formicidae) Biodiversity on Immaculata University's Campus

Author: Brianna Hong & Diana Geditz
Faculty/Mentor: Dr. Kelly Orlando & Dr. Stephen Mason

IMMACULATA UNIVERSITY

Abstract

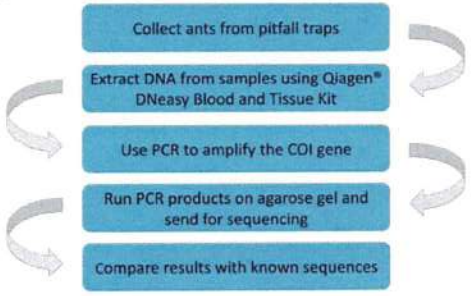
There are approximately 16,000 ant (Hymenoptera: Formicidae) species in the world. **Immaculata University can hold up to 100 species of ants**, however, there has yet to be a systematic insect study for the campus. We start this study by using DNA barcoding to determine the different ant genera and species that have formerly been collected at Immaculata. Ant specimens have been collected from pitfall traps by Immaculata's 2022 Biodiversity and Ecology class. We extracted DNA from 11 specimens and used PCR to amplify the COI gene, and ran the PCR products on agarose gels for verification. We sent our samples for sequencing and compared them to known sequences to identify the ant species. We first used the whole organism for DNA extraction, but as we perfected the DNA extraction technique we determined we could extract DNA from one ant leg so that we could keep the rest of organism to confirm its species and potentially pin for long-term storage. We have been able to sequence and identify **four ant species** from the pitfall trap: Winnow Ant (*Aphaenogaster rudis*), Ferruginous Carpenter Ant (*Camponotus chromaiodes*), Wood Ant (*Formica subaenescens*), and Winter Ant (*Prenolepis imparis*). We will **continue** to use DNA barcoding methods to learn more about the ant biodiversity for Immaculata University.

Introduction

- Biodiversity is the amount of different species in the world.
- Having a large amount of biodiversity is important for healthy ecosystems; specifically, different ant species interact with and support many plants, animals, fungi, and microbes (Biodiversity, n.d; Parker & Kronaur, 2021).
- Ants are important for underground processes that alter the physical and chemical environments which affect plants and other micro and soil organisms (Folgarait, 1998).
- It can be difficult to determine different ant species solely based on their morphology, so DNA barcoding can be used instead.
- DNA Barcoding is a process used for species identification in which DNA is extracted, a specific gene is amplified through PCR, the sequence is analyzed, and then compared to reference sequences. DNA barcoding has been used for identifying ant species in the past (Siddiqui et al., 2019).
- We can use the genetic sequence of the mitochondrial gene COI (cytochrome c oxidase subunit I) from each of our samples and compare it to similar sequences of known organisms to identify the species.

Methods

Figure 1. Description of methods. For the majority of the samples, we used a mortar and pestle to break up the sample while extracting the DNA.



Results

Table 1. Four species of ants have been identified. All 11 samples were collected in a pitfall trap between 9/23-9/30, 2022 on Immaculata University's campus by the 2022 Biodiversity and Ecology class. The habitat was on an edge of a broad-leaf, deciduous forest. Elev 198m. (M&P = used mortar and pestle during DNA extraction). Bugguide.net was used to find species common name and representative image.

Genus	Species	Sample	Description of Sample	Common Name	Accession #	Alignment length	Bit Score	e	Mismatches
Aphaenogaster	*rudis*	4	leg- M&P	Winnow Ant	KP730107.1	381	535	6.00E-149	34
Camponotus	*chromaiodes*	15	leg- M&P	Ferruginous Carpenter Ant	MN078209.1	679	1225	0	0
Camponotus	*chromaiodes*	12	leg- M&P	Ferruginous Carpenter Ant	MN078209.1	680	1219	0	0
Formica	*subaenescens*	7	leg- M&P	Wood Ant	MK037284.1	681	1215	0	3
Prenolepis	*imparis*	3a	Entire insect - m&p	Winter Ant/ False Honey Ant	MZ603102.1	603	1051	0	10
Prenolepis	*imparis*	5	leg- M&P	Winter Ant/ False Honey Ant	MW280444.1	657	1126	0	13
Prenolepis	*imparis*	8	leg- M&P	Winter Ant/ False Honey Ant	MW280444.1	681	1191	0	5
Prenolepis	*imparis*	10	leg- M&P	Winter Ant/ False Honey Ant	MW280444.1	679	1196	0	2
Prenolepis	*imparis*	9	leg- M&P	Winter Ant/ False Honey Ant	MW280444.1	683	1229	0	1
Prenolepis	*imparis*	13	leg- M&P	Winter Ant/ False Honey Ant	MW280444.1	686	1233	0	1
Prenolepis	*imparis*	3b	Entire insect - no m&p	Winter Ant/ False Honey Ant	MW280444.1	687	1227	0	1

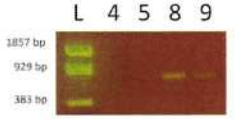


Figure 2. Sample gel showing PCR products from DNA extracted from ants. L = DNA ladder, pBR322/BstNI. PCR product is approximately 700 bp. Sample 4 was not visible with SYBR staining, suggesting there was less PCR product, and for this sample only the forward sequencing reaction provided a sequencing result.

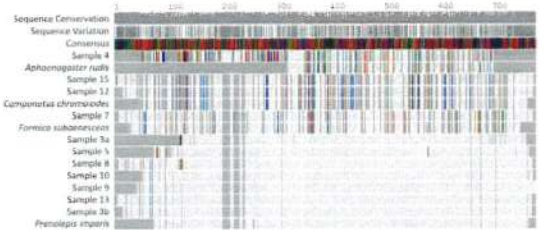


Figure 3. DNA Barcode for samples and known species. Each colored vertical line represents a mismatch compared to the consensus sequence. Similar patterns of colored lines are seen in samples that are most similar to each other; for example, samples 12 and 15 match the *Camponotus chromaiodes* reference sample.

Figure 4. Representative images of the four different species of samples collected.

- We were able to extract DNA from whole ants as well as from a single limb of an ant (Table 1, Figure 2).
- We were able to get sequences even for samples that showed no visible bands on the gel (Figure 2, Figure 3).
- We found four different ant species within our samples (Figure 3, Figure 4).

Conclusions

We were able to extract and sequence DNA in both whole ants and single legs, even when bands in the gel were not prominent. From the samples chosen, we found **four ant species**: Winnow Ant (*Aphaenogaster rudis*), Ferruginous Carpenter Ant (*Camponotus chromaiodes*), Wood Ant (*Formica subaenescens*), and Winter Ant (*Prenolepis imparis*). We will continue to use DNA barcoding methods to learn more about the ant biodiversity for Immaculata University.

Further Research

- Extract DNA from the rest of the ants in the pitfall traps (and re-run the PCR for samples that did not give accurate results in the first sequencing round)
- Potentially extract DNA from other organisms from the pitfall traps.
- Set up pitfall traps ourselves to collect ants around campus.
- Learn to pin the ant samples from the pitfall traps.

Acknowledgements

We would like to thank the Department of Natural Sciences for allowing us the opportunity and providing us with the resources to work on this project. We would like to especially thank Dr. Orlando for guiding us through the process along with Dr. Mason and the 2022 Ecology class for providing us with the samples to work with.

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Supplemental Data 1: