



# Ants' Finding: Identification Of Ant Species Using DNA Barcoding

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## Abstract

This project is about whether the ant-like ants are ants and ant-non-like ants are ants. I collected totally 17 samples in VAEI and 4 samples in DNALC, and used Silica method to get the DNA from the insects, PCR method the amplified the col part, sequenced and got the results. At last, I found that not all ant-like ants are col to ants.

## Introduction

In daily, it is hard for people to make sure the classification of an unknown insect. My question whether the ant-like ants are ants and ant-non-like ants are ants because through research I found not all ant-like insects are ants such as termites and Plecoptera (stone flies).

A group of fire ants		Scientific classification	
Kingdom:	Animalia	Kingdom:	Animalia
Phylum:	Arthropoda	Phylum:	Arthropoda
Class:	Insecta	Class:	Insecta
Order:	Hymenoptera	Cohort:	Polyneoptera
Superfamily:	Formicoidea	Superorder:	Dictyoptera
Family:	Formicidae	Order:	Blattodea
	Latreille, 1809	Infraorder:	Isoptera

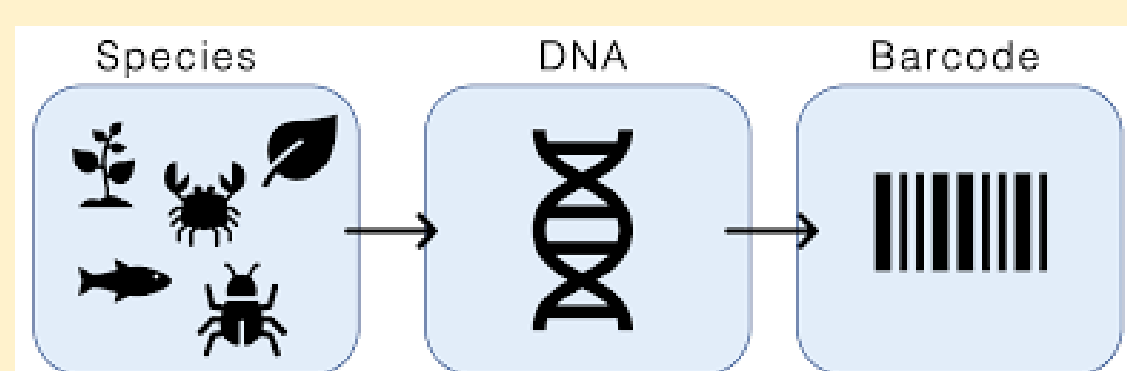
Figure 1: Ants' classification. We know that the Family of ants is *Formicidae*.

Figure 2: Termites' classification. We know that Termites are not ants because they are belongs to *Dictyoptera*.

Scientific classification	
Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Insecta
Subclass:	Pterygota
Branch:	Metapterygota
Infraclass:	Neoptera
Superorder:	Exopterygota
Order:	Plecoptera
	Burmeister, 1839

Figure 3: Plecoptera's classification. We know that they are not ants because they are belongs to *Exopterygota*.

Image 8: The process of DNA Barcoding



## Materials & Methods

In sample collection, Sample collection lasted for seven days. To collect, I put cracked biscuit (Qu Duo Duo) on the paper on the ground (about -4 altitude) from 8am to 9am, trying to catch ants. Totally, I got 15 ants (001,002,003,004,005,006,008,009,010,012,013,014,015,016,020,021), one spider-like insect (018), an unknown one (011). In DNALC, I got a butterfly-like one (007) and two bee-like ones (017,019). My sample collection has 21 samples total. During the week, after insects were stuck on the board, I poured alcohol into the small tube and put insects inside of it for storage. After one week, I took all the tubes to DNALC, but there's a problem. Cause the alcohol would evaporate, the label of first time would disappear, so that the location's information couldn't match to each sample. However, the lucky thing is that I found the problem on time so that I dry the surface and label again according to the faint number. And then, I put part of the body on petri dish using the way of amputation.

- Material:
- insects' samples
  - solution: wash buffer, lysis, PCR, Deionized water, silica resin and gel.
  - DNeasy Kit method: Buffer AL, Buffer ATL, ethanol, Buffer AW1, Buffer AW2, proteinase K microcentrifuge tube and 2ml collection tube.

- Method:
- DNeasy Blood&Tissue Kit method
  - Neasy Blood & Tissue Kits are designed for rapid purification of total DNA
  - Silica Method
  - Gel electrophoresis

Figure 10 silica method  
Gel electrophoresis is a technique used to separate DNA fragments (or other macromolecules, such as RNA and proteins) based on their size and charge. Electrophoresis runs a current through a gel containing the molecules, and the molecules will travel in different directions and speed based on the size and charge.

