

## Ant Biodiversity Within the Holtsville Wildlife and Ecology Center Serving as an Indicator of Environmental Pollutants

Carlo Costigliola

### Abstract

The purpose of this study was to examine the physical and genetic variations of ants at a former landfill, the Holtsville Wildlife and Ecology Center (HWEC), to infer if the biodiversity of these organisms, and thus the site, has been influenced by potential pollutants present at the center that could harm humans. Methods of phenotypic and genotypic analysis of the ants were used to understand species diversity at the HWEC. Ants are known as an indicator species, which made them viable organisms for use in this study and to view the impact of pollutants on human health. It was hypothesized that the presence of pollutants at the HWEC would be signaled by low ant biodiversity at different collection locations since biodiversity corresponds to ecosystem stability. Ants were collected from three distinct locations at the HWEC. Out of twenty-seven collected ants, twenty were selected for analysis. Using a silica extraction method, DNA was isolated and sequenced from these specimens. After sequencing, ten samples were removed from consideration due to poor results. Sequencing the ants from site 1 resulted in the species *Tetramorium caespitum* to be found. Ants from site 2 were the most diverse, containing the species *Lasius alienus* and *Lasius neoniger*. At site 3, ants returned as the species *Tetramorium immigrans*. Considering the entire HWEC's biodiversity, the presence of four species of ants suggests high biodiversity, indicating overall stability. Since approximations of this stability came from ant biodiversity measurements and ants are described as excellent bioindicators, it is defended further, supporting the conclusion that there are no harmful pollutants in the center. This refutes this paper's hypothesis, relieving concerns that the status of this specific center (HWEC) as a landfill-turned-park could cause human health issues, and illustrating that ant biodiversity can be indicative of pollutant presence at former landfills.

### Introduction

The status of ants as an indicator species, a species that is representative of the potential effects of the conditions of the environment it lives in, means these organisms are favorable selections for viewing the impact of pollutants and harmful substances on human health. By using ants as an indicator species, the biodiversity of different locations of inferred variation in pollutant concentrations can be measured. The purpose of this study is to examine the physical and genetic variations of ants at a former landfill, the Holtsville Wildlife and Ecology Center (HWEC), to infer if the biodiversity of these organisms, and thus the site, has been influenced by potential pollutants present at the center. Ant genomes were sequenced using DNA barcoding technology and analyzed, allowing for inferences on biodiversity to be made (Marizzi et al., 2018). Ants themselves have been widely advocated as indicators of ecological change because they are highly abundant, easily sampled, and closely connected with ecosystem function, with their responses to habitat disturbance better understood than those of other invertebrates (Lawes et al., 2017).

The HWEC in Holtsville, New York, is a large park in the Town of Brookhaven. Once a landfill, the area was rehabilitated by the local highway department after the landfill's closure in

1974 (Town of Brookhaven, NY<sup>2</sup>, n.d.). The HWEC's past presents concerns regarding its long-lasting effect on the surrounding environment, and, interestingly, its status as a landfill-turned-park on Long Island is not exclusive. At the former Merrick landfill, the Norman J. Levy Park and Preserve, a 50-acre plant and wildlife sanctuary opened in 2000, is now present (Town of Hempstead, NY, n.d.). However, in Merrick, where the park is located, the non-profit organization Environmental Working Group found harmful water contaminants, including carcinogens like hexavalent chromium, radium, and haloacetic acids (Environmental Working Group, 2021). These chemicals in Merrick's water, where a landfill was converted into a park over 20 years ago, have led to concern that the landfill could have had long-lasting negative environmental effects on its surrounding area. Consequently, although the Holtsville landfill was converted into a park 25 years earlier than the Merrick landfill, it can be inferred that similar long-lasting environmental effects are present at the HWEC since the location is two times the size of the Merrick location (Town of Brookhaven, NY<sup>2</sup>, n.d.). Thus, the former Holtsville landfill, now the HWEC, could still have similar harmful substances to the Merrick location in its environment. This provides reason to measure the area's biodiversity, and therefore the potential of any harmful substances that could adversely affect humans, with the aid of ants, an indicator species. Thus, this paper will respond to the following question: To what extent does the biodiversity of ants, an indicator species, reflect the presence of pollution and harmful substances in a former landfill?

