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ND2 as an additional genetic marker to improve identification of diving ducks involved in bird strikes

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Abstract: Knowing the exact species of birds involved in damaging collisions with aircraft (bird strikes) is paramount to managing and preventing these types of human–wildlife conflicts. While a standard genetic marker, or DNA barcode (mitochondrial DNA gene cytochrome-*c* oxidase 1, or CO1), can reliably identify most avian species, this marker cannot distinguish among some closely related species. Diving ducks within the genus *Aythya* are an example of congeneric waterfowl involved in bird strikes where several species pairs cannot be reliably identified with the standard DNA barcode. Here, we describe methods for using an additional genetic marker (mitochondrial DNA gene NADH dehydrogenase subunit 2, or ND2) for identification of 9 *Aythya* spp. Gene-specific phylogenetic trees and genetic distances among taxa reveal that ND2 is more effective than CO1 at genetic identification of diving ducks studied here. Compared with CO1, the ND2 gene tree is more statistically robust, has a minimum of 1.5 times greater genetic distance between sister clades, and resolves paraphyly in 2 clades. While CO1 is effective for identification of most bird strike cases, this study underscores the value of targeted incorporation of additional genetic markers for species identification of taxa that are known to be problematic using standard DNA barcoding.

Key words: *Aythya* spp., birds, bird strikes, CO1, diving ducks, DNA barcoding, human–wildlife conflicts, mtDNA, ND2

Bird collisions with aircraft (hereafter, bird strikes) are a daily occurrence among civil and military aircraft and pose indirect economic losses due to delays, direct damages to aircraft, and rarely, but tragically, loss of human life (Zakrajsek and Bissonette 2005, Pfeiffer et al. 2018, Dolbeer et al. 2019). Most bird strikes occur within 152 m of the ground and 3,048 m of the runway, highlighting the importance of airfield wildlife management to reduce the presence of birds (Dolbeer 2006). Correct identification of species involved in bird strikes is crucial for understanding temporal and spatial patterns in bird occurrence, management strategies for airfield habitats, and aids in the prevention of this type of human–wildlife conflict (Dolbeer et al. 2000, Dove et al. 2008, Marra et al. 2009, DeVault et al. 2011).

When diagnostic morphological evidence is available (e.g., feathers or feather fragments), it can be compared with museum specimens for rapid, accurate species identification (Dove et al. 2009). However, when only non-diagnostic or prohibitively small amounts of physical evidence remain, or when physical identification expertise is unavailable, DNA sequencing

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may be the only method for obtaining species-level identifications. In these cases, a technique called "DNA barcoding" is widely used for taxonomic identification of unknown samples (Dove et al. 2008, 2009; Waugh et al. 2011).

DNA barcoding relies on the amplification of a short, sufficiently variable portion of the genome that can provide species-level identification when compared with a library of reference sequences (reviewed in DeSalle and Goldstein 2019). In birds, as in most animals, a ~650 base pair portion of the mitochondrial (mtDNA) gene cytochrome-*c* oxidase 1 (CO1) has been widely tested and applied to species identification (Hebert et al. 2003*a*, *b*; Hebert et al. 2004; Lijtmaer et al. 2012), and extensive, publicly available reference libraries exist for comparison (Ratnasingham and Hebert 2007). The CO1 fragment can be amplified from many taxa using a small number of primers (e.g., Folmer et al. 1994, Meyer 2003) and can reliably identify >90% of avian species that have been barcoded to date (e.g., Nearctic: Kerr et al. 2007, 2009; Palearctic: Aliabadian et al. 2009; Neotropics: Chavez et al. 2015; Japanese Archipelago: Saitoh et al. 2015; New Zealand: Tizard et al. 2019). CO1 barcoding has been successfully applied to identification of bird strikes for over a decade (Dove et al. 2008, 2009; Waugh et al. 2011). However, the comparatively low mtDNA mutation rate that makes CO1 powerful for most species-level discrimination appears insufficient for distinguishing closely related species or subspecific taxa (Hebert et al. 2003b, Dove et al. 2013, Drovetski et al. 2014). When a single barcoding marker contains insufficient resolution for high-confidence taxonomic identification, the targeted use of an additional marker can maintain the high-throughput workflow of a single marker (CO1) while increasing the confidence of identifications among closely related taxa.

