Evaluating Dipteran Diversity in Farmingdale, NY Through Molecular and Morphological Identification

Authors: Khloe Erickson, Brooke Szygiel, Isabella Durante
Mentors: Dr. Oxana Livine and Peter Popov

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Abstract

Flies are hyperdiverse and play important ecological roles through pollination, decomposition, and pest suppression. However, they are underappreciated in the scientific world. This study examines the biodiversity and ecological roles of Diptera in Farmingdale, NY. Specimens were analyzed through DNA barcoding and iNaturalist. Pictures of each sample were uploaded onto iNaturalist, and the Chelex extraction method was used for DNA isolation. The DNA was amplified through PCR, visualized using gel electrophoresis, and sent for sequencing to Azenta, in NJ. The DNA sequences were analyzed using bioinformatics on DNA subway 2.0 and NIH BLAST. Our results indicated a vast biodiversity of fly species consisting of 15 families across 2 suborders; these species hold 4 major ecological roles. Additionally, DNA Barcoding provided more specific identifications, including 12 identifications to the species level, compared to iNaturalist which yielded 11. This supports our hypothesis that DNA Barcoding would provide more specific identifications.

Introduction

- Flies (Order Diptera) are insects with one pair of wings and include over 125,000 species worldwide.
- They are often overlooked but are important to biodiversity and found in nearly all ecosystems.
- Flies contribute ecologically through:
  a. pollination
  b. pest suppression
  c. decomposition of organic matter
- Flies are the second most important pollinators after bees, especially where bees are less common.
- DNA barcoding and iNaturalist are common tools for species identification and biodiversity studies.
- Gel electrophoresis is used in DNA barcoding to visualize DNA for identification.
- iNaturalist uses AI and expert review to identify organisms from uploaded observations.
- Both methods help study biodiversity and species distribution.
- This research is important because despite being overlooked in the scientific world, flies are imperative to local ecosystems and are vastly understudied.

Research Question: What Diptera species/families are present in suburban New York and what ecological roles do they hold within their ecosystem?

Hypothesis:
DNA barcoding will provide more reliable identifications because it doesn't depend on morphological identification, and these species will play essential ecological roles in the ecosystem.

Methods

Specimens collected in 2022 → Samples were preserved in ethanol in -20 °C → Specimens photographed and uploaded to the INaturalist platform

Molecular process

1. DNA extraction using the Chelex method
2. PCR (DNA amplification)
3. Visualization of DNA using Gel Electrophoresis
4. DNA sequencing and analysis

Figure 4. Red cross shows the location of fly specimens collection in Farmingdale, NY (Long Island, NY). (https://www.longislandexchange.com/towns/east-farmingdale/).

Role of Mentor

- Collected specimens
- Conducted PCR and gel electrophoresis

Role of Student

- Photographed and uploaded specimens to iNaturalist.
- Conducted DNA extraction
- Analyzed DNA BLAST sequences through bioinformatics.

Results

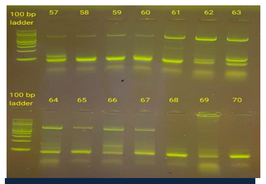

Figure 5. Gel electrophoresis samples #63-70. Sample #62 is a positive control (invertebrates).

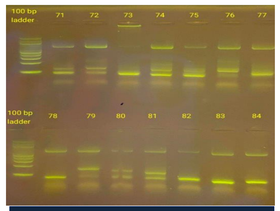

Figure 7. Gel electrophoresis samples #85-92, #93 is a positive control (invertebrates), #98 is a negative control (2 ul of water).

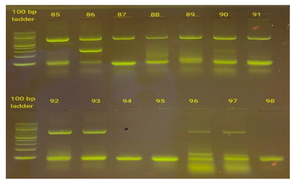

Figure 6. Gel electrophoresis samples #71-84.

- DNA samples #63–67, #71–72, #74–84, and #85–92 all produced clear ~650 bp fragments and were then submitted for sequencing.
- Gel Electrophoresis confirmed successful amplification of DNA, with both positive and negative controls also confirming success.

iNaturalist Results:
11 identifications to species, 8 to genus, 1 to subfamily, 6 to family, 1 to infraorder, 3 to zoosubsection. Only 6 specimens earned Research Grade status (samples #4, 7, 9, 18, 25, 27).

DNA Barcoding Results:
12 specimens were identified to species, 1 to genus, 6 to family.

Agreement:
Species-level agreement occurred in 2/19 samples. Genus-level agreement occurred in 1/19 samples. Family-level agreement occurred in 2/19 samples. 14/19 samples did not agree to at least the family level.

Ecological Roles Identified:
4 Ecological roles were identified using the identifications made by iNaturalist and DNA Barcoding. The distributions of the ecological roles are shown below (See figure 8 and figure 9).

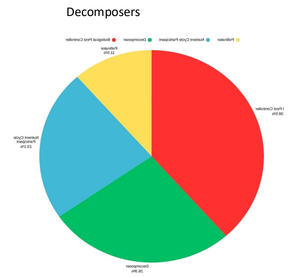

Figure 9. Ecological Roles of specimens identified in iNaturalist.

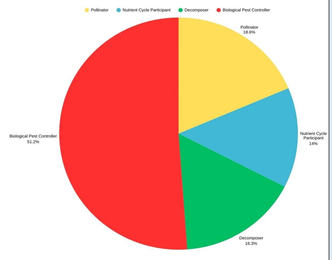

Figure 8. Ecological Roles of specimens identified in DNA BLAST.

Discussion

- Only 5/19 specimens identified using DNA BLAST and iNaturalist agreed down to at least the family level, showing limited agreement.
- Our hypothesis that DNA Barcoding would prove to be more reliable was confirmed, as it provided more specific identifications.
- Flies proved to be biodiverse, and an integral part of the ecosystem. 14 families across 2 suborders were identified and their ecological roles consisted of: minor pollinators, participants in the nutrient cycle, decomposers, and biological pest controllers.

Applications

Strengthen identification methods; highlighting limitations. → Reinforces the importance of flies within suburban settings.

Limitations and Future Research

- 11 DNA samples were lost due to low quality.
- Only 6 photos uploaded to iNaturalist achieved Research Grade status due to low quality photos.
- Our study is limited by a small sample size and single collection site.

- Improving DNA extraction methods to reduce specimen loss.
- Improving photo quality on iNaturalist to achieve more Research Grade identifications.
- Increase sample size and collection area to gain a better understanding of the biodiversity across a larger area.

References and Acknowledgements

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