Assessing the Usefulness of DNA Barcoding at Biological Field Stations

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ABSTRACT

DNA barcoding is an effective tool for discovering and documenting biodiversity and can also provide insights into species origins, and within species, evolutionary patterns. In animals, DNA barcoding involves using a 648 base-pair region of the mitochondrial gene cytochrome c oxidase 1 (CO1) to categorize and identify species. DNA barcoding using CO1 sequences has been effective in identifying bird species and, in some cases, distinct populations or subspecies. For my undergraduate thesis, I laid the groundwork for a DNA barcoding library for the bird species found at the Queen's University Biological Station (QUBS), which is linked to a collection of identified and archived specimens. Bird collections provide a map of avian genetic diversity, reveal cryptic species, and can serve as a baseline for monitoring effects of anthropogenic and climatic changes on bird populations. Accessible resources such as DNA barcoding datasets and associated avian collections can increase the use and scientific prominence of field stations and their research and better position them to address environmental challenges. I evaluate the proposal that DNA barcoding of the bird species found at QUBS will provide information on the breeding population origin for migrants and on evolutionary affinities for both migrant and resident species where there is documented phylogeographic structure.

Key words**:** DNA barcoding, cytochrome c oxidase 1, evolutionary affinity, avian collections

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LIST OF ABBREVIATIONS

BLAST: Basic Local Alignment Search Tool

BOLD: Barcode of Life Database

CO1: cytochrome c oxidase 1

mtDNA: mitochondrial DNA

PCR: polymerase chain reaction

QUBS: Queen's University Biological Station

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INTRODUCTION

The planet is facing a human-caused 6th mass extinction (Barnosky et al., 2011). According to an Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) report (United Nations, 2019), one million species globally are on the verge of extinction. Documenting biodiversity is becoming increasingly important, particularly for species on the decline which may become extinct before they are ever described or given a scientific name (Klippel et al., 2015). There are an estimated 8.7 million eukaryotic species globally, of which only around 1.2 million have been formally classified, with many listed in a central database: the Catalogue of Life. Based on this estimate, 86% of species have not yet been formally described (Mora, Tittensor, Adl, Simpson & Worm, 2011).

DNA Barcoding

DNA barcoding is an effective tool for documenting biodiversity and can also provide insight into the origins of species, and even the evolutionary history of individual conspecific populations. It allows us to translate taxonomic knowledge into widely accessible DNA sequences, which enable more people to identify specimens without formal taxonomic training (Kerr et al., 2007). Barcoding was first proposed by Hebert et al. (2003) to standardize DNA-based species identification and provide a quick and affordable tool to assess biodiversity. It involves using a short, standardized region of a genome to categorize and identify species. For animals, a 648 base-pair region of the mitochondrial gene cytochrome c oxidase 1 (CO1) is typically used (Henter, Imondi, James, Spencer & Steinke, 2016), which can be easily recovered with a limited set of primer pairs in conserved regions of targeted taxonomic groups (Kerr et al., 2007).

Because the taxonomy of birds is well-developed and birds are a major focus of ecological, evolutionary and systematics research, they are a useful group to test the efficacy of DNA barcoding (Kerr et al., 2007). DNA barcoding using CO1 sequences has proved effective for identifying bird species, and in some cases distinct populations or subspecies, across their geographical ranges (Tizard et al., 2019; Kerr et al., 2007). Hebert et. al. (2004) indicated that, of 643 North American bird species evaluated at that time, 94% of species had diagnostic barcode sequences in databases like GenBank. As of 2016, DNA barcodes were available for 41% of known bird species globally (Barreira, Lijtmaer & Tubaro, 2016).

Several studies have found that CO1 barcodes effectively identify animal species (Kerr et al., 2007). Hajibabaei et al. (2006) found that DNA barcoding could distinguish 97.9% of 521 species of tropical Lepidoptera. Depending on the family, the average within-species divergence ranged from 0.17% to 0.46%. In Australian fish species, 100% of 207 species analyzed could be differentiated using CO1 barcoding, with an average within-species divergence of 0.39% (Ward, Zemlak, Innes, Last & Hebert, 2005). In a study looking at over 260 species of North American birds, the variation of CO1 sequences between species was around 18 times greater than within species (7.93% compared to 0.43%), and no two species had the same barcode (Hebert, Stoeckle, Zemlak & Francis, 2004).

DNA barcoding data can be used to identify new or cryptic species, since large differences in mitochondrial DNA (mtDNA) sequences in animals generally indicate different species (Hebert, Stoeckle, Zemlak & Francis, 2004; Campagna et al., 2010; Mendoza et al., 2016; Winker et al., 1991). For example, most recent bird species splits (i.e. diagnosing populations once thought to be conspecific as distinct species) have been the result of molecular phylogenetic or phylogeographic studies (Kerr et al., 2007).

DNA barcoding is especially useful when morphology-based identification is not possible (Hebert, Stoeckle, Zemlak & Francis, 2004), and only requires a small amount of tissue (Mishra et al., 2017). For example, when looking at remnants in the stomachs of predators or at remains of birds that strike aircraft (Yang, Wu, Yan & Li, 2009; Dove, Rotzel, Heacker & Weigt, 2008), or at animals hit by cars (Klippel et al., 2015), identification based on phenotypic attributes may fail. DNA barcoding can also be used to tackle the illegal trade of endangered species by providing a tool to easily identify trafficked species and their likely origins (Mishra et al., 2017; Parveen, Singh, Raghuvanshi, Pradhan & Babbar, 2011). Further uses include forensic identification (Abe, Hayano & Inoue-Murayama, 2011), revealing the species identity in the blood meals of mosquitoes, distinguishing between similar juveniles or among nonbreeding adults lacking diagnostic nuptial plumage in banding work, and providing a nonlethal means to survey endangered populations (Hebert, Stoeckle, Zemlak & Francis, 2004).

One criticism of the standardized CO1 barcoding approach is that it is based on a single maternally inherited gene, instead of multiple nuclear genes. As well, early tests of the method did not compare sister species that are by definition each other's closest relative, so the efficacy of DNA barcoding for identifying species may have been overestimated (Tavares & Baker, 2008). Tavares and Baker (2008) addressed these criticisms by comparing closely related sister species of birds. They found that the CO1 gene distinguishes between sister species pairs of birds that are diverged by as little as 0.6-0.9%, and that CO1 barcoding produced similar results to multi-gene approaches.

