

Introduction

- In May - July 2021, an unusual mass mortality event occurred along the eastern coast and midwestern United States
- Thousands of birds, mostly from order *Passeriformes*, were part of the die off including blue jays (*Cyanocitta cristata*), common grackles (*Quiscalus quiscula*), European starlings (*Sturnus vulgaris*), and American robins (*Turdus migratorius*).
- Clinical signs included conjunctivitis and otitis, with seizures and trouble standing.

Study Site

Figure 1: A total of 102 birds from the United States were submitted to the New Hampshire Veterinary Diagnostic Laboratory (NHVDL) for analysis. Most birds were obtained from New Jersey, Connecticut, and Maryland. Each point on the map represents a single sample and are colored by species. Points locations were randomly generated.

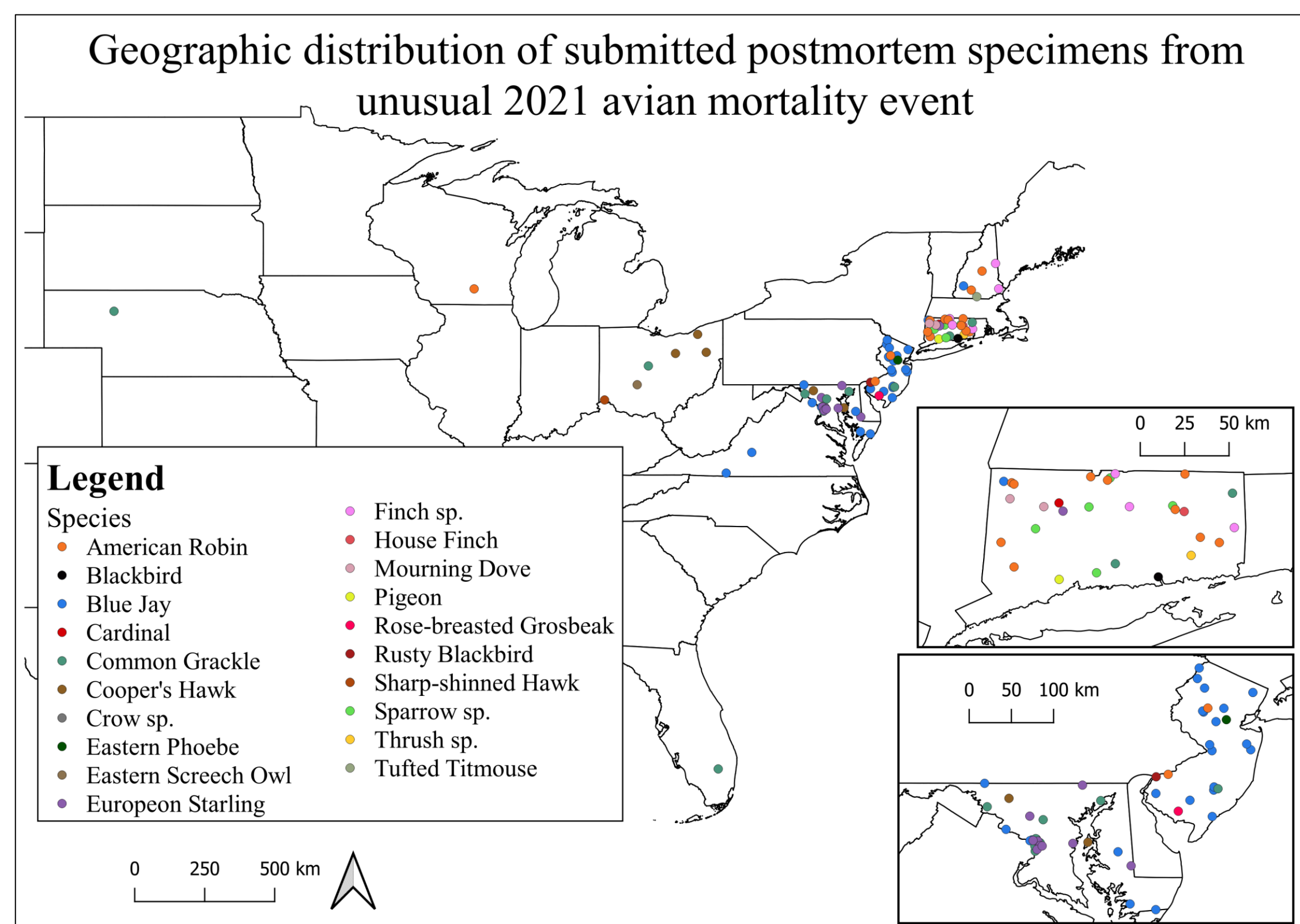
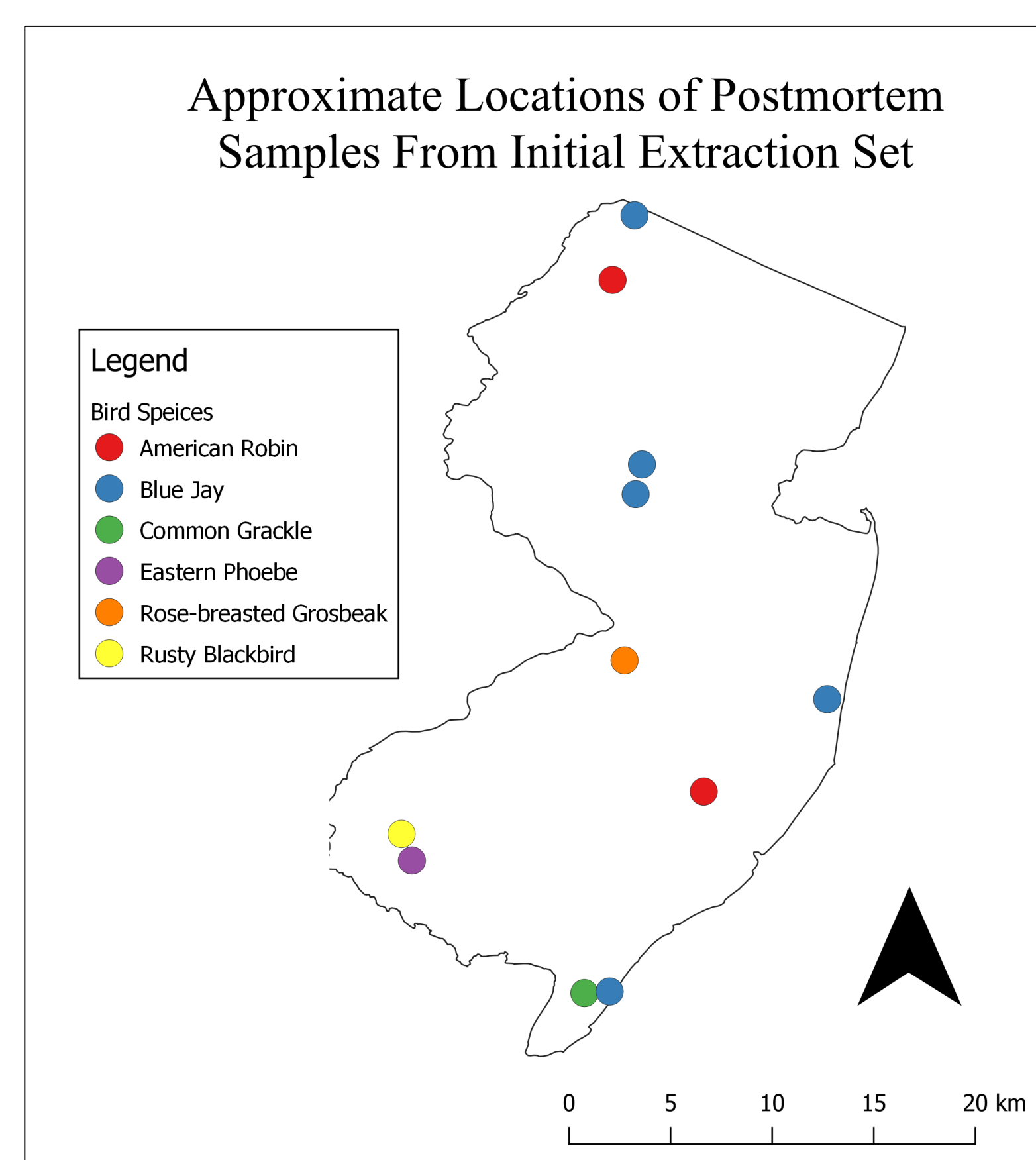


