Banisteria, Number 53, pages 22–26

Using DNA Barcoding to Identify Carcasses from Bird-window Collisions at Radford University

Claudia Y. Paniagua-Ugarte, Karen E. Powers[1], and Robert R. Sheehy

Biology Department
Radford University
Radford, Virginia 24142
[1]Corresponding author: kpowers4@radford.edu

ABSTRACT

A leading cause of avian mortality is collisions with building windows. To fully understand the impacts of bird-window collisions at Radford University, Virginia, bird carcasses (whole or in part) were collected and documented in 2018–2019. Although the majority of carcasses were identified via morphological features, the taxonomic identification of some samples was impossible due to evidence of predation, environmental degradation, and confusion in species differentiation due to sex, age, and seasonal plumage. We used DNA barcoding to identify carcasses in cases where species identification based on morphology was not possible. DNA barcoding with standard PCR primers allowed for the successful identification of five individuals across four species - two of which species had not been previously identified in this study. Our study emphasizes the application of DNA barcoding in bird-window collision studies, and its potential for use in other conservation and mitigation efforts.

Keywords: avian mortality, cytochrome c oxidase subunit 1, species identification, taxonomy.

INTRODUCTION

Bird-window collisions (BWCs) are a leading cause of mortality in the United States and word-wide, with annual mortality estimates of nearly one billion birds (Loss et al., 2014). Such collisions are suspected to be greater during peak migratory periods, as birds traverse less familiar habitats (Borden et al., 2010; Schneider et al., 2018). Borden et al. (2010) reported that migratory birds were nine times more likely to be a casualty of BWCs than resident species. While some studies report that particular species or family groups are more prone to collisions (e.g., hummingbirds, Schneider et al., 2018), it is evident that both common and rare species are susceptible to this threat.

Multiple large-scale and/or long-term studies of BWCs acknowledge that mortalities are likely under-reported (Bayne et al., 2012; Kummer et al., 2016). A major limitation is observer error, which manifests itself as a failure to detect carcasses that are present (e.g., carcasses obscured by vegetation). Carcass scavenging is a second major cause of BWC underestimates (e.g., Kummer et al., 2016). Finally, carcasses landing outside a limited search area may also affect discovery (Zimmerling et al., 2013). Recent estimates suggest that carcass recovery is 2.3–5 times less than the actual number of bird mortalities (Dunn, 1993; Zimmerling et al., 2013).

Identification is important because all bird samples collectively play a role in understanding how species migration, seasonal distribution, and density could relate to bird building collisions (Schneider et al., 2018). However, once recovered, several circumstances may limit accurate identification of the carcass. Damage to the specimen resulting from the collision, length of time between mortality and collection, and scavenger activity may all affect the ability to identify that carcass. Additionally, differentiation between juvenile and adult females may be difficult; particularly among passerines. In our BWC study (Powers et al., this volume), all of these factors impeded accurate identification of some specimens.

From February 2018 – June 2019, students at Radford University investigated myriad aspects of natural and anthropogenic influences on the number and nature of BWCs at 15 buildings on the university campus (Powers et al., this volume). Statistical analyses required the accurate identification of bird carcasses. Students

identified full carcasses, partial carcasses, and multiple feather evidence from BWCs via comparisons to bird specimens within the natural history collection at Radford University. However, morphological identification of nine of 51 birds was not possible or definitive.

DNA barcoding, identifying species by comparing a short, defined DNA sequence to a DNA sequence reference database, provided an alternative approach to identifying a specimen (Kerr et al., 2007). DNA barcoding has been effective for the species identification of whole birds (Herbert et al., 2004), bird tissue (Dove et al., 2008), and eggs (Lee & Prys-Jones, 2008) from unknown samples. Of the nine unidentifiable carcasses, seven contained tissue, and thus had the potential to be identified through DNA barcoding. Our goal was to extract DNA from carcasses and use DNA barcoding to identify them to the fullest extent possible.

