Evaluating Bird Biodiversity in Brooklyn, Manhattan, and the Bronx through DNA Barcoding

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Abstract

Birds are significant to ecosystems, helping in pest control, seed dispersal, and habitat maintenance while serving as indicators of environmental health. This study explored bird biodiversity across three New York City (NYC) boroughs, Brooklyn, Manhattan, and the Bronx using DNA barcoding to identify species from feather samples collected from residential areas, recreational parks and local nature parks. We used two methods to extract DNA from the feather samples, the DNALC's Silica DNA Isolation Method and the Qiagen DNeasy Blood & Tissue Kit. The segments of the cytochrome oxidase I (COI) gene of the DNA extracts were amplified by PCR using the COI primers, Bird F1 and Bird R1. Electrophoresed amplicons with good bands were sent for Sanger sequencing. DNA sequences were analyzed using BLAST and alignment and tree-plotting tools in DNA Subway. BLAST results and the phylogenetic tree generated revealed that although we encountered diverse bird species in the three boroughs, all our sequenced samples were of the Rock pigeon, *Columba livia*.

Introduction

Birds have an essential role in ecosystems across the world. They help to control pests, spread seeds of various plants, and maintain habitats (Miller, 2024). They help us learn about the ecosystem conditions and are indicators of ecosystem health (US Geological Survey, 2022).

New York City, containing thousands of acres of diverse types of ecosystems like forests and islands, is a major hub for bird species (New York City Bird Alliance, n.d.). New York City (NYC) is on the path of the Atlantic Flyway, a major bird migration route where birds stop by; this makes NYC an ideal area to study bird diversity (Audubon Society, 2024). A variety of birds inhabit various areas across NYC during winter to seek refuge from the harsh conditions (New York City Bird Alliance, n.d.). As NYC residents, we also find it valuable to know what birds we can enjoy as part of our environment and what birds we can also observe that are not part of our usual ecosystem.

We used a non-invasive method for our study, specifically using DNA barcoding of feather samples collected from three NYC boroughs, Brooklyn, Manhattan, and the Bronx to identify bird species. DNA extraction from birds is not a new concept as feathers have been used as a source of DNA in the past 20 years (Olsen *et al*., 2012). DNA has been successfully extracted from vertebrate tissue and feathers using protocols involving guanidine hydrochloride (Amin *et al.*, 2024) and Qiagen DNeasy Blood and Tissue kit (Amin *et al*, 2024; Peters *et al.*, 2020).

An advantage of using feathers for obtaining DNA is that it is non-invasive as it does not disturb or harm the organism being studied especially if the feathers are molted and not plucked from the bird (Bayard De Volo *et al*., 2008). Molted feathers are also used as indices of bird presence in a habitat or area. An advantage of using barcoding is that DNA barcoding translates expert taxonomic knowledge of diagnostic morphologic characters into a widely accessible format, DNA sequences, enabling more people to identify specimens (Kerr et al., 2007). The cytochrome oxidase I, COI gene is the standard for DNA barcoding in animals. A 658-bp fragment of the gene is used as a "DNA barcode" to tag animal species (Hebert *et al.*, 2003).

Specific aim

The purpose of our research is to learn about the biodiversity of bird species in NYC based on the DNA barcoding of feather samples collected from the three boroughs. We expect to find common species in NYC such as rock doves, *Columbia livia*, and mourning doves, *Zenaida macroura*. We are also interested in the potential collection of feathers of species that are not regular residents of New York. Although the prime months for fall migration for birds occur between mid-August to mid-October, some migrating species over-winter in NYC (New York City Department of Parks & Recreation, 2024). Since our data collection begins in January 2025, we expect to encounter feathers of over-wintering birds in the parks that we will collect from.

Materials & Methods

Our team collected feather samples between January and March 2025 from different localities (built-up residential, recreational parks and nature parks) within each borough. Each member sought to collect at least fifteen presumably different feather samples from each borough. Photos of feather samples were taken, and the location, habitat type and condition, and time and date of collection, were recorded on data collection sheets/forms. At the time of collection, each feather sample was placed in a separate Ziploc bag labeled by borough and sample number. To avoid contaminating the bird DNA, gloves were worn when handling the bird feathers. The samples were stored in our mentor's research lab at City Tech for subsequent processing.