Diving ducks in the genus *Aythya* that are involved in bird strikes are a good example of taxa in which CO1 barcoding has limited effectiveness (Kerr et al. 2009). In this study, we explore the usefulness of an additional molecular marker, NADH dehydrogenase subunit 2 (ND2), for distinguishing *Aythya* spp. frequently involved in bird strikes. We focus specifically on 4 closely related sister species pairs where intra-specific differences in CO1 sequences are similar to inter-specific differences (greater scaup [*A. marila*]/lesser scaup [*A. affinis*], common pochard [*A. ferina*]/canvasback [*A. valisineria*], ferruginous duck [*A. nyroca*]/hardhead [*A. australis*], and ring-necked duck [*A. collaris*]/redhead [*A. americana*]). We chose the ND2 gene as an additional marker for molecular identification of these birds because it has the fastest substitution rate (0.029 substitutions/site per Ma; 95% CI 0.024–0.033) among the mtDNA genes at nearly twice the rate of the CO1 gene (0.016 substitutions/site per Ma; 95% CI 0.014–0.019; Lerner et al. 2011). Like CO1, ND2 can be amplified across a wide variety of avian taxa using a small set of primers (Hackett 1996, Sorenson et al. 1999, Drovetski et al. 2004). We chose to sequence the entire ND2 gene (1,041 base pairs) to obtain the highest possible inter-specific divergence. To make our work applicable to the industry standard, we compare ND2 to the typical CO1 vertebrate barcoding region (~650 base pairs), which relies on the 5′ region of the CO1 gene. Previous studies have successfully used ND2 in subspecies level identifications in passerine birds and in the phylogenetic reconstruction of dabbling duck (Anatidae, Anatini) genera (e.g., Johnson and Sorenson 1998, Drovetski et al. 2005, Drovetski and Fadeev 2010, Dove et al. 2013).

Methods

We analyzed 96 sequences from both genes (CO1 n = 58; ND2 n = 38). We obtained 45 sequences of CO1 (n = 35) and ND2 (n = 10) from online searches of GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and Barcode of Life Database (http://www.boldsystems.org/) and generated 51 new sequences of CO1 (n = 23) and ND2 (n = 28) using frozen tissue from vouchered museum specimens (Table 1). These sequences represent 9 of 12 *Aythya* spp. The 3 species that we did not include are unlikely to be involved in bird strikes due to their conservation status and range restrictions. They include 2 endangered species (Baer's pochard [*A. baeri*], native to southeast Asia, and Madagascan pochard [*A. innotata*], Madagascar endemic) and 1 New Zealand endemic species (New Zealand scaup [*A. novaeseelandiae*]). We used red-crested pochard (*Netta rufina*; 1 sequence for each CO1 and ND2, obtained from GenBank) as an outgroup.

Table 1. Sequences used to build phylogenetic gene trees of 9 diving duck species (*Aythya* spp.), including unique identifiers for sequences available on public online databases.