Avian Collections

The value of a DNA barcode is enhanced by an associated preserved and identified study skin (Stoeckle & Winker, 2009). Avian study skin collections preserve the avian record (e.g. locality

where a species occurs, plumage variants across the range) and are a valuable resource for both present and future research and education (Winker et al., 1991). Historically, the primary purpose of avian collections has been to document avian diversity and its distribution (Winker, 2005). They have served as a foundation for much of our understanding of birds and help us document individual, geographic, and temporal variation (Winker et al., 1991; Winker, 2004). On a large scale, these collections provide a map of avian genetic diversity, reveal cryptic species, and can serve as a baseline for monitoring effects of anthropogenic and climatic changes on bird populations (Stoeckle & Winker, 2009).

Winker (2005) argues that avian collections are likely the most dependable shared resource in ornithology. Their useful lifespan is much longer than most other resources; properly preserved specimens are useful for centuries (Winker et al., 1991). They are continuing sources of data that repeatedly provide information about population and environmental conditions (Winker, 2005). Birds are excellent bioindicators of environmental conditions and specimens that have been preserved for over a century can provide information about the environments in which they lived (e.g. via stable isotope analysis) (Winker, 2005).

Avian collections document geographic space, biodiversity space, and time for each species (Winker, 2005), with each specimen representing a unique point in time and space (Winker et al., 1991). The continued sampling of birds is necessary to assess changes over time (Winker, 2005). The immediate research value of a specimen does not necessarily predict its future importance (Remsen, 1995). Indeed, its scientific value increases over time (Winker, 2005). Avian collections provide baselines from which hypotheses are developed and tested and provide answers to questions that were never imagined by those who originally built the collections (Winker, 2005). Archiving specimens enables the future implementation of

retrospective studies and historical specimens are being increasingly used to assess population and environmental changes (e.g. in food, habitat, diseases) (Winker, 2005).

There is strong evidence that archiving specimens will enhance future wildlife management and conservation efforts (Winker, 2005). Conservation priorities can only be established reliably if the genetic boundaries between species are established correctly, and the continued collecting of specimens has been critical in classifying birds to the species level (Remsen, 1995). For example, conservation efforts to establish priorities for land acquisition depend on accurate knowledge of the taxa present in the area, which in many cases requires the analysis of specimens in a regional collection (Remsen, 1995). In regions where habitat has been extensively altered, the use of older specimens may be the only way to determine which bird species would have once lived there (Remsen, 1995). Further, changing taxonomies and studies of migration and distribution often rely on the examination of specimens, and changes in the characteristics of populations can be monitored using bird collections (Winker et al., 1991).

Specimens also provide an inspiration for research; the examination of avian collections has stimulated many research ideas (Remsen, 1995). When these collections are a shared resource (i.e. when specimens in the collections can be loaned to others for research), researchers have the opportunity to do work that they may not have otherwise been able to do, which provides increased knowledge about that resource (Winker, 2005). Individual institutions, like research stations, universities, and museums, can have a great impact by building their resources, making them apparent to the global community, and contributing to the number of avian collections across the world (Stoeckle & Winker, 2009).

Purpose of my Research

As of 2017, 496 bird species have been recorded in Ontario (OFO, 2017). Of those, 228 are found at the Queen's University Biological Station (QUBS), of which about 50% are migrants (Schoepf, Conboy & Martin, 2018). Within a species, there may be several different mitochondrial lineages (e.g. Common yellowthroat, *Geothlypis trichas*, Ball & Avise, 1992). On average, mtDNA evolves more rapidly than nuclear DNA, so differences between closely related species accumulate, even between populations that have only been separated for a relatively short time. Divergences provide a record of evolutionary history within species and, via phylogeographic approaches, can provide insights into the processes that have shaped contemporary patterns (Hebert, Stoeckle, Zemlak & Francis, 2004). Thus, DNA barcoding of individuals from a particular location, like QUBS, can enrich our understanding of migration patterns (e.g. breeding population origin) and evolutionary histories (i.e. affinity to different intraspecific lineages).

QUBS has a collection of bird specimens (kept in freezers) that were killed by either road or window collisions. The bird species in the QUBS collection are a mix of migrants (spring and fall) and residents (year-round). For my project I began to establish a DNA barcoding library for the bird species found at QUBS, linked to identified and archived specimens. I predict that DNA barcoding can be used to diagnose breeding population origin of migrant species. For example, one of my specimens is a Tennessee warbler (*Leiothlypis peregrina*), caught in the fall on Queen's main campus. Its origin is unknown, but by obtaining a CO1 DNA sequence for it and comparing it to sequences of other Tennessee warblers in the Barcode of Life Database (BOLD) and GenBank, I predict that I will be able to determine intraspecific evolutionary relationships and thus its breeding population. For both resident and migrant species, I predict that DNA

barcodes will provide an evolutionary context in cases where there is documented phylogeographic structure or multiple sequences in one of the online databases. I am testing these predictions by collecting tissue samples from different bird species (from road mortality or window collisions), using standardized published primers to amplify and sequence the CO1 gene, and then comparing my sequences with existing sequences in BOLD and GenBank.

I prepared study skins for all individuals from which I took tissue samples so that each DNA sequence I obtain will match a study skin in the QUBS collection with catalogue number, collection date, and provenance. A DNA barcoding library that is linked to identified specimens is a powerful tool for identifying species (Hebert, Stoeckle, Zemlak & Francis, 2004) and a valuable resource for future research projects, conservation efforts, and educational purposes. Further, natural history collections are repositories of the diversity of life and are a record of former taxonomic interpretations of biodiversity (Puillandre et al., 2012).

METHODS

Obtaining Bird Specimens

The birds used in this thesis were killed by either window or road collisions and were either collected by QUBS researchers or brought to the station by community members. After death, tissue begins to decompose and DNA begins to degrade (Winker, 2000). Freezing immediately after death helps preserve the birds until it is time to prepare them (Winker, 2000). Birds were kept in freezers at QUBS and each bird was labelled with the date and location it was found, and any other relevant information such as the collector. This information can be found in *Appendix D.*

Preparing Study Skins

Tools for skinning: scalpel, scissors, tweezers, blunt probe, cotton, thread, needles, pins, tubes, Styrofoam, sticks, ethanol, borax.