## Literature Review

In the literature review to follow, the status of ants as an indicator species and significance of ant biodiversity measurements, the presence of environmental pollutants at former landfills, the phenotypic and genotypic analyses of ants, and the relationship these factors have with inferring the existence of pollutive substances at a landfill-turned-park will be discussed. The contribution of physical comparisons between ants (phenotypic analysis) and DNA barcoding (genotypic analysis), particularly for the measurement of biodiversity, will be investigated to determine how ant biodiversity is affected by pollutant presence.

### **Ants as an Indicator Species and the Significance of Ant Biodiversity**

Understanding the status and use of ants as indicator species is vital when considering how the effects of pollutive substances on human health can be inferred via measurements of ant biodiversity. An indicator species is a species that reflects the environmental conditions of where it lives, being used to assess an ecosystem's responses to these conditions (Andersen, 1997). The use of ants as an indicator species is reflected in several studies, where ants have been analyzed phenotypically to indicate pollution. In one study, ants were found to have smaller head sizes as pollution levels increased. This meant that ants acted as indicators of their polluted environment through their physical traits, allowing them to be called an indicator species (Grześ et al., 2014). Other studies similarly analyzed the physical traits of ants, finding that certain ant species responded to heavy metal pollution with decreased body mass and decreased melanization (darkening) of the head. Since pollution affects the physical characteristics of collected ants, the role of these organisms as bioindicators is again highlighted (Skaldina et al., 2018).

The classification of ants as an indicator species is further reinforced by detailed descriptions of the evidence behind this classification that has been presented in past research.

Tiede et al. (2017) explained that for ants, data on their abundance, species composition, and richness of functional traits demonstrates the status of an entire ecosystem. Likewise, researchers have also expressed that because ants are widespread, abundant, sufficiently sensitive, tolerant, and highly involved in food chains, they are proper indicators of an environment's conditions (Skaldina et al., 2018). Once more, ants have been deemed viable bioindicators due to their abundance, ease of sampling, close connection to ecosystem function, and their responses to habitat disturbance that are more understood than other invertebrates (Lawes et al., 2017). Based on the common evidence presented in these studies, the abundance and key role of ants in different environments certainly allows them to be classified as an indicator species, while other pieces of information, such as species composition and ease of sampling, also aid in cementing this role (Lawes et al., 2017; Skaldina et al., 2018; Tiede et al., 2017).

Consequently, the measurement of ant biodiversity can be used to signify the stability of environments, as ants are described as ecological indicators. Researchers assert that biodiversity relates to the stability of ecosystems and that losses in biodiversity cause losses in stability (Tiede et al., 2017). Moreover, this stabilizing effect of biodiversity is crucial, as it suggests biodiversity is critical to the long-term sustainability of ecosystems facing environmental change (Loreau & De Mazancourt, 2013). Since ants are classified as bioindicators, ant biodiversity therefore represents an ecosystem's biodiversity, which can be measured to evaluate the stability of this same ecosystem. Even further, biodiversity can be used to infer the presence of pollutants that alter stability in these environments, which is concerning since these substances could be harmful to humans.

Areas of interest when considering the potential presence of pollutants include former landfills or landfill-turned-parks, which will be subsequently discussed.

### **Presence of Environmental Pollutants at Former Landfills**

Former landfills are areas of interest for measuring biodiversity, as there is a potential presence of environmental pollutants in these areas. Various researchers have shown that former dumps still contain some degree of harmful substances. Díaz Rizo et al. (2011) documented that, in a former landfill in Cuba, heavy metal detection was conducted, and certain heavy metals, such as cobalt, zinc, and lead, were found to be above the permissible level for agriculture to be performed. Similarly, de Rosa et al. (1996) recorded the presence of heavy metals and leachate, or liquid pollution that seeps through a landfill when it rains or snows, in a former landfill's environment. These studies demonstrate how environmental pollutants can still be found in former landfills even when they are not in use. Further, Pukkala & Pönkä (2001) describe how in Finland, houses were constructed in the 1970s near a former dump that contained industrial and household waste, and after analysis of results, it was noted that the relative risk of disease increased with the number of years lived in the measured area, with contracted diseases including cancer and asthma. Moreover, the Wisconsin Division of Public Health (2001) asserts that common contaminants found at former landfills pose a risk for harmful diseases like cancer. The potential presence of pollutive substances at former landfills is thus indicated by these studies through the harmful effects that can occur when humans are exposed to these pollutants. Additionally, Burlakovs & Vircavs (2012) point out past landfills in Latvia that have been used for a variety of disposal techniques, like solid, liquid, and uncontrolled periodic waste disposal. These practices contaminated groundwater and increased

