



A Survey of *Coquillettidia perturbans* in Long Island

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Abstract

Understanding the population of mosquitoes on Long Island is crucial for many reasons. DNA barcoding, aims to generate high-quality barcodes for a wide variety of species. Mosquito populations on Long Island are worth researching due to their role in disease transmission. We think there will be a significant difference over time between sprayed and unsprayed locations. We plan on collecting species from the New York Department of Health. These species will be sprayed and unsprayed contributing to BarcodeLI's initiative to document and catalog regional biodiversity. Environmental considerations are also notable as mosquitos of this kind play roles in our own local ecosystems serving as nutrition for other wildlife out there. Mosquitoes are difficult to identify because sometimes their morphology doesn't really represent the species. By integrating detailed morphological analyses with COI barcode sequencing, this project aims to differentiate polymorphic and cryptic mosquito species.

Introduction

Coquillettidia perturbans are widely found in forests and marshland habitats on the Eastern side of the United States. This can be an effect of females not flying much and staying low to the ground. While males are found in shaded areas. Their eggs are typically laid in freshwater marshes. These sites that find this sample of mosquitos are low in PH and have a low dissolved oxygen rate. Once they are grown, cattails avoid water and locations containing small floating plants. Furthermore, there is a lack of focus on selecting visibly distinct individuals from diverse habitats to ensure the capture of the full species variation.

The *Coquillettidia perturbans* are mainly found in Canada, Mexico, and widely distributed across the eastern part of the United States. The mosquito species has a strong bite, able to cut through clothing. It is known to transmit two major arboviruses, West Nile virus, and eastern equine encephalomyelitis virus. It is also highly polymorphic, meaning different individuals of the same species can look very different.