Summary of skinning process (for more details, see *Appendix A.1*): I weighed (using an electronic scale) and measured total length for each specimen prior to preparing the study skins. I took a tissue sample from the breast of each specimen. I separated the skin from the muscle and fat tissue and removed the tissue and organs. I rolled cotton onto a stick to make a body, arranged the skin around it and then sewed the skin back together. I rearranged the feathers and then pinned the study skin to a Styrofoam board to allow it to dry.

Obtaining DNA Sequences

1) DNA Extraction

I used salt-extraction to extract DNA from each tissue sample (Aljanabi & Martinez, 1997) (see *Appendix A.2*). Before beginning, I sterilized the tools (tweezers, forceps) using flaming 95% ethanol and distilled water. I labelled each tube containing the tissue sample.

2) Analysis of DNA

I used a 2% Tris Borate EDTA (TBE) agarose gel electrophoresis stained with RedSafe Nucleic Acid Staining Solution (iNtRON Biotechnology Inc., Seongnam, Gyeonggi, South Korea) to assess the quality of the DNA (i.e. whether the DNA was of high-molecular weight, and non-sheared). I assessed DNA purity using the A260/A280 ratio on a Nanodrop spectrophotometer.

3) Polymerase Chain Reaction

I used polymerase chain reaction (PCR) to amplify the DNA using standard avian CO1 primers BirdF1 (5′-TTCTCCAACCACAAAGACATTGGCAC-3′) and CO1birdR2 (5′-ACGTGGGAGATAATTCCAAATCCTGG-3′) following the method of Kerr, Lijtmaer, Barreira, Hebert & Tubaro (2009).

PCR reactions were done in 25 μL volumes containing 2 μL of DNA, 4.35 μL of H_2O, 1.2 μL of 10 mM BirdF1 forward primer, 1.2 μL of 10 mM CO1birdR2 reverse primer, 3.75 μL of 1 mg/ml Bovine Serum Albumin (BSA) and 12.5 μL of 2X Taq FroggaMix Master mix (FroggaBio, Toronto, ON, Canada). Amplifications were done in an Eppendorf 5331 MasterCycler Gradient Thermal Cycler (Eppendorf, Hamburg, Germany). The amplification cycling parameters were: an initial denaturing step at 94 °C for 5 min, followed by 40 cycles of denaturing at 94 °C for 30 s, annealing at 51 °C for 45 s, and extension at 72 °C for 60 s, then a final extension phase at 72 °C for 7 min. I visualized amplicons using a 2% Tris Borate EDTA (TBE) agarose gel stained with RedSafe Nucleic Acid Staining Solution (iNtRON Biotechnology Inc., Seongnam, Gyeonggi, South Korea) to test for successful PCR amplification.

4) Clean-up, Sequencing and Analysis

I further purified the DNA using a Beads cleanup protocol adapted from Rohland, & Reich, 2012. I then sent the DNA to the Centre for Applied Genomics for Sanger sequencing. After receiving the DNA chromatogram files, I checked their quality visually. A BOLD (http://v3.boldsystems.org/) and Basic Local Alignment Search Tool (BLAST) (https://BLAST.ncbi.nlm.nih.gov/BLAST.cgi) search of each sequence was done to identify

sequences with the closest affinities. For species with multiple sequences from across their range, this allowed the most similar sequence to be determined and for the discovery of regionally significant affinities.

RESULTS

The species included in this study are: Dark-eyed junco (SPECIES: *Junco hyemalis*; ORDER: Passeriformes; FAMILY: Passerellidae); Pileated woodpecker (SPECIES: *Dryocopus pileatus*; ORDER: Piciformes; FAMILY: Picidae); American goldfinch (SPECIES: *Spinus tristis*; ORDER: Passeriformes; FAMILY: Fringillidae); Black-billed cuckoo (SPECIES: *Coccyzus erythropthalmus*; ORDER: Cuculiformes; FAMILY: Cuculidae); Blue jay (SPECIES: *Cyanocitta cristata*; ORDER: Passeriformes; FAMILY: Corvidae); American yellow warbler (SPECIES: *Setophaga petechia*; ORDER: Passeriformes; FAMILY: Parulidae); White-crowned sparrow (SPECIES: *Zonotrichia leucophrys*; ORDER: Passeriformes; FAMILY: Passerellidae); Baltimore oriole (SPECIES: *Icterus galbula*; ORDER: Passeriformes; FAMILY: Icteridae); Black-capped chickadee (SPECIES: *Poecile atricapillus*; ORDER: Passeriformes; FAMILY: Paridae); Hermit thrush (SPECIES: *Catharus guttatus*; ORDER: Passeriformes; FAMILY: Turdidae); Tennessee warbler (SPECIES: *Leiothlypis peregrina*; ORDER: Passeriformes; FAMILY: Parulidae); Yellow-bellied sapsucker (SPECIES: *Sphyrapicus varius*; ORDER: Piciformes; FAMILY: Picidae); Barred owl (SPECIES: *Strix varia*; ORDER: Strigiformes; FAMILY: Strigidae). All the specimens in my thesis were collected in the Kingston and Rideau Lakes areas (see *Appendix B*, Figure 1 for a map of the area). *Appendix D* provides additional details on individual specimens.

I compared the published CO1 barcode sequence of each species to sequences contained in GenBank (see *Appendix C* for the full CO1 sequences of the specimens in this thesis). Table 1

shows the accession numbers in GenBank that most closely matched my sequences and details about their origins.