heavy metal, nitrate, and chloride concentrations, indicating how former landfills can be areas where harmful substances are located. A paper by Somani et al. (2020) contributes to this notion as well, detailing how in India, after analysis of former landfills that were 3-5 and 20-25 years old, no significant difference between the waste areas could be found, with similar levels of pollutants present in each waste area, showing how former landfills can contain harmful substances even after extended periods. Lastly, two studies conducted in Brazil comparably defend how former landfills can be polluted areas. A study done by Mendes et al. (2017) explains the presence of groundwater pollution in a city near a former landfill, meaning families in the city cannot drink water from the area due to the increased coliform and nitrate concentrations that make the water dangerous. Moreira et al. (2017) propose similar claims, describing a former landfill where analysis revealed contaminants present in fractured granite. The study also discussed that polluted areas of the former dump were affecting nearby groundwater by flowing into nearby aquifers. By detailing the specific types of pollutants that can be found at former landfills, these studies support how former landfills are areas where environmental pollutants can be present. All of the aforementioned studies, when considered in conjunction with one another, bolster the same idea that former landfills can contain pollutive substances, with examples from the studies including heavy metals, leachate, nitrates, chlorides, and coliforms (Burlakovs & Vircavs, 2012; de Rosa et al., 1996; Díaz Rizo et al., 2011; Mendes et al., 2017; Moreira et al., 2017; Pukkala & Pönkä, 2001; Somani et al., 2020; Wisconsin Division of Public Health, 2001).

With the notion that former landfills can contain pollutive substances supported, it becomes clear that former landfills can be used as research sites to measure the impact of pollutive substances on ant biodiversity and therefore the environment itself. One such site where research was conducted for this study was the HWEC, a former landfill that was rehabilitated after its closure in 1974 (Town of Brookhaven, NY<sup>2</sup>, n.d.). One addition to the area following its closure as a landfill was a composting center, where people may provide leaves to be recycled into compost, turning waste into a gardening additive (Town of Brookhaven, NY<sup>1</sup>, n.d.). This composting center means the HWEC is of greater promise as a research site due to evidence from a 2019 study, in which it is stated that the presence of pollutants in waste composting can produce varying degrees of pollution hazards (Chu et al., 2019). This indicates that because a composting area exists at the HWEC, there may exist greater degrees of pollution if this composting area itself is polluted, which is supported by the notion that former landfills can contain environmental pollutants. Therefore, the HWEC is an even stronger choice of location for biodiversity measurements to be conducted.

Methods of phenotypic and genotypic analysis, which allow for measurements of ant biodiversity, will be described in the following section.

### **Phenotypic and Genotypic Analyses of Ants**

The phenotypic and genotypic methods by which ants can be analyzed allow for measurements of ant biodiversity to be collected. In studies mentioned previously, ants were used as bioindicators due to changes in their physical characteristics resulting from exposure to pollutants, with examples of these effects being smaller head sizes, lighter (less melanized) heads, and decreased body mass (Grześ et al., 2014; Skaldina et al., 2018). With these effects in mind, ants can be phenotypically analyzed to measure the presence of pollutants in an environment, which allows for biodiversity measurements.