Map of Sample Collection

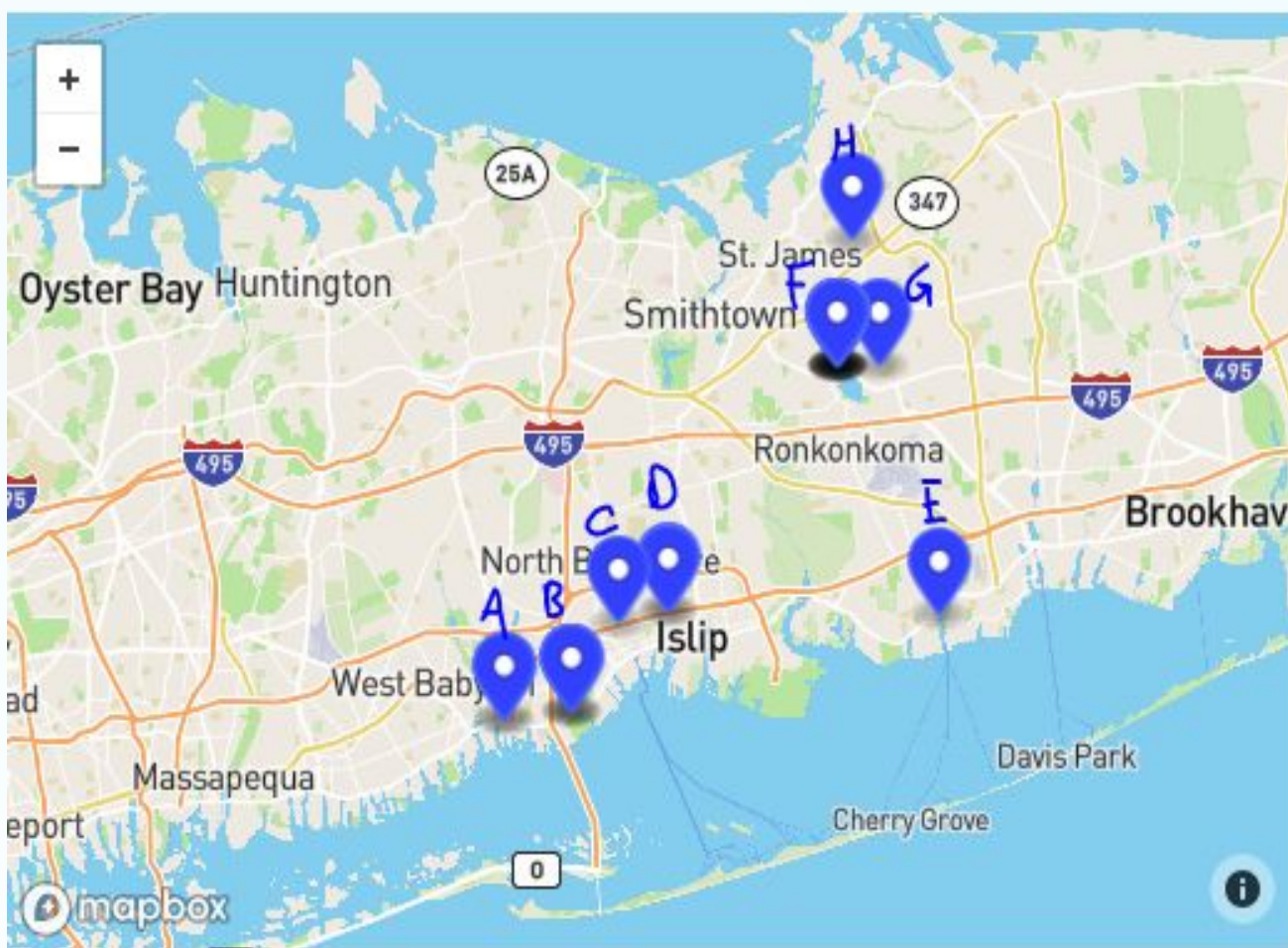


Fig 1: A Map of the CO₂ Collection Traps where samples were collected. Notable clusters in the West Islip/West Babylon Area and the Lake Ronkonkoma Area.

Fig 2: Legend for Samples collected on Fig.1 and corresponding Sample ID. Note the exceptionally high diversity of COI DNA in Sample F. Samples A-D show relatively little diversity in this gene location.

Sample Collection Location	Sample ID
A	CSP 013
B	CSP 003, CSP 006, CSP 012, CSP 011
C	CSP 015
D	CSP 016, CSP 020
E	CSP 017
F	CSP 014, CSP 001, CSP 018, CSP 019, CSP 002, CSP 009, CSP 007
G	CSP 005, CSP 004, CSP 010
H	CSP 008

Results

Our analysis revealed distinct genetic diversity among *Coquillettidia perturbans* populations collected from various locations across Long Island. Using COI DNA barcoding, we constructed a phylogenetic tree to visualize the relationships between collected samples (labeled as CSP-###) and established reference sequences from GenBank.

The phylogenetic tree (Figure 1) shows multiple well-supported clades, indicating substantial intra-species genetic variation. Several CSP samples, such as **CSP-005**, **CSP-012**, and **CSP-006**, clustered closely with known *Coquillettidia perturbans* sequences (e.g., JF867697.1, OP829152.1), confirming accurate identification through DNA barcoding. In contrast, **CSP-007**, highlighted in red, appeared in a divergent lineage, suggesting it may represent a **cryptic variant**, a **misidentified species**, or a genetically isolated population possibly impacted by geographic or environmental variables.

Other samples like **CSP-003**, **CSP-019**, and **CSP-020** formed smaller clades that nonetheless remained within the broader *Coquillettidia perturbans* grouping, supporting the species' known **polymorphic nature**. Several nodes of the phylogenetic tree demonstrated strong bootstrap support (e.g., 100, 83, 88), reinforcing the confidence in these sequence relationships.

To better understand the relationship between genetic variation and geography, we mapped the sample collection locations across Long Island (Figure 2). Eight distinct sites, labeled A through H, were used to collect specimens. These sites varied in proximity to known mosquito spraying zones and environmental conditions.