- DNA Barcoding

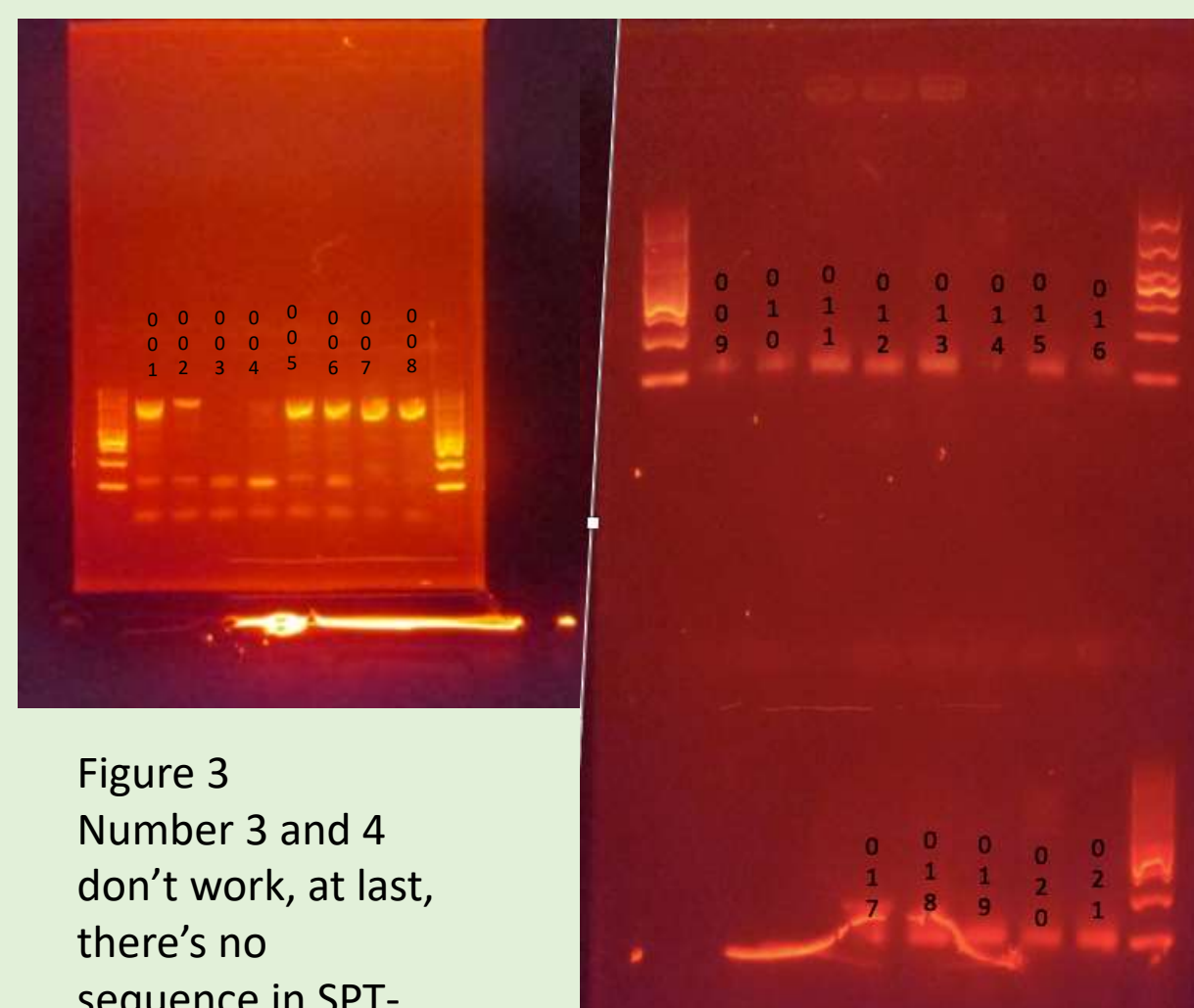


Figure 3  
Number 3 and 4 don't work, at last, there's no sequence in SPT-003R (Figure 3)



Figure 4  
There's no results in these ones except SPT-018

Figure 5: tree-NJ. We know some generations in SPT-005,006,001,008,002,007,004.