Ants can also be analyzed genotypically. DNA is useful for identifying species, as described by Pereira et al. (2008) in their study. Their research contends that DNA is a stable and long-lived biological molecule that can be recovered from biological material that has been subjected to stress conditions. The study also conveys that DNA is found in all biological tissues or fluids with nucleated cells, enabling its analysis from most biological substrates. The study adds that DNA can provide more information than proteins due to the degeneracy of the genetic code and the presence of large non-coding stretches. Therefore, this means that DNA and genetic material can be used to identify species. Multiple methods of genetic analysis exist and have developed as science has advanced. Heather & Chain (2016) explain an early form of DNA sequencing called the dideoxy chain-termination method (Sanger sequencing). The accuracy, robustness, and ease of use of this sequencing technique led it to become a common technology used to sequence DNA after its invention in the 1970s. This method, however, has been surpassed by other methods of sequencing since its creation. A more advanced method of DNA sequencing was employed by Martins et al. (2014) in their study, where they analyzed ants genetically to obtain viable genetic information. The researchers used DNA extraction, PCR (Polymerase Chain Reaction) Amplification, and DNA sequencing to examine the ants. These methods represent the evolution of DNA sequencing technology after Sanger sequencing and the development of genetic analysis methods that lead to better data after the collection and processing of organisms like ants.

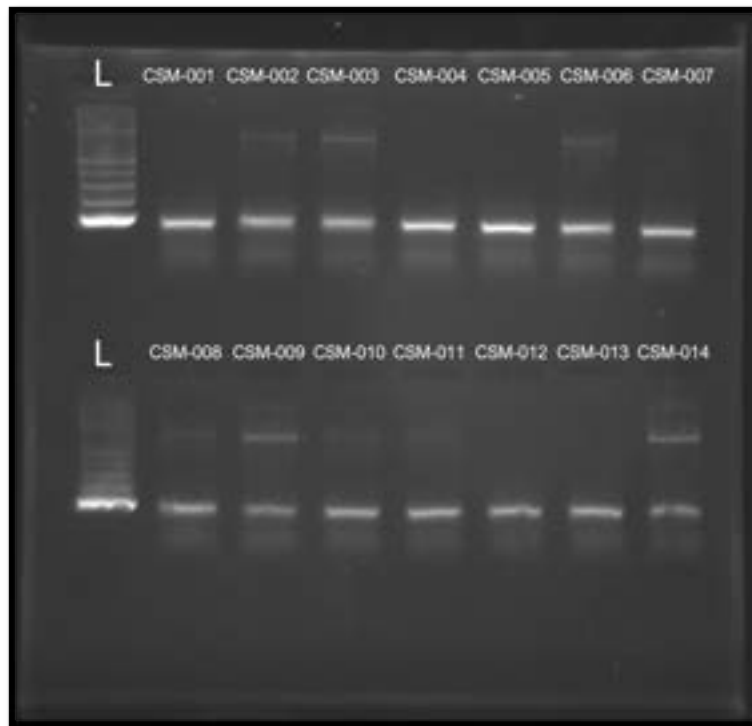
Another advanced method of sequencing is DNA barcoding, an emerging method of species identification that has great benefits and has been used by several researchers in their studies. This method uses information within a single gene region common across all taxa and accesses that information by DNA sequencing under universal conditions. These features mean the method can be standardized across species and labs, thus providing a platform for global exchange of homologous data on genetic databases and capitalizing on community-wide efforts to strengthen a flexible system for this exchange (Armstrong et al., 2005). DNA barcoding's positives indicate its attractiveness as a method of genotypic analysis, which is furthered by other studies. Marizzi et al. (2018) reveal that the accessibility of DNA barcoding technology means students using it can make real contributions to ecosystem assessment with accuracy, as the technology is easy to use. The researchers also detailed that students using the technology in a NYC park identified two unreported species of ants and published twelve novel barcodes in a genetic database called GenBank. The notions in this study are supported by another, where DNA barcoding was used in Costa Rica to genetically analyze ant specimens and add the collected data to databases (Smith et al., 2013). As this other study was conducted in Costa Rica and DNA barcoding technology was still used, the legitimacy and reliability of the technology is strengthened. The study, when considered with Marizzi et al. (2018), underscores the accuracy and ease of use of DNA barcoding technology, again supporting its utilization for ant species identification and measurement of ant biodiversity at chosen locations. It is also important to recognize which method of analysis, phenotypic or genotypic, is more accurate and should be relied on more heavily. A response to this dilemma is presented by Friedheim (2016), who concludes that DNA barcoding and genotypic analysis are the better methods of species identification because of the ease of adding a species to a genetic database for comparison, the older status of phenotypic species identification, and the intricacies of the genetic code. This supports the superiority of genotypic analysis to phenotypic analysis, but not the complete nullification of the latter method of species identification. However, base-pair variations in DNA