Figure 2: Points were randomly generated for the subset of samples that went into the initial extraction. These samples were analyzed at a deeper coverage level of 20-40 million reads.



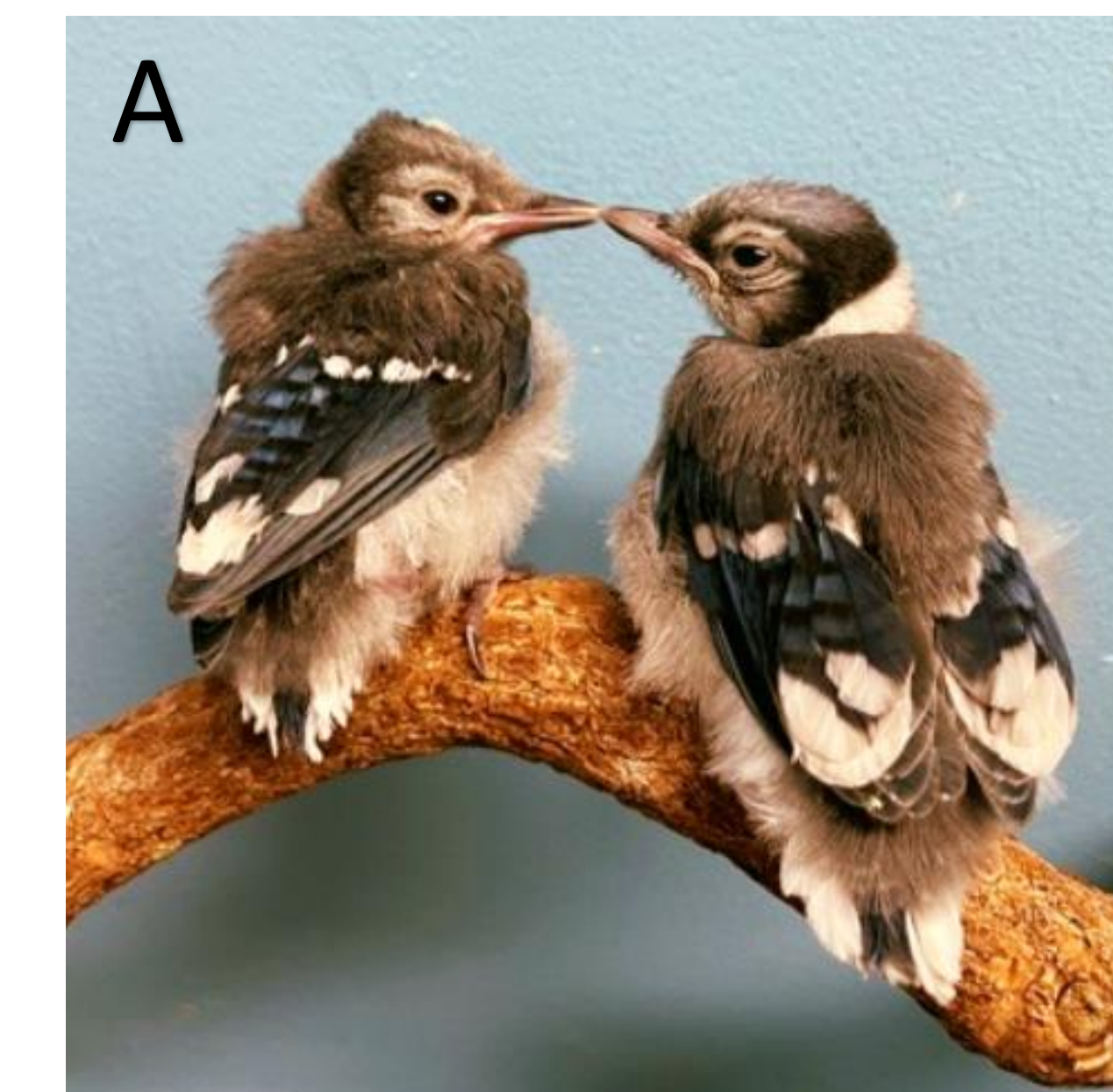
Methods

Sample Collection:

- Prior to submission, all birds were deceased, and then frozen and shipped overnight.
- Once received at the NHVDL, they were thawed for tissue collection.
- A portion of conjunctiva, ear canal, lung, liver, and feces were collected from 87 birds. Fifteen additional samples of conjunctiva were collected in the field and submitted to the NHVDL.
- 20 mg of conjunctiva was subsampled from the total of 102 birds. DNA was extracted using the MagMax DNA Multi-Sample Ultra 2.0 kit.

Metagenomic Sequencing:

- Library preparation was performed using the Kapa HyperPrep kit with TruSeq adapters
- Sequencing was completed on an Illumina NovaSeq 6000 instrument which produced 250 bp paired-end reads for two different datasets.
- One set consists of twelve genomes with coverage for 20-40 million reads each, and the other set has 1-8 million reads each.
- Reads were individually analyzed using blobtools. Datasets were first compared to the most closely related reference genome to filter out at avian reads. Then, the unmapped reads were assembled following a standard whole genome sequencing workflow.



<https://www.cedarrun.org/2021/08/songbird-mortality-updates/>

Photo credit: National Parks Service photographer Leslie Frattaroli
<https://news.delaware.gov/2021/06/30/scientists-investigate-mysterious-songbird-deaths-in-delaware-several-other-states-and-d-c-area/>