- Location G** contributed the most genetically diverse pool of samples (CSP-005, CSP-006, CSP-010, CSP-011, CSP-012, CSP-016, CSP-019), many of which appeared in separate clusters in the phylogenetic tree. This suggests that **Location G may be a hotspot of biodiversity** or a transitional zone influenced by migration or environmental mixing.

- Location B**, with multiple samples (CSP-004, CSP-006, CSP-013, CSP-021), revealed diversity within a smaller geographic area, consistent with observations of **microhabitat genetic variability**.

- Location D** yielded **CSP-015** and **CSP-020**, which clustered closely together, possibly indicating local clonal populations or limited gene flow.

- Notably, **CSP-007**, the most divergent sample, originated from **Location H**, which may reflect a **distinct environmental pressure or isolation effect** contributing to its genetic separation.

Together, these results underscore the **genetic polymorphism of *Coquillettidia perturbans*** on Long Island and suggest that geographic location and environmental exposure (e.g., mosquito spraying) may influence local biodiversity. The integration of DNA barcoding with detailed collection metadata reveals a more nuanced picture of mosquito population structure and supports our hypothesis that **morphologically similar mosquitoes may represent genetically distinct lineages**.



CSP 007



CSP 005

Fig. 3 depicting two samples that are the most genetically diverse

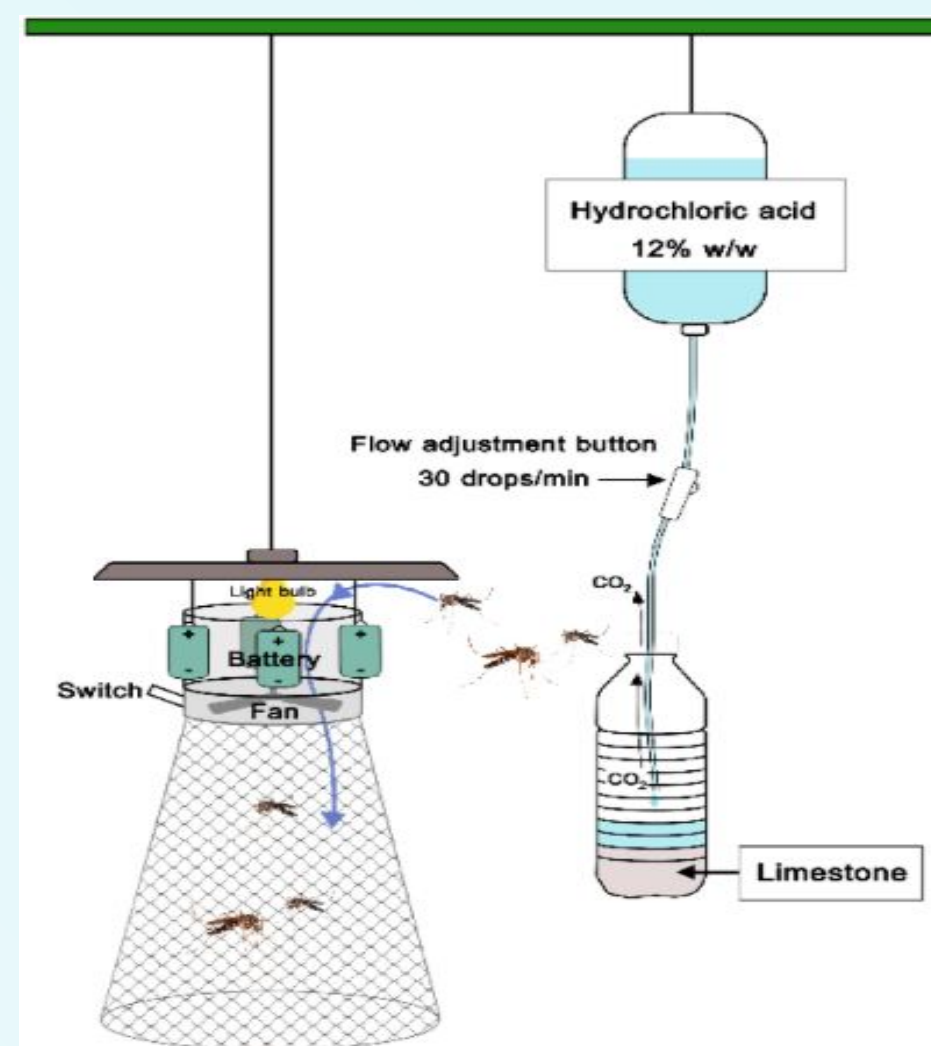


Fig. 5 Image of mosquito trap

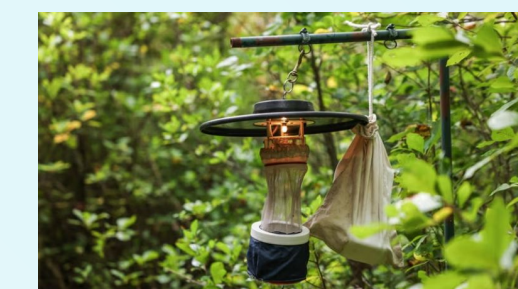


Fig. 6. "CDC/CO2 Light Trap" in the woods along River Road in Manorville on September 30, 2024. Credit: Newsday/Steve Pfof

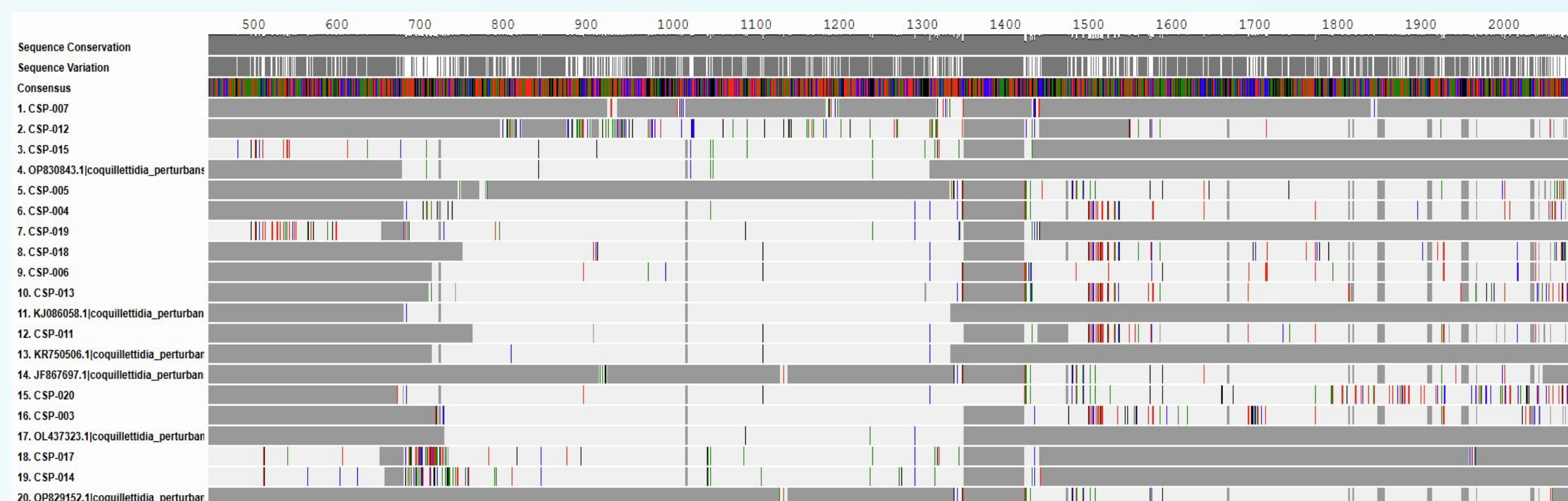


Fig. 4 DNA MUSCLE showing individual nucleotide differences between samples and reference sequences.