sequencing can also occur, signaling potentially inaccurate results. These variations occur due to the incompleteness and lack of accuracy of molecular reference databases, hindering conclusions drawn from sequencing results with these variations (Pawlowski et al., 2018).

This research attempts to study the impact potential pollutive substances have on ant biodiversity measurements at the HWEC, thus indicating the environment's biodiversity. The lack of ant biodiversity data for the HWEC represents a gap in the collected body of knowledge that, when addressed, will further the understanding of the strength of ants as indicator species and the relationship between ant biodiversity and pollution at former landfills. In previous works studied, it is said that the biodiversity of an environment is indicative of that environment's stability (Loreau & De Mazancourt, 2013; Tiede et al., 2017). It is therefore hypothesized that the presence of pollutants at the landfill-turned-park will be signaled by the low biodiversity of ants at the different collection locations. By phenotypically and genotypically examining the ants of the former landfill, their biodiversity can be observed.

### Methods

To answer the research question presented in this paper, an experimental study was implemented to examine the relationship between ant biodiversity and pollution. Twenty-seven samples of ants, later finalized to twenty samples ( $n = 20$  ants) after the removal of samples 4, 7, 15, 18, 19, 20, and 23 from sequencing consideration due to poor imaging, were collected randomly from three different collection sites in the HWEC and tested. When collected, ant samples were taken from each site using Keebler Pecan Sandies Cookies as bait, as research has underscored that the carbohydrate composition of similar cookies acts as successful bait for ants (Kolenda et al., 2020). The cookies were placed in the three sites for two hours before being stored and covered in plastic containers to ensure that larger organisms would not steal them. For the prevention of any damage to collected specimens, ants were stored in a freezer until they were used. Ants were then photographed with a microscopic camera and stored again in a freezer for DNA preservation. DNA from every sample was isolated using a silica extraction method (lab materials identified in Appendix A) and sent to be sequenced. The silica extraction technique utilizes lysis solution to break down tissues in a collected sample, releasing DNA that can then bind to added silica resin in a test tube. This silica-DNA mixture can then be centrifuged and washed with a buffer to remove excess amounts of contamination from the collected DNA (Cold Spring Harbor Laboratory [CSHL], n.d.). PCR Amplification of this DNA then occurs, in which DNA is denatured into single strands by elevated temperatures. After this, the temperature is lowered, allowing primers to anneal, or base-pair, to their complementary sequences. The temperature is then raised slightly, and *Taq* polymerase attaches to each priming site to synthesize a new DNA strand identical to the original strand. This process then repeats, amplifying the DNA samples of collected ant specimens (CSHL, n.d.). After this amplification, gel electrophoresis is used to separate DNA fragments using an electric current that divides smaller DNA fragments from larger ones. Smaller fragments of DNA travel toward the positive pole of the electrophoresis chamber due to the overall negative charge of DNA molecules, leaving larger fragments behind (CSHL, n.d.). A gel electrophoresis of collected ant specimens is shown in Figure 1. Finally, DNA sequences were referenced to the BLAST database to determine the species of each ant through the DNA Subway program. From there, the biodiversity of each site can be examined.



*Figure 1: Gel electrophoresis results of ant specimens 1–14 confirming the presence of species DNA after extraction and amplification.*

The latitude and longitude of each site, all located at the HWEC, were obtained using Google Maps. Site 1, a composting center, is located at 40.798889, -73.034167; site 2, an exercise pathway, at 40.800000, -73.032500; and site 3, a construction pathway, at 40.801944, -73.037222. All locations are shown in Figure 2. As described in the review of the collected body of knowledge, if waste pollutants are present in the composting center while materials are composting, varying degrees of further pollution can be created (Chu et al., 2019). This means that sites 2 and 3 can be considered control locations in comparison to site 1. In terms of which sites ants were collected from, samples 1–8 were from site 1, samples 9–17 from site 2, and samples 18–27 from site 3.