Genus	Species	Common name	NCBI (ND2)	NCBI (CO1)	BOLD (CO1)	ID/Catalog number*	Date	Lat	Lon
Aythya	*affinis*	Lesser scaup		DQ434306	BRDC155-5	CWS149010151	Oct. 27, 2003	50.08	-95.59
Aythya	*affinis*	Lesser scaup		DQ434307	BRDC156-5	CWS149020192	May 11, 2004	44.16	-79.30
Aythya	*affinis*	Lesser scaup		DQ434308	BRDC157-5	CWS149020200	Aug. 11, 2004	42.36	-80.29
Aythya	*affinis*	Lesser scaup	MW151590	MW151618		USNM601772	Aug. 24, 2000	64.70	-147.13
Aythya	*affinis*	Lesser scaup			BROMB434-6	ROM93289	Mar. 23, 2003	43.38	-79.22
Aythya	*affinis*	Lesser scaup	MW151591	MW151619		USNM638910	Nov. 10, 2007	58.36	-134.58
Aythya	*affinis*	Lesser scaup	MW151592			USNM643706	Jan. 5, 2011	38.93	-76.30
Aythya	*affinis*	Lesser scaup	EU585684			IPMB22515			
Aythya	*americana*	Redhead	AF090337	AF090337	CYTC4103-12			26.66	-97.40
Aythya	*americana*	Redhead		DQ434315	BRDC144-5	CWS146010097	Sep. 25, 2003	53.19	-112.42
Aythya	*americana*	Redhead		DQ434316	BRDC145-5	CWS146010098	Nov. 9, 2003	51.19	-113.28
Aythya	*americana*	Redhead		DQ434313	BRDC147-5	CWS146010100	Sep. 22, 2003	54.25	-113.36
Aythya	*americana*	Redhead		DQ434314	BRDC148-5	CWS146010169	Oct. 21, 2003	50.51	-112.34
Aythya	*americana*	Redhead	MW151593	MW151620		USNM639463	Jan. 26, 2008	39.15	-77.52
Aythya	*americana*	Redhead	MW151594	MW151621		USNM644365	Jun. 17, 2010	40.79	-111.98
Aythya	*australis*	Hardhead	MW151595	MW151622		ANWC B28997	Apr. 22, 1999	-24.11	143.33
Aythya	*australis*	Hardhead	MW151596	MW151623		ANWC B50371	Jun. 7, 2004	-30.64	115.67
Aythya	*australis*	Hardhead	MW151597	MW151624		ANWC B50372	Jun 7, 2004	-30.64	115.67
Aythya	*australis*	Hardhead	MW151598	MW151625		ANWC B50722	Oct. 4, 2004	-17.77	122.85
Aythya	*australis*	Hardhead	MW151599	MW151626		ANWC B51147	Feb. 23, 2005	-29.23	149.11
Aythya	*australis*	Hardhead			BROMB853-7	ROMMKP2221	Mar. 23, 1994	-19.15	121.29
Aythya	*australis*	Hardhead	EU585685			IPMB46900			
Aythya	*collaris*	Ring-necked duck		DQ434323	BRDC161-5	CWS150010817	Jan. 10, 2003	45.27	-65.56
Aythya	*collaris*	Ring-necked duck		DQ434322	BRDC160-5	CWS150010800	Jul. 10, 2003	45.50	-64.12
Aythya	*collaris*	Ring-necked duck		DQ434324	BRDC162-5	CWS150010901	Oct. 24, 2003	44.23	-64.39

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Aythya	*collaris*	Ring-necked duck	MW151600	MW151627		USNM626573	Jan. 15, 2001	39.05	-77.30
Aythya	*collaris*	Ring-necked duck		DQ434325	TZBNA074-3	ROM1B-209		44.60	-79.80
Aythya	*collaris*	Ring-necked duck	MW151601	MW151628		USNM626574	Jan. 20, 2001	39.05	-77.30
Aythya	*ferina*	Common pochard	EU585686			IPMB7012			
Aythya	*ferina*	Common pochard	NC024602	NC024602					
Aythya	*ferina*	Common pochard	MW151602	MW151629		USNM641837	Jan. 1, 2009	34.14	132.23
Aythya	*ferina*	Common pochard	MW151603	JF499098	SIBJP034-10	USNM641838	Jan. 29, 2009	34.14	132.23
Aythya	*ferina*	Common pochard	MW151604	MW151630		USNM651599	Sep. 30, 2013	31.50	65.85
Aythya	*fuligula*	Tufted duck	EU585687			IPMB1		47.20	8.26
Aythya	*fuligula*	Tufted duck	KJ722069	KJ722069		JS04			
Aythya	*fuligula*	Tufted duck		GU571274	BONSC029-8	NHMO11847	Oct. 1, 2006	63.55	10.20
Aythya	*fuligula*	Tufted duck		JF499099	SIBJP037-10	USNM641849	Apr. 8, 2009	34.14	132.23
Aythya	*fuligula*	Tufted duck	MW151605	MW151631		USNM641850	Apr. 8, 2009	34.14	132.23
Aythya	*fuligula*	Tufted duck		DQ433344	KKBNA350-5	UWBM56543		63.48	74.87
Aythya	*fuligula*	Tufted duck		DQ433345	KKBNA617-5	UWBM63666	Jun. 13, 1997	68.02	68.60
Aythya	*fuligula*	Tufted duck		GU571273	BON184-07	NHMOBC184	Sep. 14, 1999	60.33	5.00
Aythya	*marila*	Greater scaup		DQ434331	BRDC150-5	CWS146010097	Jun. 12, 2003	42.18	-82.17
Aythya	*marila*	Greater scaup		DQ434332	BRDC152-5	CWS148020038	Nov. 11, 2004	42.04	-83.07
Aythya	*marila*	Greater scaup		DQ434333	BRDC153-5	CWS148020162	Mar. 12, 2004	44.16	-79.30
Aythya	*marila*	Greater scaup		DQ434334	BRDC154-5	CWS148020164	Nov. 27, 2004	42.04	-83.07
Aythya	*marila*	Greater scaup	MW151606	MW151632		USNM622668	Aug. 24, 2000	39.23	-76.37
Aythya	*marila*	Greater scaup	EU585688			IPMB6992		Captive	Captive
Aythya	*marila*	Greater scaup		GU571275	BONSC028-8	NHMO11846	Oct. 1, 2006	63.55	10.20
Aythya	*marila*	Greater scaup	MW151607	MW151633		USNM638742	Apr. 5, 2006	51.87	-176.65
Aythya	*marila*	Greater scaup	MW151608	JF499102	SIBJP042-10	USNM641825	Apr. 8, 2009	34.14	132.23
Aythya	*marila*	Greater scaup	MW151609	JF499101	SIBJP043-10	USNM641826	Apr. 8, 2009	34.14	132.23
Aythya	*marila*	Greater scaup		GQ481387	KBPBU048-6	UWBM59568	Aug. 6, 1997	68.02	68.60