DNA was extracted from each feather sample using both the DNALC's Silica DNA Isolation Method and the DNEASY Qiagen Blood and Tissue kit following the protocols outlined in the DNALC handbook (https://dnabarcoding101.org.). Extracted DNA samples were stored at -20 °C pending subsequent analysis. DNA extracts were amplified by PCR using the forward and reverse COI primers, Bird F1 and Bird R1. The amplicons were analyzed using 1% agarose gel electrophoresis, following the steps in the DNALC manual and using the GeneRuler 100 bp DNA Ladder to estimate the size of the bands. Amplicons with clear bands were sent to Genewiz for Sanger sequencing.

Sequences were processed and further analyzed using the tools in the DNA Subway handbook/manual (https://dnasubway.cyverse.org.). The uploaded sequences were trimmed, and we ran BLAST search in the DNA Subway Blue Line for each sample sequence to match them with those of existing sequences found within the databases. We added reference sequences from these databases and aligned all the sequences using the MUSCLE multiple alignment software and then generated a phylogenetic tree using the Maximum Likelihood Method to show the evolutionary relationships among our samples and the added sequences.

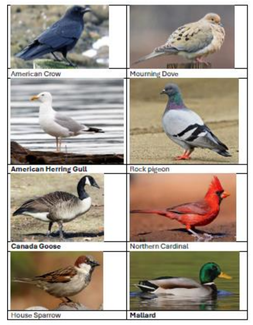

Figure 2. Birds seen in New York City boroughs and Parks during the study period. Birds whose names are written in bold were observed in the Canarsie Pier and Prospect Park (Brooklyn). Other birds were observed in neighborhoods and urban areas.

Results

The BLAST results showed that all our DNA barcoded feather samples were of only the rock pigeon, *Columba livia* (Figure 1). Bird species that we commonly encountered during the study including the rock pigeon are listed in Table 1 and shown in Figure 2.

Sequences of our barcoded samples and reference sequences from databases accessed through the DNA Subway Blue Line were used to construct a phylogenetic tree which shows the relationship between our samples and the reference sequences (Figure 3).

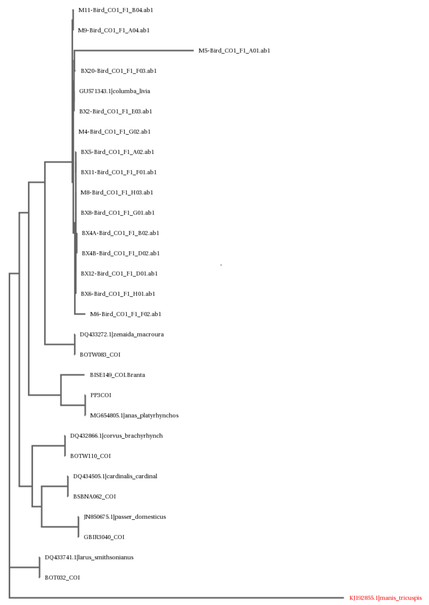

Figure 2. Phylogenetic tree of our bird samples and BLAST results.

Figure 1. The rock pigeon, *Columba livia*

	Common name	Order/family	Scientific name
1.	Rock pigeon	Columbiformes/Columbidae	Columba livia
2.	American crow	Passeriformes/Corvidae	Corvus brachyrhynchos
3.	Mourning dove	Columbiformes/Columbidae	Zenaida macroura
4.	Northern cardinal	Passeriformes/Cardinalidae	Cardinalis cardinalis
5.	House sparrow	Passeriformes/Passeridae	Passer domesticus
6.	American herring gull	Charadriiformes/Laridae	Larus smithsonianus
7.	Canada goose	Anseriformes/Anatidae	Branta canadensis
8.	Mallard	Anseriformes/Anatidae	Anas platyrhynchos

Table 1. List of birds seen in the three New York City boroughs of Brooklyn, Bronx and Manhattan.

Discussion

- In our daily observations, we saw species like the mallard, American crow, and American Herring Gull and expected to find these species in our DNA sequences. However, this was not the case, the only bird species we identified was the Rock dove.
- Our bird feather samples from both the Bronx, Brooklyn, and Manhattan consistently identified the species, Rock dove.
- We had difficulties in obtaining samples due to the weather as we were not able to find many feathers in the colder months. The colder weather also affects birds molt so although there were many bird species we thought we observed, the differences in molting processes and periods impacted what bird feather samples we could find.
- We collected our samples in local parks and streets, focusing on urban areas. Often, similar species inhabit the same area and because we visited areas with similar features, this affected our diversity of samples. Although we observed other species in the areas, different habits impacted the range of our samples.

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