

BARCODING THE BIRDS OF NORTH AMERICA

Kevin C.R. Kerr¹, Mark Y. Stoeckle², Carla J. Dove³, Lee A. Weigt³, Charles M. Francis⁴, Paul D.N. Hebert¹

¹Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada, ²Program for the Human Environment, The Rockefeller University, New York, NY, USA,

³National Museum of Natural History, Washington, DC, USA, ⁴National Wildlife Research Centre, Canadian Wildlife Service, Ottawa, Ontario, Canada



INTRODUCTION

- Birds are obvious and ubiquitous organisms, popular both to science and the general public. As such, they have been extensively studied and their taxonomy has been well established. This makes the class a valuable test group to determine the efficacy of DNA barcodes in species delimitation.
- We are compiling barcodes for all 693 bird species that regularly breed within North America, with adequate replication for each species. Our list of species was constructed using information from the American Ornithologists Union.

METHODS

- DNA was extracted from frozen tissue samples (blood, organs, or breast muscle) as well as from feathers collected by banding stations. Working with a single breast feather, the proximal tip of the calamus (the 'root' of the feather shaft) is cut off and the rest of the feather discarded. The isolated segment, typically only a few millimetres in length, bears enough residual cellular material for barcode analysis.
- DNA extractions were carried out using either the GenElute™ DNA miniprep kit (Sigma-Aldrich) or a solution of ProK and Chelex (BioRad).
- PCR was performed in a 10 µL volume, but otherwise followed the protocol described by Hebert *et al.* (2004), as did sequencing reactions.



Fig. 1. Minimal amounts of tissue are required to generate COI barcodes. Pictured here is a breast feather from a Magnolia Warbler (*Dendroica magnolia*). Only the tip of the feather shaft is required.

RESULTS

- Two primer sets have successfully recovered the COI barcoding region from more than 99% of the species sampled.
- COI barcodes have now been generated for two thirds of the target species. Total species coverage has nearly doubled since publication of the PLoS paper, while the sample size has tripled to more than 1500 individuals.

TAXON COVERAGE

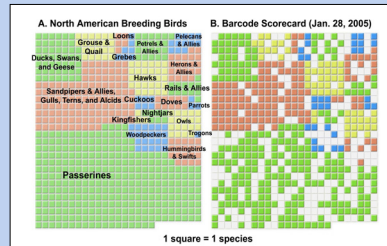


Fig. 2. This barcoding 'scorecard' provides a visual representation of our progress. The grid on the left (A) represents the 693 species of North American breeding birds. The grid on the right (B) shows those species that have been barcoded.

TAXON RESOLUTION

- Although primarily a tool for species identification, barcode-based ID trees regularly show cohesion of genera, families, and orders, enabling the 'draft' taxonomic placement of newly discovered species.



Fig. 3. This neighbour-joining taxon ID tree, constructed using a p-distance model, illustrates the patterning of COI sequence divergence for all 467 species of North American birds that have now been barcoded.

SEQUENCE DIVERGENCES

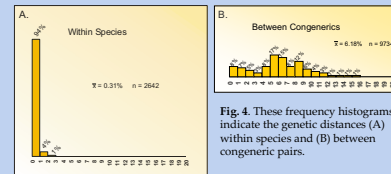


Fig. 4. These frequency histograms indicate the genetic distances (A) within species and (B) between congeneric pairs.

- These results confirm that divergences are low within species and high between species. Mean sequence divergence at the intraspecific level is 0.31% versus 6.18% divergence between congeners, a 20-fold difference.

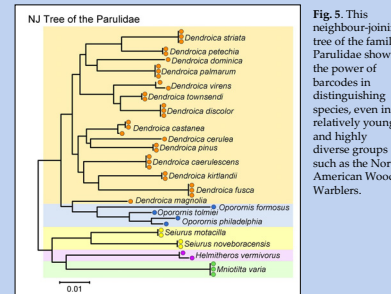


Fig. 5. This neighbour-joining tree of the family Parulidae shows the power of barcodes in distinguishing species, even in relatively young and highly diverse groups such as the North American Wood Warblers.

BARCODING CHALLENGES

- 1523 congeneric pair sets of birds have now been examined. Difficulties in species resolution occur in only 31 of these cases (2.0%).
- Three cases involve pairs of ducks (which exhibit low divergences) - Mallard (*Anas platyrhynchos*) and American Black Duck (*A. rubripes*), Blue-winged Teal (*A. discors*) and Cinnamon Teal (*A. cyanoptera*), and King and Common Eider (*Somateria spectabilis* and *S. mollissima*). The first two pairs frequently hybridize where their ranges overlap.
- The other 28 cases involve eight species of large white-headed gulls, centering around the *Larus argentatus* species complex. These taxa either share barcodes or show extremely low divergences. However, the taxonomy of this group is often debated; they certainly represent a complex of recently diverged, incompletely isolated taxa.



Fig. 6. Some species, such as this Iceland Gull (*Larus glaucoideus*) are not separable from close allies through COI barcodes, likely due to recent divergence and ongoing hybridization between species.

CRYPTIC SPECIES

- One great value of DNA barcodes lies in their ability to detect cryptic species. Four potential cryptic species were revealed during a preliminary investigation involving 260 North American bird species (Hebert *et al.* 2004). As geographical coverage increases, more cases are expected to emerge.
- Some bird species differ geographically in their behaviour, song, or plumage; barcodes may help distinguish clinal variation from actual species differences.

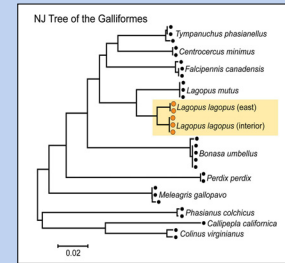


Fig. 7. This neighbour-joining tree of the order Galliformes demonstrates how relatively deep genetic divergences may be revealed through barcodes. In this case, the Willow Ptarmigan (*Lagopus lagopus*) shows more than 2% divergence between representatives of its eastern and interior populations.

CONCLUSIONS

- Progress on the birds of North America is advancing rapidly toward draft closure (5x coverage for all species), but we are aiming for 10x coverage. Even now, it is clear that DNA barcodes will be a highly effective approach to bird species identification.
- Taxonomic groups where barcode resolution is complex, such as the ducks (Anatinae) and gulls (Larinae), warrant deeper investigation.
- The organizational structure employed to execute this study, with two analytical centres sequencing specimens from numerous sites, may be a useful model for large-scale barcoding efforts on other taxonomic groups.

ACKNOWLEDGEMENTS

- This study was greatly facilitated by tissue samples provided by the Burke Museum, Field Museum, Royal Ontario Museum, Smithsonian Institution, and the Canadian Wildlife Service. We also thank the banding stations across Canada that provided feather samples.
- This work was supported by grants from the Gordon and Betty Moore Foundation, the Canadian Wildlife Service, and the Federal Aviation Administration.

REFERENCE

- Hebert, P.D.N., M.Y. Stoeckle, T.S. Zemlak and C.M. Francis. 2004. Identification of birds through DNA barcodes. PLoS Biol 2:1657-1663