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Aythya	*nyroca*	Ferruginous duck	EU585689			IPMB7474		Captive	Captive
Aythya	*nyroca*	Ferruginous duck	MW151610	MW151634		USNM623224	Jan. 4, 2002	Captive	Captive
Aythya	*nyroca*	Ferruginous duck	MW151611	KP252170	SIBIQ121-12	USNM645915	Aug. 27, 2010	33.94	44.36
Aythya	*nyroca*	Ferruginous duck	MW151612	MW151635		USNM645980	Apr. 23, 2011	33.94	44.36
Aythya	*nyroca*	Ferruginous duck	MW151613	MW151636		USNM646143	Aug. 2011	33.94	44.36
Aythya	*nyroca*	Ferruginous duck	MW151614	MW151637		USNM646150	Jun. 2011 to Aug. 2011	33.94	44.36
Aythya	*nyroca*	Ferruginous duck		GQ481388	KBPBU049-6	UWBM46236	May 18, 1993	44.89	75.12
Aythya	*valisineria*	Canvasback			BROMB744-7	ROM1B-1035	Jan. 1, 1981		
Aythya	*valisineria*	Canvasback			BROMB440-6	ROM93509	Dec. 1, 1999	42.55	-80.07
Aythya	*valisineria*	Canvasback	MW151615	MW151638		USNM641640	Jan. 2008	38.31	-122.72
Aythya	*valisineria*	Canvasback	MW151616	MW151639		USNM641770	Jan. 1, 2008	38.31	-122.72
Aythya	*valisineria*	Canvasback	MW151617	MW151640		USNM643733	Jan. 22, 2011	38.34	-75.91
Netta	*rufina*	Red-crested pochard	KC466568						
Netta	*rufina*	Red-crested pochard		GQ482234		UWBM56423	May 22, 1996	46.20	47.37

*Acronyms for voucher institutions: ANWC = Australian National Wildlife Collection, CWS = Canadian Wildlife Service, IPMB = Institut fuer Pharmazie und Molekulare Biotechnologie, JS = Anhui University, ROM = Royal Ontario Museum, NHMO = Natural History Museum University of Oslo, USNM = United States National Museum, UWBM = University of Washington Burke Museum.

We extracted genomic DNA using a phenol-chloroform reaction on an AutoGen© (Holliston, Massachusetts, USA) Gene Prep DNA extraction system according to the manufacturer's protocols. We measured the concentration of the extracted DNA using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts) and diluted or dried down the DNA extract when possible to achieve an ideal range of 10–20 ng DNA for polymerase chain reaction (PCR; actual range of DNA used 0.1–21.2 ng).