Specimen 03R_DEJU-CO1 (Dark-eyed junco) has a 100% identity match to ten other individuals of the same species. These individuals were sampled throughout the United States and Western Canada across a significant span of the species' range. Specimen 04F_PIWO-CO1 (Pileated woodpecker) has a 99.70% identity match to another individual of the same species, whose origin was not reported. It also has a 99.54% identity match to two other Pileated woodpecker individuals whose origins are Florida and not reported. Specimen 05F_AMGO-CO1 (American goldfinch) has a 99.85% identity match to four other individuals of the same species. Their origins are California, Ontario, New Brunswick, and not reported. Specimen 06F_BBCU-CO1 (Black-billed cuckoo) has a 99.85% identity match to another individual of the same species, which originated in Ontario. Specimen 01_BLJA_CO1 (Blue jay) has a 99.55% identity match to another individual of the same species, which originated in Ontario, and a 99.54% identity match to two other individuals of the same species, whose origins were not reported. Specimen 02_YEWA_CO1 (American yellow warbler) has a 99.85% identity match to two other individuals of the same species, whose origins are Ontario and Nova Scotia. Specimen 03_WCSP-1_CO1 (White-crowned sparrow) has a 98.35% identity match to another individual of the same species, which originated in Ontario. Specimen 08_BAOR_CO1 (Baltimore oriole) has a 95.78% identity match to two other individuals of the same species, which originated in Ontario and Quebec. Specimen 04_BCCH-1_CO1 (Black-capped chickadee) has a 97.44% identity match to another individual of the same species, which originated in the Yukon. Specimen 06_HETH_CO1 (Hermit thrush) has a 100% identity match to eight other individuals of the same species. These originated in Ontario, Nova Scotia, and British Columbia. The origins

of two of the individuals were not reported. Specimen 09_BBCU-1_CO1 (Black-billed cuckoo) has a 98.95% identity match and a 98.94% identity match to two other individuals of the same species, both originating in Ontario. Specimen 11_TEWA_CO1 (Tennessee warbler) has a 100% identity match to another individual of the same species, which originated in Quebec. Specimen 12_YBSA_CO1 (Yellow-bellied sapsucker) has a 99.85% identity match to another individual of the same species, originating somewhere in the United States. Specimen 02R_BADO_CO1 (Barred owl) does not match with any individual of the same species.

I also extracted DNA from the tissues of two other Black-capped chickadee specimens, and from two unlabelled tissue samples that had been previously collected from prepared specimens. The PCR did not work for these tissues and the sequences were of poor quality, so they were not included in the results of this study.

DISCUSSION

Along with other researchers, I prepared study skins from 13 different bird species from four orders and ten families. Reflecting their diversity and relative abundance in nature but also the fact that they are most often killed by collisions with windows, most specimens were perching birds (ORDER: Passeriformes). Of the 13 species, five are residents at QUBS and eight are migrants (see Table 1).

The BLAST searches of the CO1 sequences that I obtained provided varying degrees of insight. The usefulness of a BLAST search depends very much on the number of specimens that have been deposited in GenBank, the details that authors provide (e.g. provenance of the specimens), the thoroughness of sampling, the DNA markers used, and the scope of research that generated these sequences.

The BLAST searches were successful in identifying all specimens to the species level, except for the Barred owl. In some instances, the individuals from Rideau Lakes or Kingston and environs matched most closely with other individuals from Ontario or Quebec. The Black-billed cuckoos, Blue jay, and White-crowned sparrow specimens matched most closely with specimens on GenBank sampled in Ontario. The Baltimore oriole matched most closely with specimens sampled in Ontario and Quebec and the Tennessee warbler matched most closely with a specimen sampled in Quebec. In other instances, the BLAST searches gave matches for individuals that were sampled at distant locales in Canada or the United States and did not have any regional significance. These include Dark-eyed junco, Pileated woodpecker, American goldfinch, American yellow warbler, Black-capped chickadee, Hermit thrush, and Yellow-bellied sapsucker.

Studies have found high levels of intraspecific mtDNA variation among bird populations (Bates et al., 1999; Milá et al., 2012). Regional genetic differences within species may indicate distinct evolutionary lineages between different populations (Bates et al., 1999). These differences can provide insights into the processes that have shaped contemporary distribution patterns and genetic structure (Hebert, Stoeckle, Zemlak & Francis, 2004). My results support this finding, but further sampling and analysis are needed to determine whether DNA barcoding using the CO1 gene can distinguish between distinct lineages within the species listed above and to determine breeding population origins for migrants at QUBS. This thesis has laid the groundwork for future research on the evolutionary significance of intraspecific variation in the CO1 gene as well as for a DNA barcoding library associated with preserved specimens in the QUBS collection.

Future Work and Prospects for the Development of this Resource

Once we have a QUBS catalogue number for each specimen, we will submit sequence data to BOLD and GenBank, to establish a reference library for the bird species found at QUBS. For sequences in GenBank with a close affinity but where the origin is not reported (see Table 1), we will write to the authors asking for details on the origins of those specimens. We will use this information to gain insight on evolutionary affinities of the specimens in this study.

As QUBS' barcoding library and associated study skin collection grow, their usefulness as research resources will increase. Once the library is established in GenBank, therefore, the focus should be on expanding the library and the associated specimen collection. To do this, we will use social media to spread awareness about the library and associated collection and their importance as resources for research and ask community members to bring dead bird specimens they find to QUBS. As QUBS receives more bird specimens, we will train more people to prepare study skins and take tissue samples for DNA extraction. Having more people trained to prepare study skins will ensure that DNA does not degrade before analysis occurs (Winker, 2000). Members of Dr. Lougheed's lab will extract DNA from the tissue samples and prepare them for sequencing. Sequences will then be deposited in GenBank and BOLD to be added to QUBS's barcoding library.

Significance

As more species become endangered and face extinction, it is increasingly important to be able to quickly diagnose species. DNA barcoding is an effective conservation tool for documenting biodiversity. Conservation and research are core mandates of QUBS (https://qubs.ca) and a DNA barcoding library for QUBS can aid its conservation efforts and serve as a basis for present and

future research projects (Francis et al., 2010), both at QUBS and elsewhere. As well, this library and associated skin collection create a genetic record of what species are present at QUBS and strengthen its research collection resources. Finally, increased access to research data from field stations enables others to use the data (Brunt & Michener, 2009) and contributes to collaborative global efforts to assess and understand environmental change (Tydecks, Bremerich, Jentschke, Likens & Tockner, 2016). In this way, accessible resources such as DNA barcoding datasets and bird collections can increase the use and scientific prominence of field stations like QUBS and their research and better position them to address environmental challenges (National Research Council, 2014).

