

## Introduction



Photo credit: Planet Voice

Earthworms are soil engineers that can impact the structure and chemistry of the soil. Earthworm secretions can change soil pH; earthworm waste can add significant amounts of nitrogen and phosphorus to the soil; and earthworm movement through the soil creates paths for new plant roots to penetrate deeper in the soil. Earthworm species have preferred habitat characteristics which include vegetation, soil moisture and amount of sunlight. This project focuses on the impact of proximity to water on the biodiversity of earthworms



Figure 1. Location of collection (Photo credit: Google maps)

Adjacent to pond is the area of collection for the first 10 samples and the soccer field is the second collection site for the last 4 samples. It was expected that there would be more biodiversity in the pond area.

## Methods and Materials

### Sample Collection

14 earthworms were collected from two locations on the Shoreham-Wading River High School campus. Small shovels were used to collect worms from just below the grass layer in each location. The worms were carefully cleaned off with water, photographed, then stored in the freezer for later identification. Identification of species was done using field guides based on color, size, and banding patterns.

### DNA Barcoding

DNA was extracted from the tissue sample, and a small portion of the COI gene was amplified by PCR. The amplified DNA was confirmed through gel electrophoresis, then sent out for sequencing in both directions.

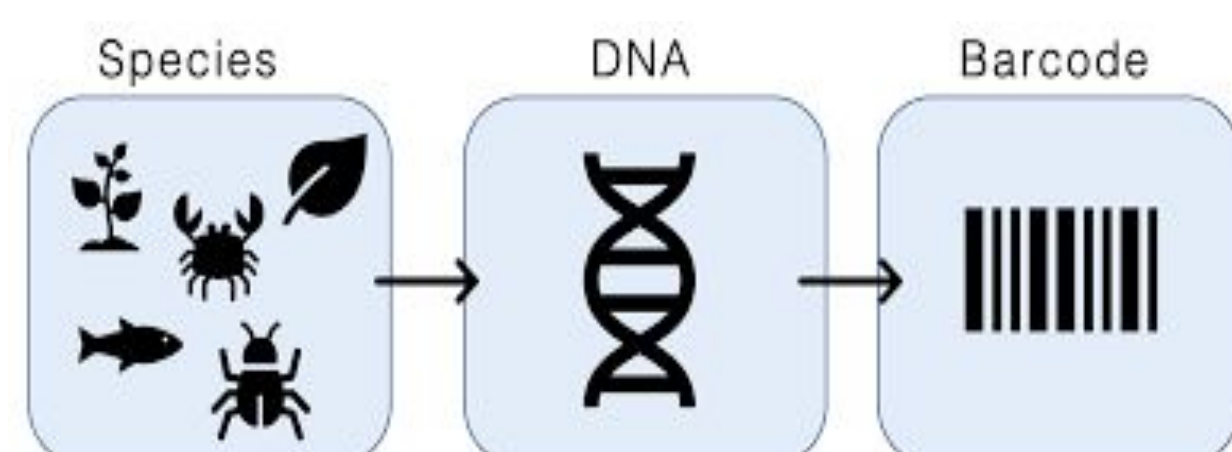


Photo credit: Wikipedia

## Results

Number	Scientific Name	Common Name	Genetic ID	Location
CYB1	<i>Anecic</i>	Deep-Burrowing earthworm		pond
CYB2	<i>Lumbricus terrestris</i>	Nightcrawler		pond
CYB3	<i>Aporrectodea longa</i>	anecic worms	<i>Aporrectodea sp.</i>	pond
CYB4	<i>Octolasion cyaneum</i>	Blue-gray worm		pond
CYB5	<i>Aporrectodea rosea</i>	Rosy-tipped worm	<i>Aporrectodea caliginosa</i>	pond
CYB6	<i>Aporrectodea rosea</i>	Rosy-tipped worm		pond
CYB7	<i>Lumbricus rubellus</i>	Red head worm		pond
CYB8	<i>Allolobophora chlorotica</i>	Green worm		pond
CYB9	<i>Octolasion cyaneum</i>	Blue Gray worm	<i>Aporrectodea caliginosa</i>	pond
CYB10	<i>Octolasion cyaneum</i>	Blue-gray worm		pond
CYB11	<i>Octolasion cyaneum</i>	Blue-gray worm		Soccer Field
CYB12	<i>Octolasion cyaneum</i>	Blue-gray worm		Soccer Field
CYB13	<i>Lumbricus rubellus</i>	red worm		Soccer Field
CYB14	<i>Allolobophora chlorotica</i>	Green worm		Soccer Field

Table 1: This table shows where our worms were collected what their scientific and common names and their ID number.

	Qualitative chemical data
<b>pH</b>	Pond- Neutral/Slight acid Soccer field - Neutral
<b>Potash</b>	Pond- N1 Deficient/Depleted Soccer field - Depleted/Sufficient
<b>Nitrogen</b>	Pond K3 sufficient/depleted soccer field K4 sufficient
<b>Phosphorus</b>	Pond P4 surplus Soccer field P4 surplus

Table 2: The table shows the soil samples which we collected our worms from. We used a soil test kit in which we found the Nitrogen, Potash, pH, and Phosphorus qualitative chemical data in the soil.

