



Biodiversity is the variety of organisms and species in a given ecosystem., which can be tested in many ways, including DNA barcoding. DNA barcoding is the sequencing of an organism's genome or part of its genome by means of collection, isolation, amplification, and finally analysis. The goal was to analyze biodiversity in Sans Souci county park by means of barcoding as many possible samples of plants, fungi, and insects/animals. It has been hypothesized that biodiversity is relatively high, as it is necessary for any successful ecosystem. Samples were collected, their DNA was isolated, and specific primers were used during PCR to amplify target genes (rbcl, COI, ITS). These amplified genes were then sequenced for analysis in DNA subway. When compared with known sequences in the database our results showed that two of our samples, NYZ-002 (plant) and NY-009 (insect), showed little to no variation with species already in the database. The remaining two samples, NYZ-008 and NYZ-012 (both insect), showed significant variation from other known sequences in the database that they were compared too. Variation from samples already in the database could mean that samples NYZ-008 and NYZ-012 are members of species that have not yet been sequenced and uploaded. Although further investigation is needed, it can be concluded that these samples may be novel sequences.

#### **Introduction:**

Biodiversity, or a large variety of different organisms, allows for stable and resistant ecosystems that can last much longer than ecosystems with low biodiversity. Without this essential component ecosystems would be less stable, as species depend on each other for survival. Many methods can be used to analyze biodiversity, such as DNA barcoding. This shows biodiversity by analyzing different DNA sequences of different organisms. Our sample sequences can be compared to known sequences that indicate what our samples are most genetically similar to. By identifying our samples, we can take a census of the variety of different organisms that contribute to the genetic diversity of Long Island. Sans souci, the location of interest for this project is a combination of an estuary where salt and freshwater meets, as well as a forested section. The goal was to analyze this diversity in Sans Souci County Park through the Barcode Long Island program by comparing sample sequences to those in the database in order to identify our samples.

Barcode LI is a four-year study program that allows students to set up DNA barcoding projects to analyze the biodiversity of Long Island ecosystems. This is done through specific means of collecting samples, then extracting, isolating and amplifying their DNA. This process begins with collecting small pieces of specimens and then using particular primers in order to replicate the desired gene. Samples that are isolated and amplified correctly can be sent to be sequenced. Sequencing will produce the DNA code of the organism, so it can be compared to other organisms. Similarities in DNA sequences show that organisms are closely related and have a recent common ancestor. The most significant outcome of this sequencing technique is the ability to document species living on Long Island and therefore illustrate its biodiversity.

# **Census of Biodiversity in Sans Souci County Park**

## **Ciara Joseph and Frank Giacchetto**

### **Materials and Methods:**

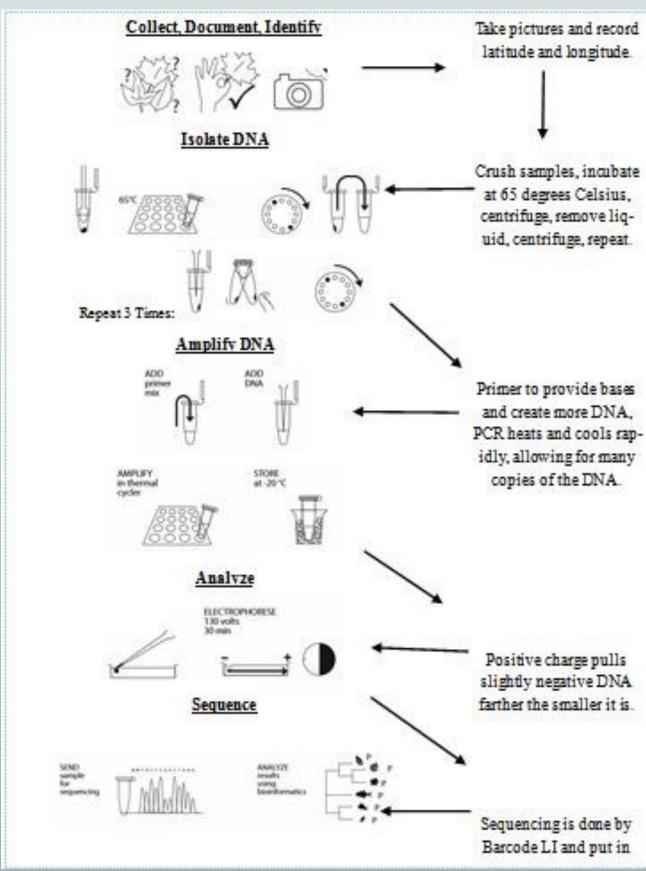


Figure 1: diagram of DNA isolation, extraction, amplification, and analysis.