METHODS

Seven bird carcasses that contained tissue but were physically unidentifiable were collected and stored in a standard chest freezer at 20° C. One additional BWC specimen collected in October 2016 at Radford University was included in this project, increasing our sample size to eight.

DNA was extracted from tissues using the Qiagen DNeasy blood and tissue kit (Qiagen, Austin, Texas). We amplified a 708 bp fragment of the mitochondrial encoded cytochrome c oxidase subunit I (COI) gene using tailed primers BirdF1_t1 and BirdR1_t1 (Kerr et al., 2007). Each 50 µl PCR reaction contained 25 µl of 2x Quick-Load Taq master mix (NEB, Ipswich, Massachusetts) 2.3 mM $MgCl_2$, 0.5 mM each of forward and reverse primers, 5 µl of DNA template (approx. 200 ng), and distilled water. PCR amplification was performed with an initial denaturation (3 min at 94° C) followed by 35 cycles of 94° C for 1 min, 51° C for 1 min, and 68°C for 1 min. This was followed by a final extension step at 68° C for 10 min. PCR reactions were held at 4° C for 6 to 8 h until storage at -20° C.

We identified successful amplification by running 5 µl of each sample on 2% agarose/TBE/EtBr gel. Successfully amplified samples were sent to GENEWIZ (South Plainfield, New Jersey) for sequencing. Both strands of our amplicons were sequenced using the Sanger dideoxy chain termination method (Sanger & Coulson, 1975). We used the DNA subway (DNA Learning Center, https://dnasubway.cyverse.org) as a means to align forward and reverse sequences of each sample and to evaluate/correct discrepancies between the sequences of the forward and reverse strands and to construct a consensus sequence of the forward and reverse strands. We used the "Identification Engine" (http://boldsystems.org/index.php/IDS_OpenIdEngine) to identify the taxonomic origin of unknown specimen DNA using the consensus sequence as a query. We assume "correct" species identification for samples that show ≥99.0% sequence similarity with bird species already in the database.

RESULTS

Of the eight carcasses tested, we successfully amplified and identified five to species level (Table 1). We confidently identified one Cedar Waxwing (*Bombycilla cedrorum*), one House Sparrow (*Passer domesticus*), two Cape May Warblers (*Setophaga tigrina*; one was the 2016 collection), and one Swainson's Thrush (*Catharus ustulatus*). The thrush and the Cape May Warblers had not been previously identified in the BWC project. The specimens whose DNA did not amplify remain unidentified via morphological features.

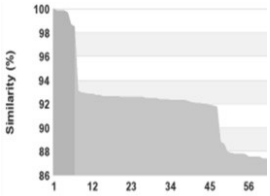
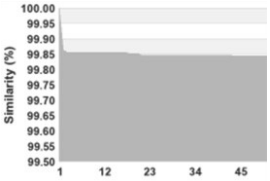
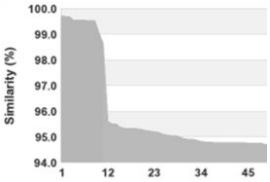
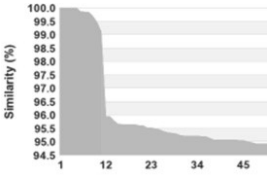
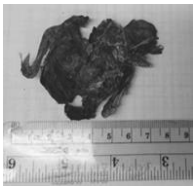
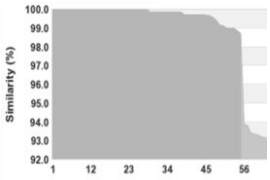
DISCUSSION

The use of DNA barcoding allowed for the identification of five of the eight unidentifiable bird carcasses. All five of these carcasses had a sequence similarity of ≥99.0% to reference species and are considered to be accurately identified. Unsuccessful amplification may be due to the degradation of DNA that results with age and/or the environmental conditions experienced before collection. There are, however, various methods that may allow for the analysis of degraded DNA such as the PCR amplification of shorter sequences using internal primers.