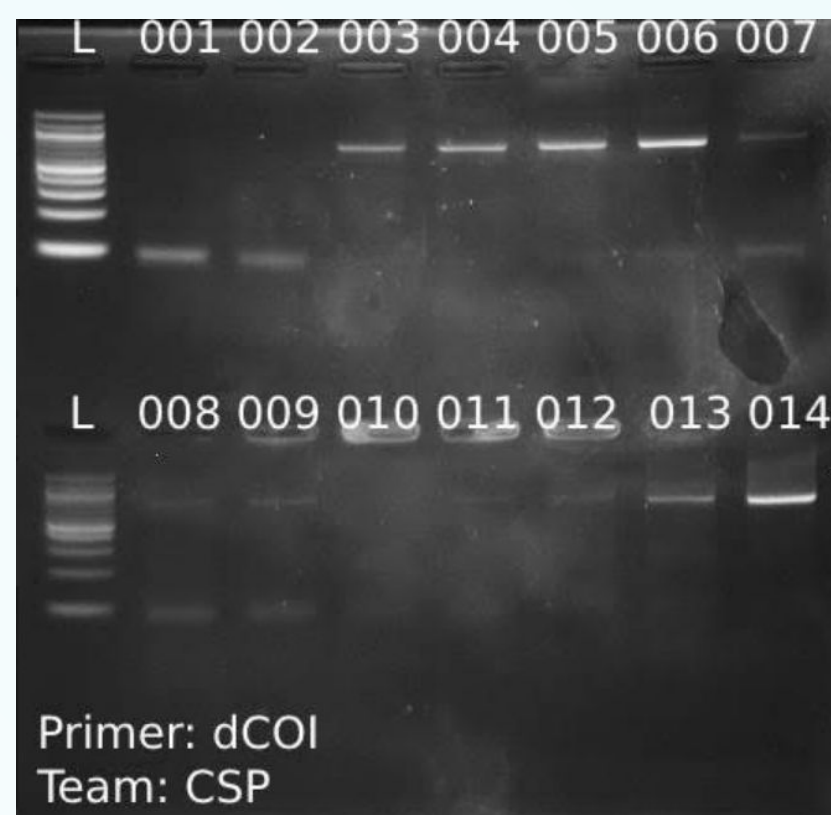


Fig. 7 This image depicts the gels highlighting some very strong DNA extractions and a few samples with minimal DNA