## Earthworm Photos

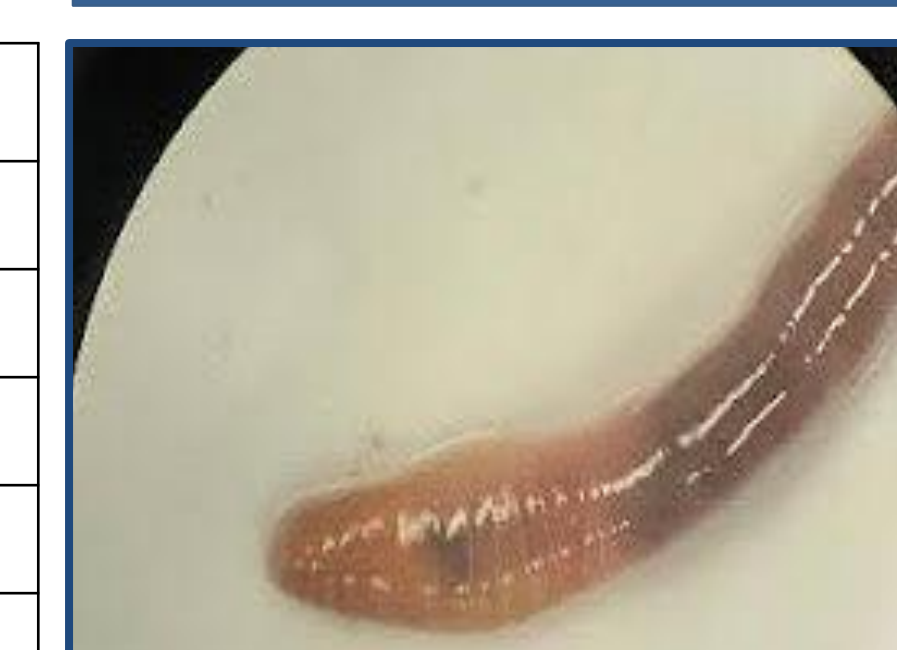


Figure 1: Blue-Gray worm in Petri dish under microscope (predicted species *Octolasion*) Photo credit: student researchers Worm:(CYB10)



Figure 2: *Octolasion cyaneum* Photo credit: Hampshire & Isle of Wight Wildlife Trust



Figure 3: Blue-Gray worm in Petri dish under microscope (predicted species *Octolasion*) Photo credit: student researchers Worm:(CYB4)

## Discussion & Conclusions

The pH comparison between our two locations is around 6.5-7.0 which shows that both soils are neutral. Worms enjoy soil that is a neutral non acidic pH. The phosphorus is both in surplus for our locations. Phosphorus helps the new tips of plants grow. In the end we found a greater biodiversity of our worms in our pond area. We believe that the reason for the greater biodiversity in earthworms in our pond area is because of having a more moist soil that worms have been shown to preferably enjoy in order to confirm this we would need to confirm the soil moisture. We have found out that the best NPK (Nitrogen, Potash, Phosphorus) should be a ratio of 4:2:1. Still we found a greater biodiversity on the pond even though the soccer field has a better overall soil content.