While we used primers designed to amplify the complete 708 bp "Folmer region" of the COI gene (Folmer et al., 1994), these primers prove ineffective at amplifying all samples. Often, this results from the fragmentation of DNA due to environmental degradation which results in the lack of a full-length DNA template. Future research will be aimed at designing internal primers which will allow the amplification of shorter target sequences and/or the application of DNA mini-barcodes (Meusnier et al., 2008) to these problematic specimens. This should allow us to apply DNA barcoding to a more degraded template.

The results from the five successfully identified samples provide useful data to help us understand the relationships of particular bird species with BWCs. Of greatest interest were the two species (Swainson's Thrush and Cape May Warbler) that had not previously been identified as BWC casualties in this project. Both are migratory species in the region and not considered

Table 1. Bird-window collision carcasses collected from Radford University (2016, 2018-2019) that were identified with DNA barcoding. Presented is a photo of each original carcass, the range of sequence similarity to members of the top matched species, and the DNA percent match, broken down by family, genus, and specific epithet. All samples fall into the order Passeriformes.

SAMPLE	TAXON ASSIGNMENT	PROBABILITY OF PLACEMENT (%)	SEQUENCE SIMILARITY TO TOP 100 DATABASE MATCHES
	Bombycillidae *Bombycilla* *cedrorum* Cedar Waxwing	100 100 100	
	Passeridae *Passer* *domesticus* House Sparrow	100 100 100	
	Parulidae *Setophaga* *tigrina* Cape May Warbler	100 100 99.7	
	Parulidae *Setophaga* *tigrina* Cape May Warbler	100 100 100	
	Turdidae *Catharus* *ustulatus* Swainson's Thrush	100 100 100	

summer residents. Radford University's campus abuts the New River, a migratory pathway. Therefore, it is not entirely unexpected to find these species. While both species are uncommon, they have been documented along the New River in Radford. The Cape May Warbler has been observed in late September and early October, and the Swainson's Thrush has been observed most often in September through mid-October with sporadic observations in May (e.g., eBird data for Bisset Park and Riverway Trail, Radford, Virginia; https://ebird.org/atlasva). The Swainson's Thrush was collected on 30 June 2018, providing the first local record of this taxon outside regular migration periods. To date, only one June record in the region has been reported (Giles Co., VA/Monroe Co., WV line, C. Kessler, pers. comm.). Powers et al. (2019) review these species in greater detail. The identification of the Swainson's Thrush demonstrates the power of DNA barcoding as this potentially significant observation would have otherwise gone unnoticed.

We have demonstrated the power of DNA barcoding in providing an alternative means for the taxonomic identification of specimens where, for numerous and varied reasons, traditional means of identification based on morphology may be inconclusive or impossible. While we have applied this approach to the identification of avian taxa, it is easy to see where this approach would be useful in other studies as well. DNA barcoding could easily be applied to the taxonomic identification of tissues from roadkills, prey remains found in association with predatory animals, and cryptic species where the identification based on morphological differences is problematic. These methods could be particularly useful in regulatory projects, like surveys around wind turbines (for birds and bats), where identification to species level may have greater implications for mitigation efforts.

Applying DNA barcoding as an approach for species identification should not fall outside the realm of many studies. With the exception of DNA sequencing, the molecular techniques necessary to complete this project are common among introductory biology and genetics college-level courses. Myriad of companies are available to perform the Sanger dideoxy sequencing reaction if these are unavailable in-house. The cost per sample of DNA barcoding can range from between $5.00 to $10.00 USD depending upon: in-house versus commercial sequencing, sequencing one or both strands or, the necessity for band isolation to remove primer dimers. If one is distinguishing among only a few potential taxa, a related approach, Cleaved Amplified Polymorphic Sequences (CAPS; Konieczny & Ausubel, 1993) or the related dCAPS (derived Cleaved Amplified Polymorphic Sequences; Neff et al., 1998) could successfully be applied to species identification saving time and reducing the cost of analysis to a few dollars.