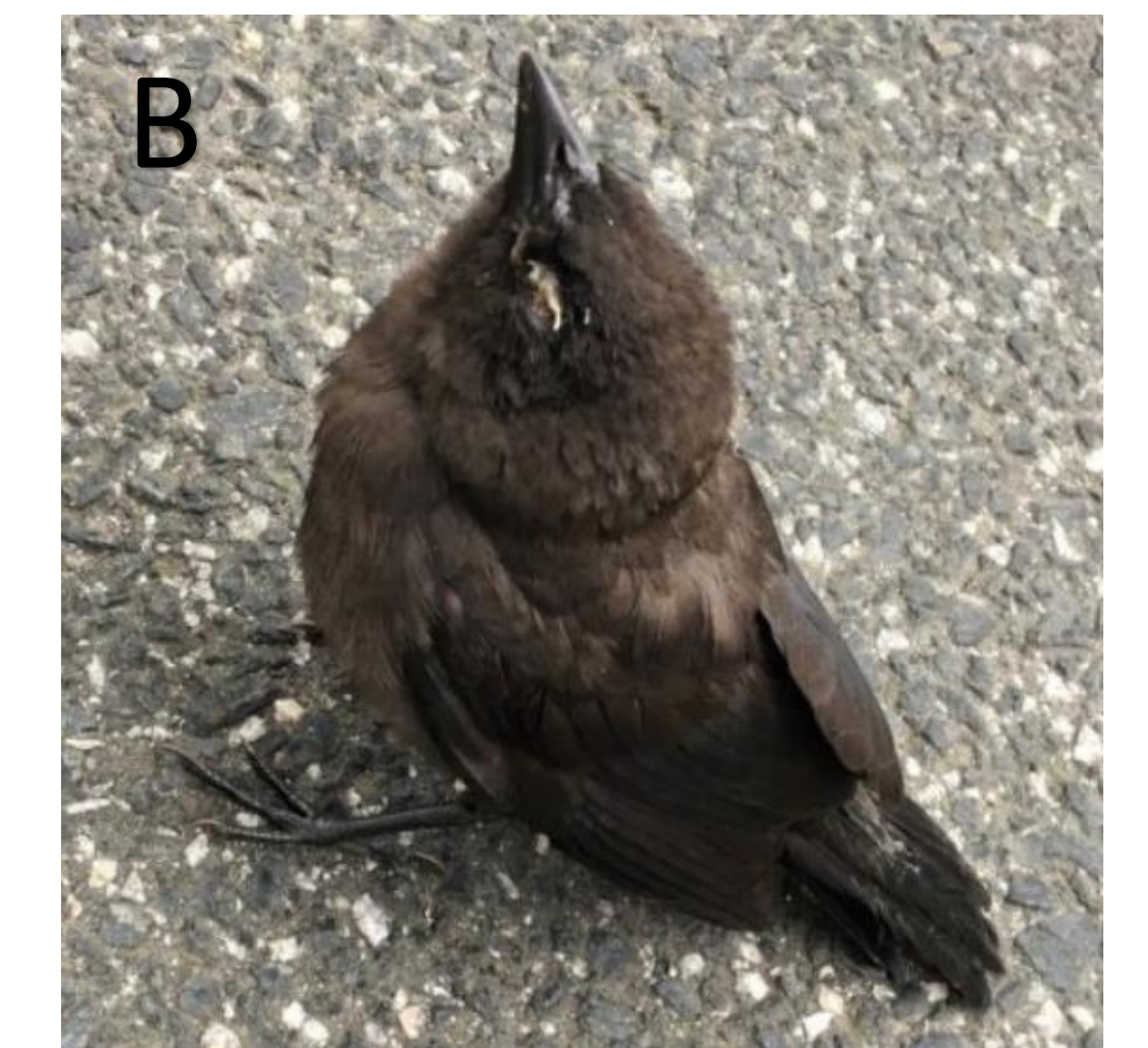


Figure 3: A) Healthy blue jay fledglings sitting on a branch posted by New Jersey Division of Fish and Wildlife. B) Sick common grackle fledgling sitting on the ground. Image posted by Delaware Fish and Wildlife.

Results

- No single common pathogen was found across all samples
- Of the viral species identified within the first batch of extractions, an average of 8.46% of the genome was labeled undefined or unresolved.
- Most identified viruses only appeared in one sample.
- *Gallid alphaherpesvirus 2*, *Murine leukemia virus-related retrovirus*, *Psittacid alphaherpesvirus 1*, and *Xestia c-nigrum granulovirus* were all found in two samples.
- *Pseudaletia unipuncta granulovirus* was found in four samples.
- An average of 3.25% of the bacterial findings were labeled as undefined or unresolved. Of the thousands of identified bacterial species, they belonged to a seven different orders.