*Figure 2: The three collection sites at the HVEC*

## Results

After the sequencing of samples, the following samples were not processed correctly and were removed from the analysis of the data: Samples 1, 2, 5, 6, 8, 10, 11, 12, 13, and 25. The remaining samples (3, 9, 14, 16, 17, 21, 22, 24, 26, and 27) were run through the MUSCLE sequence alignment software provided by the DNA Subway program and examined to find the closest match to a logged species in the BLAST database. The following figure is used to show which sequence each sample was matched most closely to, showing all base-pair variations. Each sample is labeled with its site location.



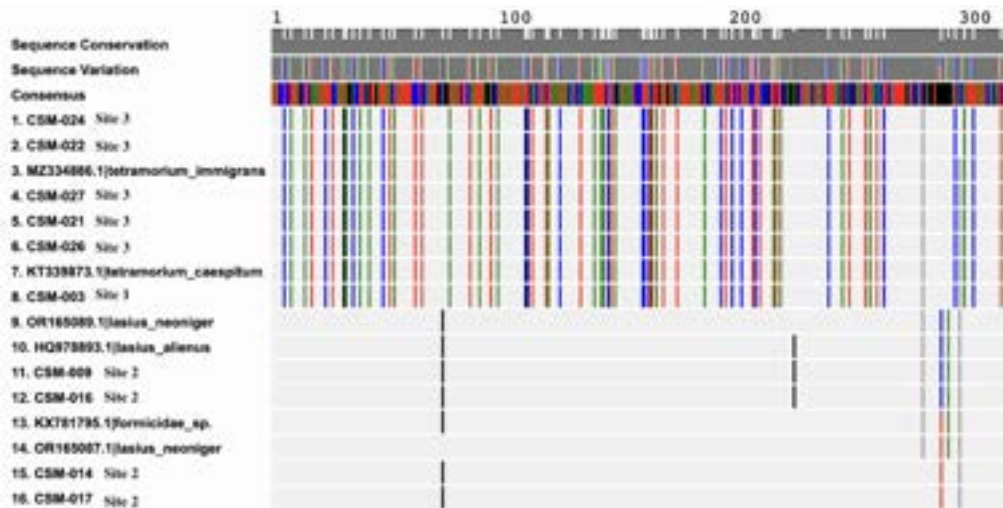


Figure 3: MUSCLE chart for correctly sequenced ant samples retrieved from DNA Subway.

According to Figure 3, CSM-003, the only sample from site 1 that was sequenced without error, corresponds most closely to the sequence KT339873.1|tetramorium\_caespitum, meaning that this sample is of the species caespitum. Site 2, after analysis of this MUSCLE chart, can be deemed the most biodiverse location, having two distinct species of the *Lasius* genus. Samples CSM-009 and CSM-016 are shown to be of the species alienus, matching with the sequence HQ978893.1|lasius\_alienus, while samples CSM-014 and CSM-017 are shown to be of the species neoniger, linking with the sequence OR165087.1|lasius\_neoniger. Site 3, made up of five correctly sequenced samples, represents the location with the most ant specimens that can be examined post-sequencing. These samples (CSM-021, CSM-022, CSM-024, CSM-026, and CSM-027) all correlated to the highest degree with the sequence MZ334886.1|tetramorium\_immigrans, indicating that they are of the same genus as CSM-003 from site 1 (*Tetramorium*) but of a varied species (*immigrans*). It is important to note that each site contained samples that matched with unique species absent at another site, showing biodiversity across these three locations of the HWEC.



Figure 4: Sample CSM-003 from Site 1 (left) and the database image for sequence KT339873.1|tetramorium\_caespitum (right)

The figure above displays the sequence image for KT339873.1|*tetramorium\_caespitum* adjacent to the collected specimen from Site 1, CSM-003. This sample, CSM-003, was found to have no base-pair variations with the sequence KT339873.1|*tetramorium\_caespitum*, further illustrating the match between the sample and sequence. As can be seen, there is a clear difference in the darkness of the ants in the images above, with the sample image having a greater melanization, or darkening, around the head, thorax, and abdomen.