Figure 2: map of Long Island, with a mark on Sans Souci Park, our area of interest and collection site

#### **Results:**

| NYZ-002  |                          |   |                  |              |                 |                   |  |  |  |
|----------|--------------------------|---|------------------|--------------|-----------------|-------------------|--|--|--|
| <b>#</b> | Accession #              | <b>♦</b> Details  | ♦ Aln.<br>Length | Bit<br>Score | \$ <del>0</del> | ♦ Mis-<br>matches |  |  |  |
| 1(1).    | gi 685847147 gb KJ841345 | Gaultheria procumbens - ribulose-1,5-<br>bisphosphate carboxylase/oxygenase<br>large subunit (rbcL) gene, partial cds;<br>chloroplast | 537              | 969          | 0.0             | 0                 |  |  |  |
| 2(2).    | gi 642969309 gb KJ593438 | Gaultheria procumbens - ribulose-1,5-<br>bisphosphate carboxylase/oxygenase<br>large subunit (rbcL) gene, partial cds;<br>chloroplast | 537              | 969          | 0.0             | 0                 |  |  |  |
| 3(3).    | gi 642989311 gb KJ593439 | Gaultheria procumbens - ribulose-1,5-<br>bisphosphate carboxylase/oxygenase<br>large subunit (rbcL) gene, partial cds;<br>chloroplast | 537              | 969          | 0.0             | 0                 |  |  |  |
| 4(4).    | gi(755573234)gb(KJ922007 | Gaultheria forrestii - large subunit<br>(rbcL) gene, partial cds; chloroplast   | 537              | 960          | 0.0             | 2                 |  |  |  |
|          |                          |   |                  |              |                 |                   |  |  |  |



| eria procumbens - ribulose-1,5-<br>phate carboxylase/oxygenase<br>ubunit (rbcL) gene, partial cds;<br>last | 537 | 969 | 0.0 |
|--|-----|-----|-----|
| erla procumbens - ribulose-1,5-<br>phate carboxylase/oxygenase<br>ubunit (rbcL) gene, partial cds;<br>last | 537 | 969 | 0.0 |
| eria forrestii - large subunit<br>ene, partial cds; chloroplast  | 537 | 960 | 0.0 |
|  |     |     |     |
|  |     |     |     |

Figure 4: picture of plant sample NYZ-002, with BLAST results. The results show that sample NYZ-002 is most likely a member of Gaultheria procumbens.

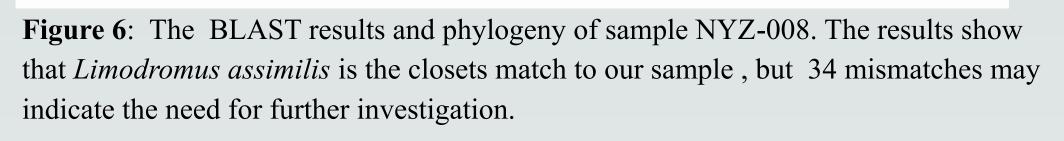


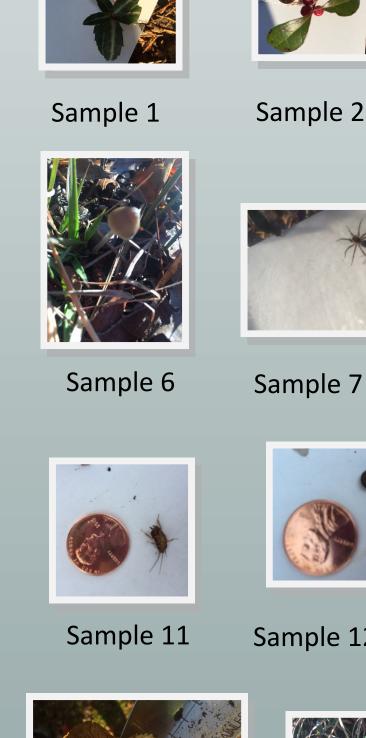


Figure 5: picture of insect sample NYZ-008 and *Limodromus* assimilis, the closest match to sample NYZ-008 in the database.