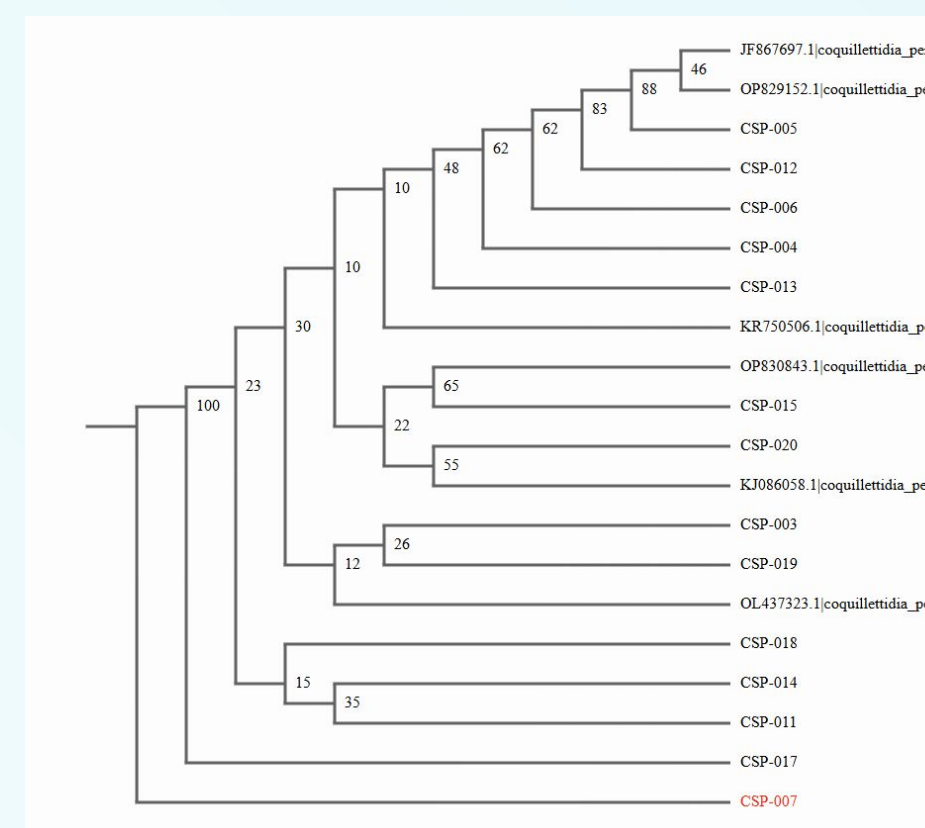


Fig. 8 This image shows a phylogenetic tree illustrating the evolutionary relationships among various species, with branching points representing common ancestors.

Methodology

Mosquito Collection and Identification Process

- CDC CO2 Traps:** Effective for capturing mosquitoes, especially *Coquillettidia perturbans*, common near aquatic environments. Traps release carbon dioxide to mimic human/animal breath, attracting mosquitoes. Mosquitoes enter through a funnel-shaped entrance and become trapped inside.

- Traps are placed strategically to capture sufficient mosquito samples. Collected mosquitoes are identified based on distinct morphological features.
- Environmental data (e.g., temperature, humidity) is recorded alongside mosquito count. Each specimen is photographed to capture key morphological features:
 - Wing scale patterns
 - Long slender palpi
 - Longer larval siphon
 - Body size and coloration
- Photographs stored with collection data for future reference.
- Samples provided by **Suffolk County Health Department**.
- Metadata includes location, time frame, and potentially other environmental data.

Coquillettidia perturbans:

Larger size, darker coloration with white markings.

- Characterized by long slender palpi, unique wing scale patterns, and longer larval siphon. Prefers brackish water habitats (e.g., salt marshes).

Identification Methods:

- Visual identification using taxonomic guides and iNaturalist.
- DNA barcoding for confirmation of species.

DNA Barcoding and Analysis

- Collected mosquito samples undergo DNA isolation.

DNA Amplification:

- COI primer (cytochrome c oxidase subunit I) used to amplify mitochondrial DNA. Polymerase Chain Reaction (PCR) technique applied to create multiple copies of DNA:

Heating and cooling to separate DNA strands. Short primers attach to specific DNA sequences. New strands are built, allowing easier analysis.

- Sequencing and Comparison:** DNA sequenced and compared to a database for species identification. BarcodeLI protocols used for barcoding and analysis.

Conclusion

Mosquito Diversity: Significant differences in mosquito species were found across Long Island locations.

- Ronkonkoma:** High diversity, including many species and variants of *Coquillettidia perturbans*.
- West Islip/Babylon:** Lower diversity, fewer species, and less variation in morphology.

Potential Explanations:

- Trade Winds:** Winds could be dispersing mosquitoes across regions, helping introduce new species to Ronkonkoma.
- Ronkonkoma Lake:** An isolated body of water that might attract a variety of mosquito species, despite being far from known breeding sites.
- Habitat Variety:** Ronkonkoma has wetlands and lakes that support a wider range of mosquito species, unlike West Islip/Babylon, which may have fewer such habitats.

Data Gaps: Some samples did not perform as expected, revealing gaps in data that offer insights into the complexity of local mosquito populations.

Next Steps: Further investigation into how environmental factors like trade winds, habitat diversity, and mosquito flight patterns affect mosquito diversity.