Figure 5: Sample CSM-009 from Site 2 (left) and the database image for sequence HQ978893.1|*lasius\_alienus* (right)

Figure 5 shows the database image of one species of the genus *Lasius* found at Site 2, *alienus*, along with the image of specimen CSM-009 that matched with this species. CSM-009 had no base-pair variations with the *alienus* specimen, with the other specimen from Site 2, CSM-016, sharing this property. The absence of variations strengthens the match between these specimens and the sequence. A slightly lighter coloring can be seen in the collected specimen's thorax and abdomen compared to the database image, with the heads of both images being dark. CSM-009 also had a lighter abdomen and thorax compared to the database image above, but a darker head, again having a relationship with the database image like that of the CSM-009 specimen.



Figure 6: Sample CSM-014 from Site 2 (left) and the database image for sequence OR165087.1|*lasius\_neoniger* (right)

The database image for the other species found at Site 2, *neoniger*, is displayed above, with the image of specimen CSM-014 also presented. CSM-017, the other specimen from Site 2 that matched with this species, and CSM-014 had two base-pair variations with the *neoniger*

sequence, creating concern for the strength of this match. Comparing the images of CSM-014, CSM-017, and the database image of the neoniger species, a darkening of the heads and abdomens of both CSM-014 and CSM-017 is visible, while this darkening is not present in their corresponding database image.



Figure 7: Sample CSM-024 from Site 3 (left) and the database image for sequence MZ334886.1|*tetramorium\_immigrans* (right)

Figure 7, shown above, highlights both the sample CSM-024 from Site 3 and the image for the sequence MZ334886.1|*tetramorium\_immigrans*. The specimens from Site 3, which all corresponded with this sequence, had no variations of base-pairs with the sequence, reinforcing the match between it and these specimens. The sample and sequencing images represented above also demonstrate a closer visual relationship than that of CSM-003 and its corresponding sequence image, even though the pairs are of the same genus, *Tetramorium*. For the immigrans species, a dark head, thorax, and abdomen are present in its photo, matching the darkened head, thorax, and abdomen of CSM-024. This same increased melanization is also present in images for CSM-021, CSM-022, CSM-026, and CSM-027, the other specimens from Site 3.

## Discussion

The results of this study indicate that site 2, the exercise pathway, had the most biodiversity within a single site, containing ant specimens from two distinct species of the same genus (*Lasius alienus* and *Lasius neoniger*). Genetically, this difference in species demonstrates the stability of this specific site within the larger HWEC since biodiversity corresponds to ecosystem stability (Loreau & De Mazancourt, 2013; Tiede et al., 2017). It is also possible that this site's biodiversity was impacted by its status as an exercise pathway, where human foot traffic from visitors may have influenced the ants and measurements taken. This is supported by the fact that this site was found to be the most biodiverse internally among all sites and is also the site humans traverse through the most. This notion, though, requires more studies at related sites to be conducted to gain increased support. Furthermore, the two base-pair variations in the sequencing for specimens CSM-014 and CSM-017, which were linked to the species *Lasius neoniger*, raise some concerns as to the accuracy of their correlation. These variations occur due to the incompleteness and lack of accuracy of molecular reference databases, hindering the match between these specimens from site 2 and the neoniger species (Pawlowski et al., 2018). This limitation lessens support for site 2 being a location of stability, with the variations challenging the site's true biodiversity, then causing the strength of a conclusion regarding its stability to decrease.

At site 1, the composting center, only one specimen, CSM-003, was returned after sequencing all samples. This means that biodiversity within the site cannot be measured as there are no other correctly sequenced site 1 samples to compare to CSM-003. Consequently, further studies should be conducted at sites similar to the composting center, as research has shown that the presence of pollutants in waste composting can create differing degrees of pollution hazards, which could affect the biodiversity of ants at this location (Chu et al., 2019).