| NYZ-0      | 08                       |   |                  |              |     |                           |
|------------|--------------------------|---|------------------|--------------|-----|---------------------------|
| <b>‡</b> # | Accession #              | ≑ Details   | ♣ Aln.<br>Length | Bit<br>Score | ≜ θ |                           |
| 1(1).      | gi)695083252 gb KJ962312 | Limodromus assimilis - cytochrome oxidase<br>subunit 1 (COI) gene, partial cds; mitochondrial | 494              | 738          | 0.0 | 34                        |
| 7(7).      | gi 695091509 gb KJ966429 | Limodromus assimilis - cytochrome oxidase<br>subunit 1 (COI) gene, partial cds; mitochondrial | 494              | 733          | 0.0 | 35                        |
| _          |                          | 100   |                  |              |     | gi 695083252 1<br>NYZ-008 |
|            |                          |   |                  |              |     | <del>1</del> 1322409027#  |

gi|322409027|brachychaeteuma\_bradeae

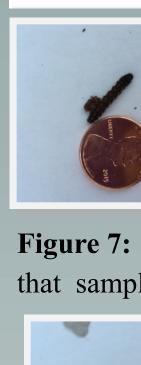






Sample 16

Sample 17





| NYZ-012     |                          |   |                  |              |             |                 |
|-------------|--------------------------|---|------------------|--------------|-------------|-----------------|
| <b>\$</b> # | Accession #              | <b>‡</b> Details  | ♦ Aln.<br>Lenath | Bit<br>Score | <b>\$</b> 8 | Mis-<br>matches |
| 1(1).       | gi 322409027 gb HQ966191 | Brachychaeteuma bradeae - ZSM MYR 00274<br>cytochrome oxidase subunit 1 (COI) gene, partial cds;<br>mitochondrial |                  | 628          | 1e-<br>177  | 70              |
| 2(2).       | gi 333826917 gb HM245889 | Pseudotremia barri - cytochrome c oxidase subunit I<br>(cox1) gene, partial cds; mitochondrial                    | 523              | 623          | 1e-<br>175  | 71              |
| 3(3).       | gi 333826919 gb HM245890 | Pseudotremla barrl - cytochrome c oxidase subunit I<br>(cox1) gene, partial cds; mitochondrial                    | 523              | 623          | 1e-<br>175  | 71              |
| 4(4).       | gi 333826935 gb HM245898 | Pseudotremia minos - cytochrome c oxidase<br>subunit I (cox1) gene, partial cds; mitochondrial                    | 523              | 623          | 1e-<br>175  | 71              |
| 5(5).       | gi 333826937 gb HM245899 | Pseudotremia minos - cytochrome c oxidase<br>subunit I (cox1) gene, partial cds; mitochondrial                    | 523              | 623          | 1e-<br>175  | 71              |
| 6(6).       | gi 333826939 gb HM245900 | Pseudotremia minos - cytochrome c oxidase<br>subunit I (cox1) gene, partial cds; mitochondrial                    | 523              | 623          | 1e-<br>175  | 71              |
| 7(7).       | gi 333826945 gb HM245903 | Pseudotremia minos - cytochrome c oxidase<br>subunit I (cox1) gene, partial cds; mitochondrial                    | 523              | 623          | 1e-<br>175  | 71              |
| 8(8).       | gi 333826947 gb HM245904 | Pseudotremia minos - cytochrome c oxidase<br>subunit I (cox1) gene, partial cds; mitochondrial                    | 523              | 623          | 1e-<br>175  | 71              |
| 9(9).       | gi 333826911 gb HM245886 | Pseudotremia minos - cytochrome c oxidase<br>subunit I (cox1) gene, partial cds; mitochondrial                    | 523              | 619          | 1e-<br>174  | 72              |
|             |                          |   |                  |              |             |                 |
|             |                          |   |                  |              |             |                 |
|             |                          | _   |                  |              |             | 100             |
|             |                          |   | 100              |              |             |                 |
|             |                          |   | 100              |              |             |                 |
|             |                          |   |                  |              |             |                 |
|             |                          |   |                  |              |             |                 |
|             |                          |   |                  |              |             |                 |
|             |                          |   |                  |              |             |                 |

Figure 9: BLAST results and phylogeny of sample NYZ-012. The results show that sample NYZ-012 and Brachychaeteuma bradeae are very different and our sample is most likely not a member of *Brachychaeteuma bradeae*, so further investigation is needed.