## DNA Barcoding Results

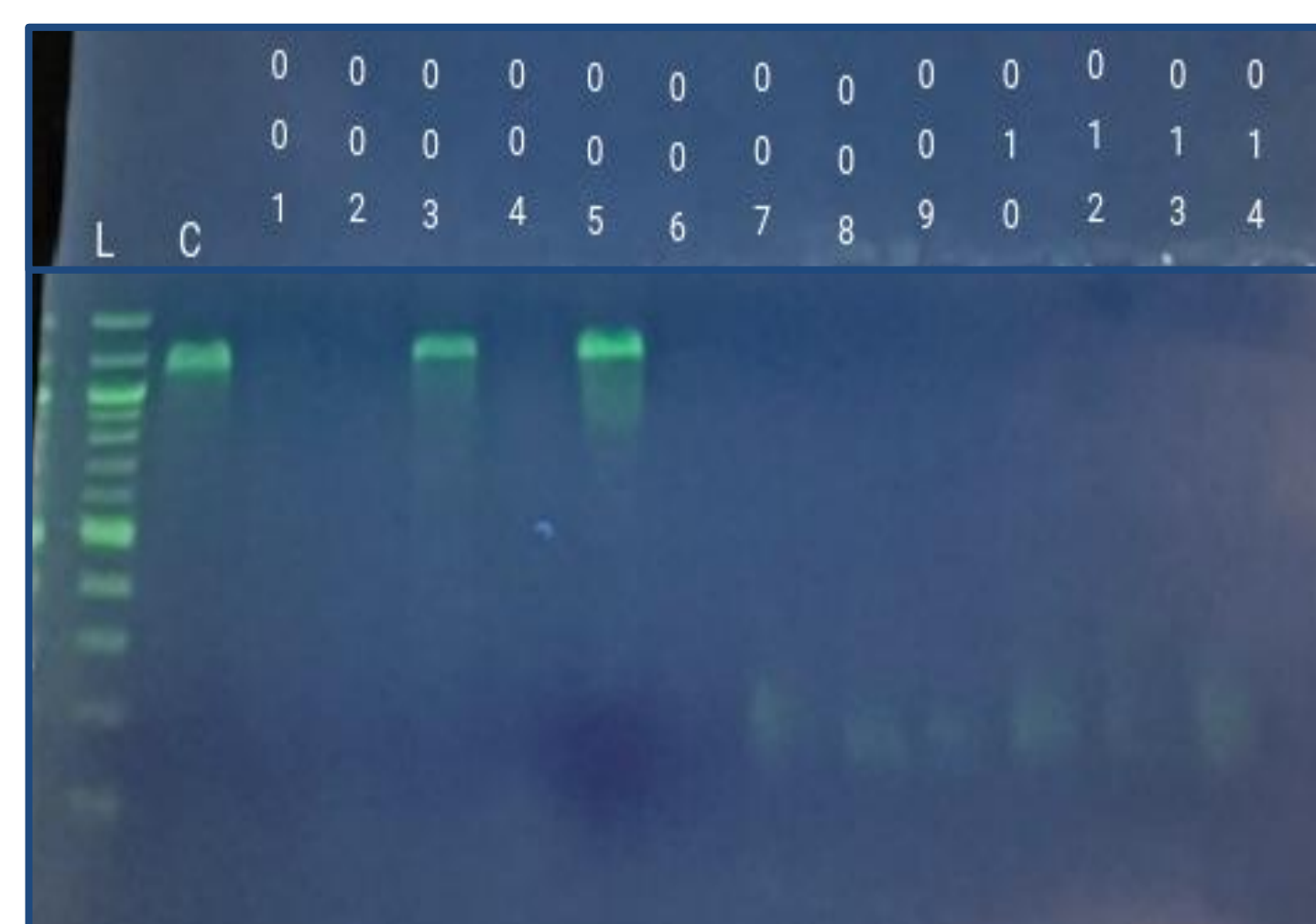


Figure 5: Gel electrophoresis of extracted and amplified earthworm DNA. 003 and 005 were sent out for sequencing.

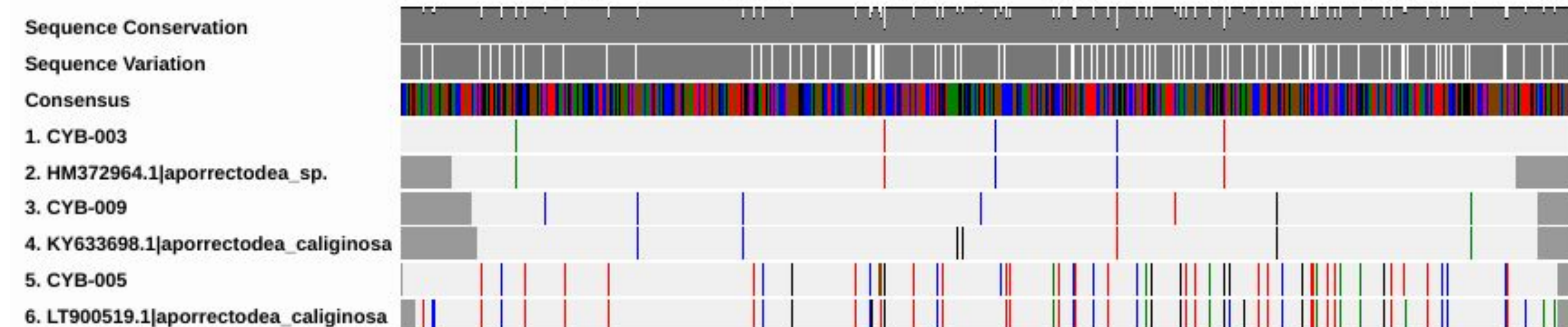


Figure 6. Sequence alignment from samples 3, 5 and 9

## References

DNA learning center barcoding 101. (n.d.). <https://dnabarcoding101.org/programs/bli/>  
 Le Bayon, R. C., Bullinger, G., Schomburg, A., Turberg, P., Brunner, P., Schlaepfer, R., & Guenat, C. (2021). Earthworms, plants, and soils. *Hydrogeology, chemical weathering, and soil formation*, 81-103.  
 Mariko Whyte Trainee Ecologist. (2018, July 31). *What can worms tell us about our soils?*. Hampshire and Isle of Wight Wildlife Trust.  
 Nitrogen cycle | soils 4 teachers. (n.d.). <https://www.soils4teachers.org/nitrogen-cycle>  
 Naas. (n.d.-a). <http://naas.org.in/Policy%20Papers/policy%20114.pdf>