Within sites 1 and 2, variations in coloring between the collected specimens and the sequence images for correlated species were found. For site 1, specimen CSM-003 was darker than its matching sequence image at its head, thorax, and abdomen. For site 2, CSM-014 and CSM-017 were darker than their sequence image at their heads and abdomens. The darker, more melanized heads compared to the sequence images had no mention in the collected body of knowledge, and further research should be conducted to gather more evidence to determine their significance. The base-pair variations for specimens CSM-014 and CSM-017, which decrease the accuracy of the match with their corresponding species, could also speak for the discrepancy in the color of these specimens with their sequence image. Also, the heads, thoraxes, and abdomens of all collected specimens from site 3, the construction pathway, were all found to be darkened, and these specimens' relation to their sequenced species is reinforced by the matches in color. This also points to the notion that the passing of construction vehicles over the site, which occurred exclusively at site 3, could have lowered its biodiversity, as all correctly sequenced site 3 specimens matched strongly with only one species. For this notion to be supported further, additional studies should be conducted at locations in which large vehicles traverse periodically. Moreover, at site 2, specimens CSM-009 and CSM-016 had lighter coloring at their thoraxes and abdomens compared to their matching sequence image, with a similar head color. This difference is important to point out, but no studies in the collected body of knowledge tell of what the lighter coloring of these specific ant regions could indicate, while other studies have stated that lighter coloring of the head is indicative of heavy metal pollution (Skaldina et al., 2018). This may still indicate pollution of this site, but further research should be conducted to measure the possibilities of the coloring's indications. Additionally, while smaller head sizes of the collected ants compared to their sequence images could produce evidence of pollution in each site, as explained by Grześ et al. (2014), these physical characteristics of the ants could not be compared accurately with their matched images due to differences in quality and scale between pictures. To draw strong conclusions regarding this facet of the ants, more research should be conducted with increased accuracy in photo documentation, allowing for comparisons of ant head sizes.

Considering the biodiversity of the entire HWEC, the presence of four distinct species of ants across its sites (*Tetramorium caespitum* at site 1, *Lasius alienus* and *Lasius neoniger* at site 2, and *Tetramorium immigrans* at site 3) suggests the center's biodiversity is high, indicating stability across its entirety (Loreau & De Mazancourt, 2013; Tiede et al., 2017). Since approximations of this stability came from ant biodiversity measurements and ants have been described as excellent bioindicators, it is defended further (Lawes et al., 2017; Skaldina et al., 2018; Tiede et al., 2017). This stability then leads to the conclusion that there are no harmful pollutants in the center, refuting this paper's hypothesis, relieving concerns that the status of this specific center (HWEC) as a landfill-turned-park could cause human health issues, and illustrating that ant biodiversity can be indicative of pollutant presence at former landfills. Limitations to this overall indication, however, do exist, since a fraction of ants (which were only

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from three HWEC locations) that were sequenced returned data, meaning that conclusions drawn in this study will need reinforcement from future studies conducted with increased sample sizes at more site locations. This could also serve as an implication to this research, as the conduct of future studies to bolster conclusions drawn in this one can further cement the status of ants as an indicator species, develop the technologies required to analyze ants phenotypically and genotypically, and increase comprehension of the complex relationship between a location's biodiversity and its stability.

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## **APPENDIX A**

### **Lab materials:**

- Lysis solution [6 M Guanidine Hydrochloride GuHCl] (720  $\mu$ l)
- Silica Resin (8  $\mu$ l)
- Specimen tissue samples (from Part 1)
- Wash buffer (2400  $\mu$ l)
- Distilled Water or TE buffer (240  $\mu$ l)
- Container with cracked or crushed ice
- Microcentrifuge
- Microcentrifuge tube rack
- 6 microcentrifuge tubes (1.5 ml)
- Micropipettes and tips (2-1000  $\mu$ l)
- Permanent marker
- 2 plastic pestles
- Vortexer (optional)
- Water bath or heating block at 65 degrees C and 57 degrees C
- Possible: 2 Razor blades, scissors, tweezers, or 2, 10  $\mu$ l pipette tips for tissue removal from specimen
- For sample storage: 96-100% Ethanol or freezer