For all new sequences generated for this paper, we sequenced one or both of the mtDNA genes CO1 (partial, 652 base pairs) and ND2 (complete, 1,041 base pairs), using 2 primer pairs per gene. We amplified the CO1 gene fragment using either: BirdF1 (TTCTCCAACCACAAAGACATTGGCAC) and BirdR1 (ACTTCTGGGTGGCCAAAGAATCAGAA; Hebert et al. 2004) or dgLCO-1490 (GGTCAACAAATCATAAAGAYATYGG) and dgHCO-2198 (TAAACTTCAGGGTGACCAAARAAYCA; Meyer 2003). We amplified the ND2 gene using either: L5215 (TATCGGGCCCATACCCCGAAAAT; Hackett 1996) and H1064 (CTTTGAAGGCCTTCGGTTTA; Drovetski et al. 2004), or L5219 (CCCATACCCCGAAAATGATG) and H6313 (CCTTTATTTAAGGCTTTGAAGGC; Sorenson et al. 1999). Our CO1 PCR contained 0.5 U Biolase Taq (New England Biolabs Inc., Ipswich, Massachusetts), 0.5 mM total dNTPs, 1.5–2.0 mM $MgCl_2$ (concentration increased for difficult reactions), 0.3 µM

each forward and reverse primer, and 0.07 μg/mL BSA (New England Biolabs Inc., Ipswich, Massachusetts). Our ND2 PCR contained HotStart DNA Polymerase master mix (Promega Corporation, Madison, Wisconsin, USA) with $MgCl_2$ concentration increased to 3.0 mM for difficult reactions, and up to 0.4 μg/mL BSA. The PCR thermal-cycle profiles for both genes included a denaturation at 95°C for 5 minutes, 35 cycles of denaturation for 30 seconds, annealing temperature for 30 seconds, and extension at 72°C for 1 minute, followed by 10 minutes of final extension at 72°C. We used the following annealing temperatures for each primer set: 48°C (dgCO1), 50°C (BirdCOI), 57°C (L5219/H6313), and 59°C (L5215/H1064).

We visualized PCR fragments on a 1.5% agarose gel to confirm successful amplification and to check the negative controls. We cleaned the amplified fragments using ExoSAP-IT (Affymetrix Inc., Santa Clara, California, USA) exonuclease enzymes to remove excess primer and dNTPs, sequenced the fragments using fluorescent dye terminators (BigDye 3.1) according to manufacturer's protocol, and removed unincorporated fluorescent dyes using Sephadex G50 before sequencing the fragments on an ABI 3730x Genetic Analyzer (Applied Biosystems Inc., Foster City, California). We aligned the resulting sequences automatically and manually edited them in Sequencher 5.4.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Complete sequence information is available on GenBank (accession numbers MW151590–MW151640).

We used the sequence alignments in the program jModeltest2 (Darriba et al. 2012) to determine a substitution model for each gene based on Bayesian Information Criteria. The top model for CO1 was HKY+I (ΔBIC = 3.34), a model that includes variable base frequencies, separate transition and transversion rates, and the proportion of invariable sites (I; Hasegawa et al. 1985). The top model for ND2 was 012232+I+F (ΔBIC = 0.18), a special case of the general time reversible model that models variable base frequencies, specified substitution rates for 3 of the 6 possible nucleotide substitutions, and the proportion of invariable sites (I; Tavaré 1986). We implemented both models using the parameters from the substitution models in BEAST 2.4.2 (Bouckaert et al. 2014) under a strict molecular clock to generate a Bayesian Maximum Likelihood phylogenetic reconstruction for each gene using 10^8 MCMC with 10^4 burn-in period under a Yule process speciation prior. Every 10^4 steps we sampled and visualized parameters in Tracer 1.6 (http://beast.bio.ed.ac.uk/Tracer) to determine the effective sample size and 95% highest posterior density interval for each parameter. We generated a Maximum Clade Credibility tree for each gene from the top 10001 output trees from the MCMC using TreeAnnotator 2.4.2 (Bouckaert et al. 2014) and visualized the consensus tree in FigTree v1.4.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

The ingroup clade depth in the ND2 gene tree was 1.5 times greater than the CO1 gene tree (0.0277 substitution/site for ND2, 0.0182 substitutions/site for CO1; Figure 1). Only 3 of 9 ingroup species had strong support for monophyly of the CO1 gene region (posterior probabilities ≥0.95). Two sister pairs were paraphyletic at CO1 (greater scaup/lesser scaup and common pochard/canvasback), and the posterior probability for CO1 of the remaining 5 monophyletic species varied between 0.25 and 0.93. In contrast, all species were monophyletic in the ND2 gene tree, with strong support for monophyly in 7 of the 9 ingroup species and posterior probability of 0.85 and 0.92 for the remaining 2 species.