REFERENCES

Abe, H., Hayano, A., & Inoue-Murayama, M. (2011). Forensic species identification of large macaws using DNA barcodes and microsatellite profiles. *Molecular Biology Reports, 39*(1), 693-699. doi: 10.1007/s11033-011-0787-1

Aliabadian, M., Beentjes, K. K., Roselaar, C. S., van Brandwijk, H., Nijman, V., & Vonk, R. (2013). DNA barcoding of Dutch birds. *ZooKeys,* (365), 25–48. https://doi.org/10.3897/zookeys.365.6287

Aljanabi, S., & Martinez, I. (1997). Universal and rapid salt-extraction of high-quality genomic DNA for PCR- based techniques. *Nucleic Acids Research, 25*(22), 4692-4693. https://doi.org/10.1093/nar/25.22.4692

Barnosky, A., Matzke, N., Tomiya, S., Wogan, G., Swartz, B., & Quental, T. et al. (2011). Has the Earth's sixth mass extinction already arrived? *Nature, 471*(7336), 51-57. doi: 10.1038/nature09678

Ball, M., & Avise, J. (1992). Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *The Auk,* 109(3). doi: 10.1093/auk/109.3.626

Barreira, A., Lijtmaer, D., & Tubaro, P. (2016). The multiple applications of DNA barcodes in avian evolutionary studies. *Genome, 59*(11), 899-911. doi: 10.1139/gen-2016-0086

Bates, J., Hackett, S., & Goerck, J. (1999). High levels of mitochondrial DNA differentiation in two lineages of Antbirds (*Drymophila* and *Hypocnemis*). *The Auk, 116*(4), 1093-1106. https://doi.org/10.2307/4089688

Brunt, J., & Michener, W. (2009). The resource discovery initiative for field stations: enhancing data management at North American biological field stations. *Bioscience, 59*(6), 482-487. doi: 10.1525/bio.2009.59.6.6

Campagna, L., Lijtmaer, D., Kerr, K., Barreira, A., Hebert, P., Lougheed, S., & Tubaro, P. (2010). DNA barcodes provide new evidence of a recent radiation in the genus Sporophila (Aves: Passeriformes). *Molecular Ecology Resources, 10*(3), 449-458. doi: 10.1111/j.1755-0998.2009.02799.x

Dove, C., Rotzel, N., Heacker, M., & Weigt, L. (2008). Using DNA Barcodes to identify bird species involved in birdstrikes. *Journal of Wildlife Management, 72*(5), 1231-1236. doi: 10.2193/2007-272

Francis, C., Borisenko, A., Ivanova, N., Eger, J., Lim, B., & Guillén-Servent, A. et al. (2010). The role of DNA barcodes in understanding and conservation of mammal diversity in Southeast Asia. *Plos ONE, 5*(9), e12575. doi: 10.1371/journal.pone.0012575

Hajibabaei, M., Janzen, D., Burns, J., Hallwachs, W., & Hebert, P. (2006). DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings Of The National Academy of Sciences, 103*(4), 968-971. doi: 10.1073/pnas.0510466103

Hebert, P., Cywinska, A., Ball, S., & deWaard, J. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(1512), 313-321. doi: 10.1098/rspb.2002.2218

Hebert, P., Stoeckle, M., Zemlak, T., & Francis, C. (2004). Identification of birds through DNA barcodes. *Plos Biology, 2*(10), e312. doi: 10.1371/journal.pbio.0020312

Henter, H., Imondi, R., James, K., Spencer, D., & Steinke, D. (2016). DNA barcoding in diverse educational settings: five case studies. *Philosophical Transactions of the Royal Society B: Biological Sciences, 371*(1702), 20150340. doi: 10.1098/rstb.2015.0340

IPBES. (2019). Global assessment report on biodiversity and ecosystem services.

Kerr, K., Stoeckle, M., Dove, C., Weigt, L., Francis, C., & Hebert, P. (2007). Comprehensive DNA barcode coverage of North American birds. *Molecular Ecology Notes, 7*(4), 535-543. doi: 10.1111/j.1471-8286.2007.01670.x

Kerr, K., Lijtmaer, D., Barreira, A., Hebert, P., & Tubaro, P. (2009). Probing evolutionary patterns in Neotropical birds through DNA barcodes. *Plos ONE, 4*(2), e4379. doi: 10.1371/journal.pone.0004379

Klippel, A., Oliveira, P., Britto, K., Freire, B., Moreno, M., & dos Santos, A. et al. (2015). Using DNA barcodes to identify road-killed animals in two Atlantic forest nature reserves, Brazil. *Plos ONE, 10*(8), e0134877. doi: 10.1371/journal.pone.0134877

Mendoza, Á., Torres, M., Paz, A., Trujillo-Arias, N., López-Alvarez, D., & Sierra, S. et al. (2016). Cryptic diversity revealed by DNA barcoding in Colombian illegally traded bird species. *Molecular Ecology Resources, 16*(4), 862-873. doi: 10.1111/1755-0998.12515

Milá, B., Tavares, E., Muñoz Saldaña, A., Karubian, J., Smith, T., & Baker, A. (2012). A trans-Amazonian screening of mtDNA reveals deep intraspecific divergence in forest birds and suggests a vast underestimation of species diversity. *Plos ONE, 7*(7), e40541. https://doi.org/10.1371/journal.pone.0040541

Mishra, P., Kumar, A., Sivaraman, G., Shukla, A., Kaliamoorthy, R., Slater, A., & Velusamy, S. (2017). Character-based DNA barcoding for authentication and conservation of IUCN Red listed threatened species of genus *Decalepis* (Apocynaceae). *Scientific Reports, 7*(1). doi: 10.1038/s41598-017-14887-8

Mora, C., Tittensor, D., Adl, S., Simpson, A., & Worm, B. (2011). How many species are there on Earth and in the ocean? *Plos Biology, 9*(8), e1001127. doi: 10.1371/journal.pbio.1001127

National Research Council. (2014). Enhancing the value and sustainability of field stations and marine laboratories in the 21st century. *National Academies Press*. doi: 10.17226/18806

OFO. (2017). Checklist of the birds of Ontario. Retrieved from http://www.ofo.ca/site/page/view/checklist.checklist

Parveen, I., Singh, H., Raghuvanshi, S., Pradhan, U., & Babbar, S. (2011). DNA barcoding of endangered Indian *Paphiopedilum* species. *Molecular Ecology Resources, 12*(1), 82-90. doi: 10.1111/j.1755-0998.2011.03071.x

Remsen, J. (1995). The importance of continued collecting of bird specimens to ornithology and bird conservation. *Bird Conservation International, 5*(2-3), 146-180. https://doi.org/10.1017/s095927090000099x

Rohland, N., & Reich, D. (2012). Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Research, 22*(5), 939-946. https://doi.org/10.1101/gr.128124.111