ACKNOWLEDGEMENTS

We thank the Department of Biology at Radford University for covering laboratory expenses associated with this bird-window collision research, and for supporting two courses in this research. We thank the more than 30 students at Radford University who contributed nearly 1,000 hours of surveys in this bird-window collision project.

LITERATURE CITED

Bayne, E. M., C. A. Scobie, & M. Rawson-Clark. 2012. Factors influencing the annual risk of bird-window collisions at residential structures in Alberta, Canada. Wildlife Research 39: 583–592.

Borden, W. C., O. M. Lockhart. A. W. Jones, & M. S. Lyons. 2010. Seasonal, taxonomic, and local habitat components of bird-window collisions on an urban university campus in Cleveland, OH. Ohio Journal of Science 110: 44–52.

Dove, C. J., N. C. Rotzel, M. Heacker, & L. A. Weigt. 2008. Using DNA barcodes to identify bird species involved in birdstrikes. Journal of Wildlife Management 72: 1231-1236.

Dunn, E. H. 1993. Bird mortality from striking residential windows in winter. Journal of Field Ornithology 64: 302–309.

Folmer, O., M. Black, W. Hoeh, R. Lutz, & R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Herbert, P. D., M. Y. Stoeckle, T. S. Zemlak, & C. M. Francis. 2004. Identification of birds through DNA barcodes. PLoS Biology 2(10): e312. https://doi.org/10.1371/journal.pbio.0020312

Kerr, K. C., M. Y. Stoecke, C. J. Dove, L. A. Weight, C. M. Francis, & P. D. Hebert. 2007. Comprehensive DNA barcode coverage of North American birds. Molecular Ecology Notes 7: 535–543.

Konieczny A., & F. M. Ausubel. 1993. A procedure for mapping *Arabidopsis* mutations using co-dominant

ecotype-specific PCR-based markers. Plant Journal 4: 403–410.

Kummer, J. A., C. J. Nordell, T. M. Berry, C. V. Collins, C. R. L. Tse, & E. M. Bayne. 2016. Use of bird carcass removals by urban scavengers to adjust bird-window collision estimates. Avian Conservation and Ecology 11(2): 12. http://dx.doi.org/10.5751/ACE-00927-11021.

Lee, P. L., & R. P. Prys-Jones. 2008. Extracting DNA from museum bird eggs, and whole genome amplification of archive DNA. Molecular Ecology Resources 8: 551–560.

Loss, S. R., T. Will, S. S. Loss, & P. P. Marra. 2014. Bird-building collisions in the United States: Estimates of annual mortality and species vulnerability. Condor 116: 8–23.

Meusnier, I., G. A. C. Singer, J. F. Landry, D. A. Hickey, P. D. N. Hebert, & M. Hajibabaei. 2008. A universal DNA mini-barcode for biodiversity analysis. BMC Genomics 9: 214–218.

Neff, M. M., J. D. Neff, J. Chory, & A. E. Pepper. 1998. dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: Experimental applications in *Arabidopsis thaliana* genetics. Plant Journal 14: 387–392.

Powers, K.E., L.A. Burroughs, B.M. Mullen, H.C. Reed, & Z.Q. Krajcirovic. 2019. Investigating campus features that influence bird-window collisions at Radford University, Virginia. Banisteria 53: 11–21.

Sanger, F., & A. R. Coulson. 1975. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. Journal of Molecular Biology 94: 441–448.

Schneider, R. M., C. M. Barton, K. W. Zirkle, C. F. Greene, & K. B. Newman. 2018. Year-round monitoring reveals prevalence of fatal bird-window collisions at the Virginia Tech Corporate Research Center. PeerJ e4562. https://peerj.com/articles/4562/

Zimmerling, J. R., A. C. Pomeroy, M. V. d'Entremont, & C. M. Francis. 2013. Canadian estimate of bird mortality due to collisions and direct habitat loss associated with wind turbine developments. Avian Conservation and Ecology 8(2): 10. http://dx.doi.org/10.5751/ACE-00609-080210