The Confluence

Volume 1 | Issue 1 Article 3

August 2021

# Barcoding US Ants: Ozarks Region - Research Highlight

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## Introduction

There are more than 12,000 ant species known to science (Bennett & Ellison, 2021) and more yet to be discovered. Ants play many important ecological roles including soil aeration, pollination, and seed dispersal (Bennett & Ellison, 2021). In their food webs, they eat a wide variety of organic matter and serve as food for birds, mammals, amphibians, other arthropods, and even pitcher plants. Ants are ubiquitous in ecosystems all around the globe, yet we know little about their species distributions or dispersal patterns (CSHL, 2020). Mapping ant distributions, using DNA sequencing, allows scientists to understand their preferred habitats and observe their patterns of movement. This can help us better understand how climate change is affecting species distributions and will also allow scientists to predict future changes in ecosystems. Additionally, contributing DNA samples to GenBank (NIH, 2020) increases our ability to identify specimens collected in the future accurately by comparing DNA sequences. We collected ants for the Barcoding US Ants Project, a citizen science project run by the DNA Learning Center at Cold Spring Harbor Laboratory that aims to map ant diversity across the country (CSHL, 2020).

## Methods

### Specimen Collection

We used Wikipedia and AntWiki to determine habitats of target ant species. We sampled ants in St Charles city and county parks in forest leaf litter, on bark and branches of trees and shrubs, in soil and herbaceous vegetation in grassy meadows, and inside of decomposing wood. To maximize species diversity of specimens, we collected ants using a variety of techniques, including aspiration from surfaces, breaking apart decomposing logs, baiting traps with canned cat food and pecan sandy cookies, and knocking ants from foliage onto a canvas sheet. Each sample was numbered and the type of habitat, location, and collection method were recorded. Collected specimens were freeze-killed and preserved in 70% ethanol for later identification and DNA extraction.

### DNA Extraction and Sequencing

Ant extraction techniques followed protocols determined by the DNA Learning Center (CSHL, 2020). We removed ants from the ethanol and allowed them to dry for 10 min. We placed a 1/8- 1/4-inch piece of ant tissue in a test tube containing Chelex and grounded the ant tissue for 2 min with a mortar and pestle to break open the cells. The ground ant tissue was incubated for 10 min in boiling water to extract the DNA. We sent the extracted DNA to the DNA Learning Center for sequencing using DNA Subway software developed by CyVerse.

## Results

We collected 8 ant species in St Charles city and county parks **(Table 1)**. Three of our specimens yielded new polymorphisms to GenBank, and three of our specimens were species that are currently not-well represented in GenBank. Polymorphisms are genetic variations within a particular gene for a particular species.

| Specimen ID | Species ID | Habitat | Method of Capture |
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| HNH-001 | *Tetramorium immigrans* | Nesting under dead tree branch | Aspiration |
| HNH-002 | *Camponotus chromaiodes*[1,2] | Sandy gravel path | Aspiration |
| HNH-003 | *Aphaenogaster rudis* | Nesting in rotting log | Break apart logs |
| HNH-004 | *Camponotus chromaiodes*[1] | Nesting in rotting log | Break apart logs |
| HNH-005 | *Formica subsericea* | Tree trunk | Aspiration |
| HNH-006 | *Prenolepis imparis* | Tree leaves | Beat sheet |
| HNH-007 | *Monomorium minimum*[1,2] | Leaf litter in riparian forest | Aspiration |
| HNH-008 | *Camponotus pennsylvanicus* | Tree trunk | Aspiration |
| HNH-009 | *Tapinoma sessile*[2] | Nesting under leaf litter | Aspiration |

**Table 1:** Information on specimens collected in St Charles, MO and submitted to GenBank. [1]Species not well-represented in GenBank. [2]A new polymorphism not previously catalogued in GenBank.

## Discussions

By submitting DNA samples of *Camponotus chromaiodes* and *Tapinoma sessile* with new polymorphisms to GenBank, we have increased scientific knowledge of genetic variation in these species. Similarly, we have increased representation of three species in GenBank, which improves confidence in the use of these sequences to identify future specimens of these species. For all of the specimens we collected, having records of the species in specific geographic locations is important for establishing their current ranges and tracking future range shifts resulting from climate change and habitat loss.

## References

AntWiki. 2020. AntWiki.org.

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