Schoepf, I., Conboy, M., & Martin, P. (2018). QUBS bird species. Retrieved from https://qubs.ca/sites/default/files/2019-01/QUBS_Birds_species_list_2018.pdf

Stoeckle, M., & Winker, K. (2009). A global snapshot of avian tissue collections: state of the enterprise. *The Auk, 126*(3), 684-687. doi: 10.1525/auk.2009.9709a

Tavares, E., & Baker, A. (2008). Single mitochondrial gene barcodes reliably identify sister-species in diverse clades of birds. *BMC Evolutionary Biology, 8*(1), 81. doi: 10.1186/1471-2148-8-81

Tizard, J., Patel, S., Waugh, J., Tavares, E., Bergmann, T., & Gill, B. et al. (2019). DNA barcoding a unique avifauna: an important tool for evolution, systematics and conservation. *BMC Evolutionary Biology, 19*(1). doi: 10.1186/s12862-019-1346-y

Tydecks, L., Bremerich, V., Jentschke, I., Likens, G., & Tockner, K. (2016). Biological field stations: a global infrastructure for research, education, and public engagement. *Bioscience, 66*(2), 164-171. doi: 10.1093/biosci/biv174

Ward, R., Zemlak, T., Innes, B., Last, P., & Hebert, P. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences, 360*(1462), 1847-1857. doi: 10.1098/rstb.2005.1716

Winker, K., Fall, B., Klicka, J., Parmelee, D., & Tordoff, H. (1991). The importance of avian collections and the need for continued collecting. *The Loon, 63*(4), 238-246.

Winker, K. (2000). Obtaining, preserving, and preparing bird specimens. *Journal of Field Ornithology, 71*(2), 250-297. doi: 10.1648/0273-8570-71.2.250

Winker, K. (2004). Natural history museums in a Post-biodiversity era. *Bioscience, 54*(5), 455. https://doi.org/10.1641/0006-3568(2004)054[0455:nhmiap]2.0.co;2

Winker, K. (2005). Bird collections: development and use of a scientific resource. *The Auk, 122*(3), 966-971. doi: 10.1093/auk/122.3.966

Yang, R., Wu, X., Yan, P., & Li, X. (2009). Using DNA barcodes to identify a bird involved in a birdstrike at a Chinese airport. *Molecular Biology Reports, 37*(7), 3517-3523. doi: 10.1007/s11033-009-9945-0

TABLES

Table 1. Results

Specimen Field Number*	Species	Migrant (M) or Resident (R) at QUBS	GenBank Accession Number	Origin of Specimen	Accession Number of Closest Individual(s) **	Percent Identity ***	Origin of Closest Individual(s)
03R_DEJU-CO1	Dark-eyed junco (*Junco hyemalis*)	M	Pending	Kingston, Ontario	FJ236293.1	100%	USA: California, Monterey County
					JN850765.1	100%	USA
					HM033487.1	100%	Canada: Alberta, Calgary, Inglewood Bird Sanctuary
					DQ434619.1	100%	Canada: Alberta
					DQ434613.1	100%	USA: North Carolina
					DQ433707.1	100%	USA: Oregon
					DQ433706.1	100%	USA: Colorado
					DQ433705.1	100%	USA: Colorado
					DQ433704.1	100%	USA: Oregon
					DQ432957.1	100%	USA: Virginia
04F_PIWO-CO1	Pileated woodpecker (*Dryocopus pileatus*)	R	Pending	Rideau Lakes, Ontario	AY666388.1	99.70%	Not reported
					MK060129.1	99.54%	Not reported
					DQ780879.1	99.54%	USA: Florida, Gainesville
05F_AMGO-CO1	American goldfinch (*Spinus tristis*)	R	Pending	Opinicon Road, Ontario	FJ236301.1	99.85%	USA: California, Marin County
					DQ434517.1	99.85%	Canada: Ontario

					AY666200.1	99.85%	Not reported
					DQ434516.1	99.85%	Canada: New Brunswick
06F_BBCU-CO1	Black-billed cuckoo (*Coccyzus erythropthalmus*)	M	Pending	Kingston, Ontario	DQ434552.1	99.85%	Canada: Ontario
01_BLJA_CO1	Blue jay (*Cyanocitta cristata*)	R	Pending	Ontario	DQ434557.1	99.55%	Canada: Ontario
					AY666459.1	99.54%	Not reported
					AY666552.1	99.54%	Not reported
02_YEWA_CO1	American yellow warbler (*Setophaga petechia*)	M	Pending	Opinicon Road, Ontario	DQ434575.1	99.85%	Canada: Ontario
					HM033394.1	99.85%	Canada: Nova Scotia, Atlantic Bird Observatory, Bon Portage Island
03_WCSP-1_CO1	White-crowned sparrow (*Zonotrichia leucophrys*)	M	Pending	Ontario	DQ434840.1	98.35%	Canada: Ontario
08_BAOR_CO1	Baltimore oriole (*Icterus galbula*)	M	Pending	Ontario	DQ434611.1	95.78%	Canada: Ontario
					HM033484.1	95.78%	Canada: Quebec, Ste-Anne-de-Bellevue, Stoneycroft Wildlife Area, McGill Bird Observatory
04_BCCH-1_CO1	Black-capped	R	Pending	Kingston, Ontario	HM033678.1	97.44%	Canada: Yukon

	chickadee (*Poecile atricapillus*)						Territory, Teslin Lake, Teslin Lake Bird Banding Station
06_HETH_CO1	Hermit thrush (*Catharus guttatus*)	M	Pending	Kingston, Ontario	EF484235.1	100%	Not reported
					AF197834.1	100%	Not reported
					DQ434525.1	100%	Canada: Ontario
					HM033258.1	100%	Canada: Nova Scotia, Atlantic Bird Observatory, Seal Island
					HM033255.1	100%	Canada: Nova Scotia, Atlantic Bird Observatory, Seal Island
					HM033254.1	100%	Canada: British Columbia, Mackenzie, Mugaha Marsh, Mackenzie Nature Observatory
					AY666565.1	100%	Not reported
					AY666295.1	100%	Not reported
09_BBCU-1_CO1	Black-billed cuckoo (*Coccyzus erythropthalmus*)	M	Pending	Ontario	DQ433515.1	98.95%	Canada: Ontario
					DQ434552.1	98.94%	Canada: Ontario
11_TEWA_CO1	Tennessee warbler (*Leiothlypis peregrina*)	M	Pending	Kingston, Ontario	HM033893.1	100%	Canada: Quebec, Ste-Anne-de-Bellevue, Stoneycroft Wildlife Area, McGill

							Bird Observatory
12_YBSA_CO1	Yellow-bellied sapsucker (*Sphyrapicus varius*)	M	Pending	Kingston, Ontario	KC813276.1	99.85%	USA
02R_BADO_CO1	Barred owl (*Strix varia*)	R	Pending	Ontario	N/A	N/A	N/A

*Full sequences can be found in Appendix C.