Figure 8 Sample collection Part of the Collections

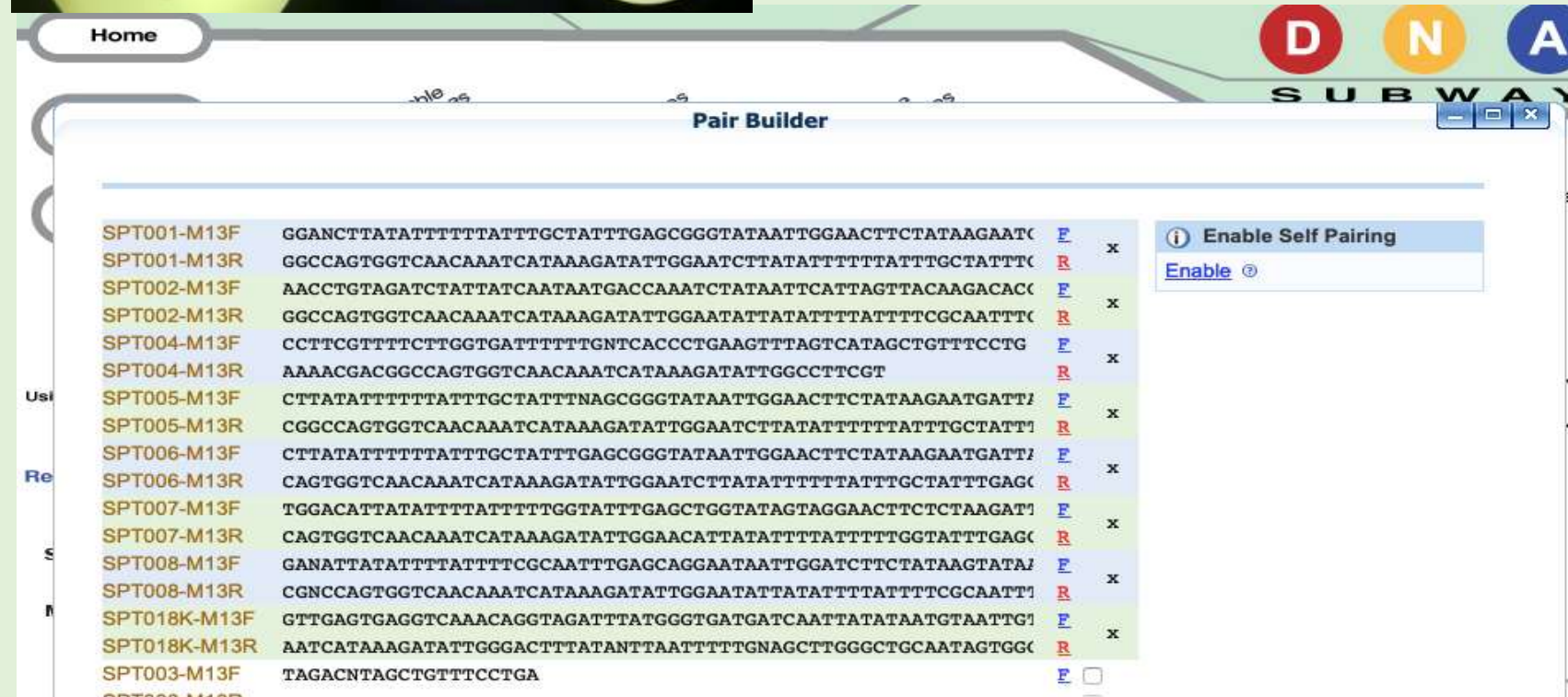


Figure 7 SPT001,002,004,005,006,007,008,018 has automatically pair in groups.

## Results

- In figure 3, I obtained PCR products for SPT-001, 002, 005-008, and I saw that SPT-003 and SPT-004 have no PCR results.
- In figure 7, I obtained Quality Sequence for SPT001,002,004-008,018, and I saw that there's no sequence in SPT-003R, and less sequence in SPT-003F, and the SPT-018 luckily survive.
- In figure 5(tree NJ) and 6(tree ML), I see that SPT-001, SPT-005 and SPT-006 are belong to *Nylanderia burbonica* (robust crazy ants), and SPT-007 is belong to *Amata fortunei* (not ants) and SPT-008 and SPT-002 are belong to *Pheidole noda* (ants), and SPT-018(not ants), and SPT-004 is most similar to *Eukrohnia hamata* (not ants).
- SPT-001,005,006 are *Nylanderia burbonica* called *robust crazy ants*, and they are *invasive species around world that damage banana crops*.
- SPT-004 *Eukrohnia hamata* is found in China.
- SPT-007 *Amata fortunei* is kind of moth from native Japan, and it may have been carried to DNALC by a recent typhoon.
- SPT-008 and SPT-002 are *Pheidole noda* from North-East China and Jiangsu Province.



Figure 11  
Where are *Pheidole noda*



Figure 12 where are *Amata fortunei*

## Discussion

The first problem is when collecting the sample, because the cold weather, there are fewer ants, so I choose to catch them, not waiting.

When analyzing the agarose gel the second time, I found there were no results from the PCR reaction. I repeated this step again and there were still no results. This suggests there are problems with the DNA extraction step., and these also use the DNeasy Kit method.

Another reason may be that PCR works not so well on insects. It may be that the primer is not able to bind to the DNA because of sequence difference. Also, some insects have GC rich regions of DNA that are difficult to amplify by PCR.

In the experiment based on research, I know that all not ant-like insects are belongs to ants(*Formicidae*), and I found *Nylanderia burbonica* ( crazy ants), *Eukrohnia hamata* (from native Chinese), *Amata fortunei* (from native Japan) and *Pheidole noda* from North-East China, Zhejiang and Jiangsu Province.

## References

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