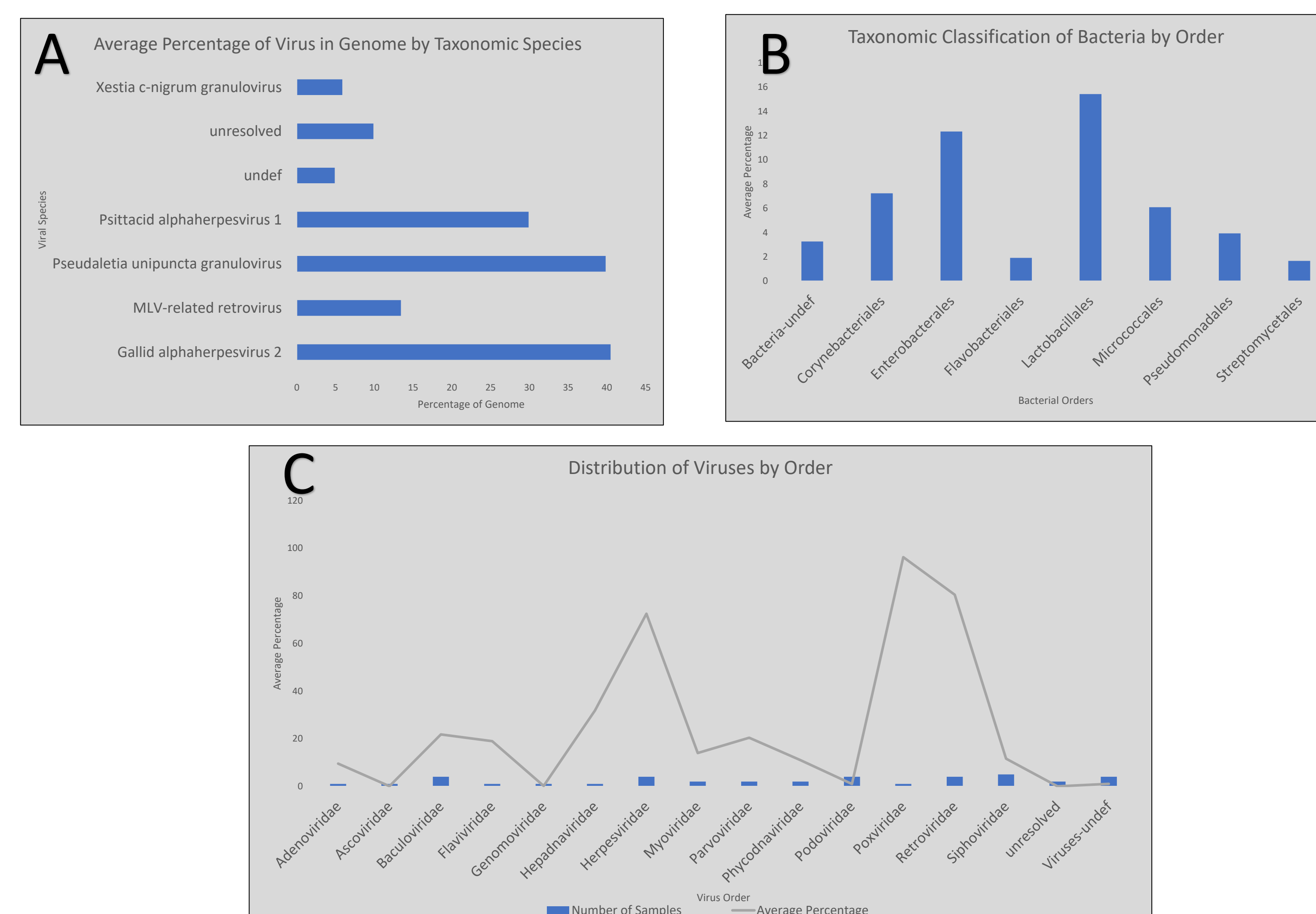


Figure 4: Identified viral and bacterial taxonomy in the first extraction set and their average percentage within the genome. A) Average percentage of each viral species present in the genome of 10 samples. B) Average percentage of each bacterial order identified in avian genomes. C) Average percentage of viral orders and the number of samples they were found in.

Discussion

- The finding of *Gallid alphaherpesvirus 2* is unusual as this virus is more commonly known as Merick's disease which is ubiquitous with chickens
- The presence of *Murine leukemia virus-related retrovirus* may be an incidental finding because similar viruses have been identified in chickens (Borysenko et al.).
- *Psittacid alphaherpesvirus 1* was of interest because this is the causative agent of Pacheco's disease which is a highly contagious respiratory illness in parrots
- *Xestia c-nigrum granulovirus* and *Pseudaletia unipuncta granulovirus* are both viruses that infect insects.
- Granuloviruses which have been used for pest management strategies (Sood et al.).

Conclusion and Next Steps

- The results of this study did not reveal a conclusive explanation for the high mortality rate of songbirds observed in 2021 but did outline possible future studies.
- Next steps include:
 - Analyzing all other samples at a deeper level to compare the viral taxonomic composition found in their genomes.
 - Investigate the ecological impacts of each virus
- A follow-up study regarding the *Psittacid herpesvirus* is currently underway with samples from new birds in New Jersey.

Acknowledgements

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