**The closest individual column contains the accession numbers of the specimens with sequences in GenBank (target sequences) most closely matched to sequences from this study (query sequences).

***Percent identity: how similar the query sequence is to the target sequence.

APPENDICES

Appendix A: Protocols

A.1: Preparation of Study Skin (Adapted from Winker, 2000)

1. Weigh and measure the overall length of each bird before removing any tissue or organs, because measurements often change upon preparation and drying.
2. Label 1.5 ml Eppendorf tubes with a waterproof marker.
3. Use water to move the breast feathers of the bird to the side. Make an incision in the skin from the furculum to the cloaca and begin to separate the skin from the body. To do this, use a blunt probe or finger to push the skin outward and push the body surface inward. Throughout the process, avoid pulling on the skin as it can tear. Work at the point where the skin is still attached to the body with a blunt probe. Here, and at each step below or when there are body fluids, add borax to keep fluids from getting onto the feathers.
4. Take a tissue sample from the breast and put in tube containing 95% ethanol.
5. Skin the knees, legs, tail, shoulders, and head. Start by working the knee until it is visible, then push the blunt probe underneath the joint and cut through the knee joint to detach the leg. The lower leg is now attached only to the leg skin. Skin the leg by removing the muscles from the leg, then push the leg back inside right. Tie the legs together if necessary, to keep them in position.
6. Separate the skin from the tissue around the pelvic area. Hold the tail base and cut the vertebrae. Be careful not to cut the bases of tail feathers, or they will fall out. Use a scalpel to free any remaining tissue so that the tail is separated from the body.

7. Push the skin away from the body down the back and make an incision at the shoulder joints to separate the wings from the body. Separate the skin from the tissue and remove the muscle tissue from the wings.
8. Continue to work the skin away from the tissue. Add water along the throat to keep it moist and flexible. Invert the head. Pinch and roll out the ears to avoid making a hole in the skin and then remove the eyes, leaving the eye ring intact. Note: for birds with large heads (e.g. owls), they cannot be inverted, and an incision should be made in the back of the head.
9. Pull the throat out of the scull. Remove the tongue. Be careful when working with woodpeckers because their tongues curl around behind their skulls. Then, remove the brain by sticking cotton into the hole where the throat was attached and cleaning out the scull.
10. Put pieces of cotton in the skull that show through the eye sockets. Make sure they are about the size of the original eyes by comparing them with the eyes that were removed. Carefully reinvert the head by pushing the bill until you can see it and pull it out.
11. Clean the tail and cut out the oil gland. Be careful not to cut off too much muscle here, particularly when working with a small bird, because the tail feathers may begin to fall out.
12. Remove any remaining meat or fat from the skin.
13. Rinse the skin in a beaker of water. Then, put it in a large closed bag containing borax and shake it to dry it out and remove any oils. Shake for 10 to 15 minutes. When it is almost dry, remove the skin and shake the borax off.

14. Make a body by rolling cotton onto a stick. Use the carcass as a model for size of cotton body and length of stick. For long-necked birds, create a neck using the cotton. Insert the stick up the neck of the skin.
15. Carefully work the skin onto the cotton body. Tie the bill together by passing a threaded needle through the nostrils and tying the thread around the bill.
16. Sew the skin together by bringing the needle up from inside of the skin through to the outside. Gaps between stitches can be left where the cotton shows because the plumage will usually cover these. Unless a hole in the skin is very large, it does not need to be sewn closed and can usually be hidden by the feathers.
17. Preen the feathers and rearrange them so that they are properly aligned.
18. Pin the skin onto a Styrofoam board, so that it lies on its dorsal surface with the wings on the back and the wingtips under the tail.
19. Write a label for the skin and pin it onto the Styrofoam board with the skin. The skin label is the complete final label that correlates with the tissue sample. Complete immediately following the preparation of the study skin to ensure that all the appropriate data are recorded.
20. Make notes of things like sex, if it can be determined, and amount of fat, if it is notable.

A.2: DNA Salt-Extraction (Adapted from Aljanabi & Martinez, 1997)

1. Add approximately 1 cm^3 or less of tissue to the following solution:
    - 80 µL of sterile TEN buffer (Tris-HCl 1M pH8, EDTA 0.5M pH8, NaCl)
    - 10 µL of 20 mg/ml proteinase K (ProK)
    - 20 µL of 20% SDS (add this last)

2. Incubate solution containing samples at 56 ℃ for at least 3 hours or overnight*. Vortex the samples every hour, or until the tissue is completely lysed.

   *If the tissue is not completely lysed after it has been incubated overnight, excessive tissue was added in the first step. Add 10 μL of ProK and incubate for at least one hour before proceeding.
3. Add 120 μL of 5M NaCl. Vortex the samples.
4. Centrifuge the samples for 5 minutes at 12,000 rpm. If after 5 minutes you cannot see any precipitation, centrifuge for an additional 5 minutes.
5. Label fresh tubes. Transfer the supernatant to the fresh tubes. Avoid collecting the precipitate. Discard the old tubes containing the precipitate.
6. Add 300 μL of ice cold 100% ethanol. Invert the tube several times to mix. In this step it is sometimes possible to see a white DNA strand.
7. Incubate the samples at -20 ℃ for at least 1 hour or overnight.
8. Centrifuge the sample for 15 minutes at 4 ℃ at 12,000 rpm.
9. Discard the ethanol by inverting the tube carefully, making sure not to dislodge the DNA (usually, the DNA is bound to the wall).
10. Add 150 μL of 70% ethanol to wash the DNA. Flick the tube to dissolve the salt. Centrifuge the sample for 5 minutes at 4 ℃ at 12,000 rpm.
11. Discard the ethanol and air-dry the samples. To dry them more quickly, put them in the incubator but be careful not to over-dry the DNA.
12. Add 75 μL of nuclease-free water.
13. Incubate the DNA for at least 2 hours at 56 ℃. Tap the tubes every half hour.