Genetic distances between the clades in 4 pairs of closely related species (greater scaup/lesser scaup, common pochard/canvasback, ferruginous duck/hardhead, and ring-necked duck/redhead) were 1.5–9.0 times greater in the ND2 gene tree than those in the CO1 gene tree, as calculated by comparing the substitution rates between species pairs (see Table 2).

Discussion

Accurate species identification is essential for reducing the likelihood of bird strike occurrence and/or damage (Dolbeer et al. 2000). Understanding which species are involved in bird strikes at or near a particular airfield can improve the effectiveness of habitat management strategies employed by airfield biologists and reduce the likelihood of collisions (Sodhi 2002, DeVault et al. 2011). Furthermore, know-

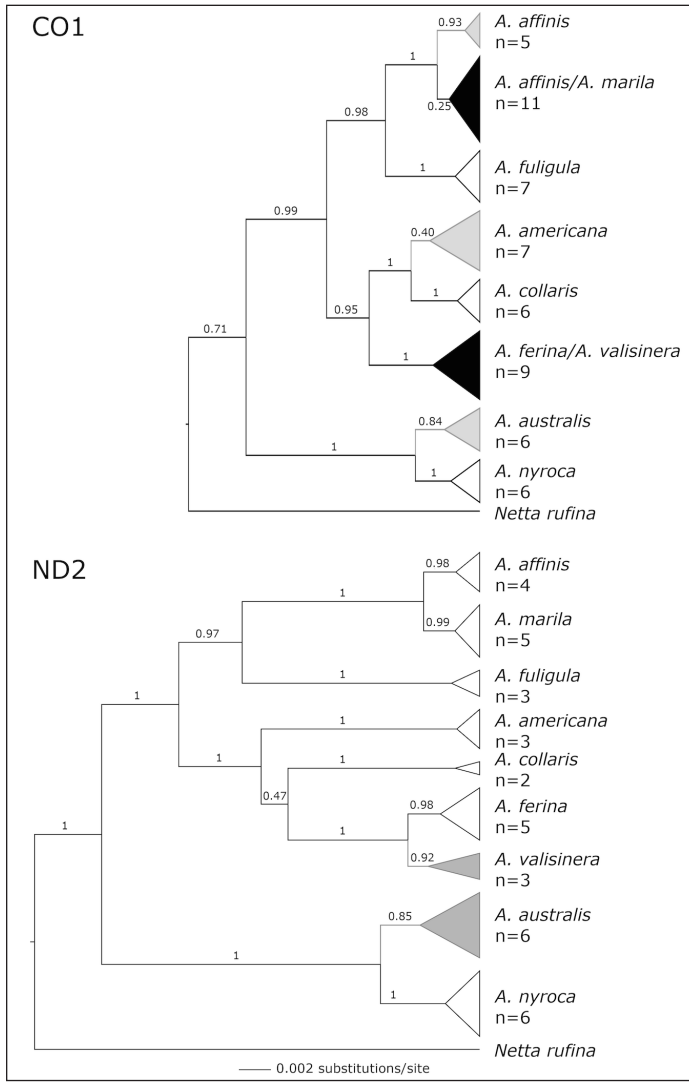

Figure 1. CO1 (top) and ND2 (bottom) gene trees drawn to the same scale. Branch lengths are scaled by substitutions per site (note the longer branch lengths of the ND2 tree). Gray triangles identify species-specific clades with poor statistical support (posterior probability <0.95). Black triangles identify clades with species-level paraphyly. Numbers above branches indicate posterior probability values. The ND2 tree has greater statistical support overall, more substitutions per site separating sister taxa and clades, and resolves all cases of paraphyly present in the CO1 tree. *Aythya affinis* (lesser scaup), *A. marila* (greater scaup), *A. fuligula* (tufted duck), *A. americana* (redhead), *A. collaris* (ring-necked duck), *A. ferina* (common pochard), *A. valisineria* (canvasback), *A. australis* (hardhead), *A. nyroca* (ferruginous duck), *Netta rufina* (red-crested pochard).

ing the mass of species involved in bird strikes is crucial when designing aircraft to be resilient to potential damage (Sodhi 2002, Jin 2018). *Aythya* spp. vary in mean body mass from 727 g (ferruginous duck) to 1,658 g (canvasback; Carboneras and Kirwan 2020, Mowbray 2020), and thus vary in potential damage each species may cause to an aircraft (Dolbeer et al. 2019).