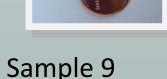
Sample 3



Sample 8









Sample 14







Sample 15



Figure 3: Samples collected in Sans Souci County Park

| NYZ-009    |                          |   |                  |              |            |                 |  |  |  |
|------------|--------------------------|---|------------------|--------------|------------|-----------------|--|--|--|
|            |                          |   |                  |              |            |                 |  |  |  |
| <b>#</b> # | Accession #              | ‡ Details   | ♣ AIn.<br>Length | Bit<br>Score | <b>≜</b> e | Mis-<br>matches |  |  |  |
| 1(1).      | gi 630046306 gb KJ375474 | Idia Iubricalis - BOLD:AAA2230 voucher<br>CNCLEP00098202 cytochrome oxidase subunit 1<br>(COI) gene, partial cds; mitochondrial | 647              | 1162         | 0.0        | 1               |  |  |  |
|            |                          |   |                  |              |            |                 |  |  |  |



Figure 7: picture of insect sample NYZ-009, with BLAST results. The results indicate that sample NYZ-009 is likely a member of *Idia lubricalis*.

Figure 8: picture of insect sample NYZ-012 and Brachychaeteuma bradeae, the closest match to NYZ-012 in the database.

#### **Discussion:**

The BLAST results for sample NYZ-002 showed that its closest match in the database is the Gaultheria procumbens, a small, lowgrowing shrub. More specifically, it is a wintergreen that is a vital food source to a handful of organisms, mainly deer. The database showed no mismatches of the rbcl DNA sequence to the same sequence of *Gaul*theria procumbens. This means that all of the nitrogenous bases for both DNA strands of the target gene, rbcl, were the same. This suggests that the samples are very similar and supports the likelihood of our sample being a member of the Gaultheria procumbens species. Sample NYZ-002 had a bit score of 969, indicating that our sample DNA and the Gaultheria procumbens rbcl sequence were moderately aligned, but not completely as the score is under 1,000. The e-score of 0 (less than 1) indicates that we can be very confident that our sample and the Gaultheria procumbens are closely related and have a very recent common ancestor. Based on this data, this sample is most likely closely related to or a member of *Gaultheria procumbens*.

Sample NYZ-009, also an insect sample, was found to be most similar to *Idia lubricalis*, a species of moth. The Bit-score of 1162, over 1000, indicates that our sample DNA and the *Idia lubricalis* DNA from the database are very well aligned. The e-score of 0 also supports that sample NYZ-009 and *Idia lubricalis* are closely related. There was only one mismatch between our sample and the Idia lubricalis DNA, suggesting that NYZ-009 and *Idia lubricalis* are very similar. Due to this information, we can conclude that sample NYZ-009 is likely to be a member of Idia lubricalis.

Sample NYZ-008, and insect sample, was most similar to Limodro*mus assimilis*, a species of ground beetle. This beetle does not have a specific environmental role, but remains active during the winter and is a food source of larger organisms. This sample had a lower Bit-score of 738, indicating that the samples were not aligned perfectly. The escore of 0 showed that we can be confident that our sample and *Limo*dromus assimilis are closely related. However, there are 34 mismatches between our sample DNA of the target gene and the DNA found in the database for *Limodromus assimilis*. This leads us to believe that our sample may be part of a species that has not yet been sequenced and uploaded into the database or could possibly be a novel species, but further investigation is needed.

Sample NYZ-012 was found to be the most similar to *Brachy*chaeteuma bradeae, a species of millipedes. The Bit-core of 628 suggests that our sample DNA sequence and the DNA sequence of *Brachy*chaeteuma bradeae were not well aligned. There were 70 mismatches between our sample and the DNA sequence of *Brachychaeteuma* bradeae, showing that they are most likely not closely related. The escore was 1e-177, meaning that our sample and *Brachychaeteuma* bradeae are distantly related and do not have a recent common ancestor. Thus, we cannot conclude that sample NYZ-012 is a member of Brachychaeteuma bradeae. Therefore, there is a possibility that with further investigation our sample may be a member of a species that has not yet been sequenced and uploaded into the database, or is a novel species.

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#### References

Invertebrate Collection Manual. (2007). Retrieved November 13, 2015 from http://australianmuseum.net.au/uploads/documents/9382/the%20invertebrate%20collection%20 manu-<u>al.pdf</u>

Leonard, P.L. (editor) (2010). A Guide to Collecting and Preserving Fungal Specimens for the Queensland Herbarium. Queensland Herbarium, Department of Environment and Resource Management, Brisbane Field Techniques Used by Missouri Botanical Garden. Retrived November 30, 2015 from http:// www.mobot.org/MOBOT/molib/fieldtechbook/pdf/handbook.pdf

Lacey, John, Sam Short, and Jeff Mosley. "How to Collect, Press and Mount Plants." Montana State University Extension. N.p., n.d. Web. 30 Nov. 2015.