Appendix B: Figures

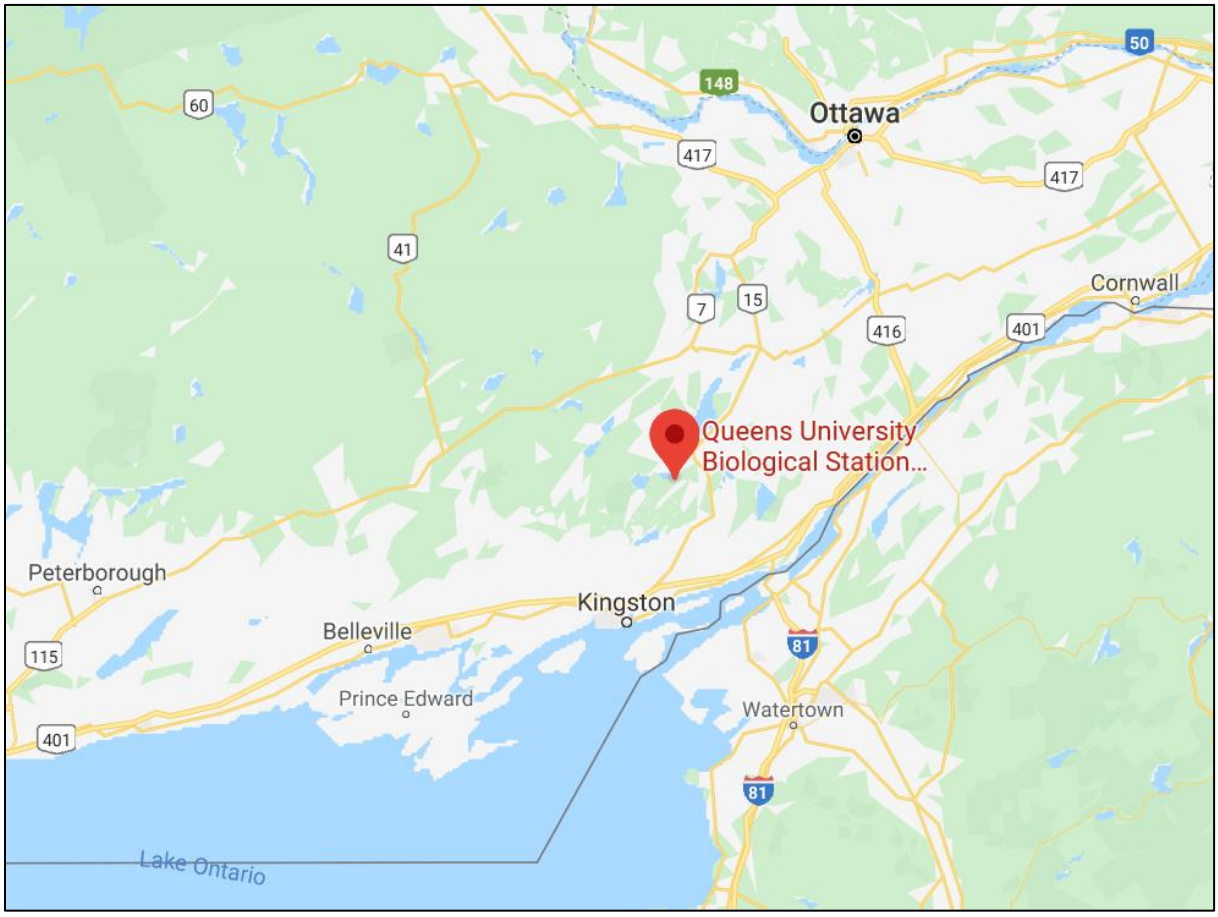

Figure 1. Queen's University Biological Station, 280 Queens University Rd, Elgin, K0G 1E0.

Appendix C: Sequences

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Appendix D: Additional information about specimens used in my thesis

Specimen Field Number	**Species**	**Date and Locality of Collection**	**Specimen Collector**	**Length (cm)**	**Weight (g)**
03R_DEJU-CO1	Dark-eyed junco (*Junco hyemalis*)	Kingston, Ontario, October 29th 2018	Stephen C. Lougheed	13.8	14.37
04F_PIWO-CO1	Pileated woodpecker (*Dryocopus pileatus*)	Rideau Lakes, Ontario, October 20th 2019	Unknown	43.0	278.80
05F_AMGO-CO1	American goldfinch (*Spinus tristis*)	Opinicon Road, Ontario, November 16th 2019	Stephen C. Lougheed	12.0	13.41
06F_BBCU-CO1	Black-billed cuckoo (*Coccyzus erythropthalmus*)	Kingston, Ontario, May 18th 2019	Adriana Lopez Villalobos	28.5	46.24
01_BLJA_CO1	Blue jay (*Cyanocitta cristata*)	Ontario	Unknown	Unknown	Unknown
02_YEWA_CO1	American yellow warbler (S*etophaga petechia*)	Opinicon Road, Ontario, May 24th 2015	Unknown	Unknown	Unknown
03_WCSP-1_CO1	White-crowned sparrow (*Zonotrichia leucophrys*)	Ontario	Unknown	Unknown	Unknown
08_BAOR_CO1	Baltimore oriole (*Icterus galbula*)	Ontario	Unknown	Unknown	Unknown
04_BCCH-1_CO1	Black-capped chickadee (*Poecile atricapillus*)	Kingston, Ontario, October 12th 2018	Matthew Macpherson	11.5	10.10
06_HETH_CO1	Hermit thrush (*Catharus guttatus*)	Kingston, Ontario,	Unknown	16.5	30.52

		April 25th 2018			
09_BBCU-1_CO1	Black-billed cuckoo (*Coccyzus erythropthalmus*)	Ontario	Unknown	Unknown	Unknown
11_TEWA_CO1	Tennessee warbler (*Leiothlypis peregrina*)	Kingston, Ontario, September 12th 2019	Unknown	10.6	9.49
12_YBSA_CO1	Yellow-bellied sapsucker (*Sphyrapicus varius*)	Kingston, Ontario, October 3rd 2018	Unknown	19.0	44.04
02R_BADO_CO1	Barred owl (*Strix varia*)	Ontario	Unknown	46.5	1144.7