The standard vertebrate DNA barcoding marker, CO1, is an efficient marker for high-throughput identification of unknown bird strike samples (Dove et al. 2008, 2009) and performs well in the vast majority of bird species studied to date (e.g., Kerr et al. 2007, 2009; Aliabadian et al. 2009; Chavez et al. 2015; Saitoh et al. 2015; Tizard et al. 2019). In a small proportion of species, including some *Aythya* ducks, the relatively low mtDNA substitution rate of CO1 (Lerner et al. 2011) limits its utility for species-level identification. In these cases, the targeted application of additional genetic markers with a higher substitution rate can aid in species identification.

Our analysis shows that ND2 is an effective additional marker for species-level identification when high-throughput protocols using CO1 barcoding identify a sample as belonging to the genus *Aythya*. Our comparison of CO1 and ND2 performance reveals 1.5–9.0 times greater divergence between sister species pairs in ND2 than CO1. In a side-by-side comparison of Bayesian Maximum Likelihood gene trees, the ND2 gene tree resolves all cases of apparent species paraphyly seen in the CO1 gene tree, demonstrating the increased statistical power of ND2 for species identification in *Aythya* spp. that is consistent with the difference in substitution rates between ND2 and CO1 (1.8 times greater for ND2) reported by Lerner et al. (2011). The continued effort to revisit and improve applied molecular methods for identification of unknown samples is crucial not just in the bird strike field but also in wildlife forensics (e.g., Iyengar 2014), identifying ecological interactions of pathogen vectors (e.g., Martinez-Dela Puente et al. 2017), identification of invasive agricultural pests (e.g., Pieterse et al.

Table 2. Genetic distances and number of segregating sites (fixed base pair differences between species in the pair) between closely related species pairs of diving ducks (*Aythya* spp.) were greater for ND2 than CO1 for all species pairs compared.

Species pair	CO1 Substitutions/site	CO1 Segregating sites	ND2 Substitutions/ site	ND2 Segregating sites	Magnitude increase in ND2 substitution rate
Lesser scaup/ greater scaup (*A. affinis*/ *A. marila*)	0.0004	0	0.0035	3	8.75
Common pochard/ canvasback (*A. ferina*/ *A. valisineria*)	0.0005	0	0.0045	3	9.00
Ferruginous duck/ hardhead (*A. Nyroca*/ *A. australis*)	0.0040	2	0.0061	5	1.53
Ring-necked duck/ redhead (*A. collaris*/ *A. americana*)	0.0043	4	0.0136	22	3.16

2010), revealing mis-labeling of species in food production (e.g., Keskin and Atar 2012), and non-invasive methods of conservation monitoring (e.g., Pfleger et al. 2016). Although we demonstrate this technique in a single genus under the specific circumstance of bird strikes, the addition of molecular markers, such as ND2, with higher mutation rates than traditional CO1 barcoding can be modified for application in other taxonomic groups. Ongoing work to determine efficient and accurate methods for genetic identifications of wildlife are critical for the bird strike community and beyond.

Management implications

According to a report on wildlife strikes to civil aircraft (Dolbeer et al. 2019), waterfowl comprise 28% of all damaging strikes in which the bird type was identified, including several of the *Aythya* spp. studied here. Understanding species-level patterns in bird strikes is crucial to proper implementation of bird strike management plans, the design of new aircraft and engines, and in keeping bird–aircraft interaction risks to a minimum. Although diving ducks congregate in mixed-species flocks during seasons of peak strike risk, species exhibit specific foraging strategies (e.g., Thornburg 1973) that may allow for targeted management opportunities. While this paper focuses on *Aythya* spp. as a case study, expanding molecular tools for species identification of unknown bird strikes is a fundamental step in understanding this human–wildlife conflict.

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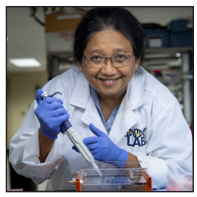

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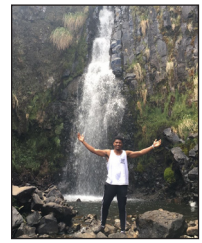

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