USING MORPHOMETRICS TO DETERMINE THE SEX OF COMMON RAVENS

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ABSTRACT—We tried using morphometrics to determine sex for a population of Common Ravens (*Corvus corax*) in northwestern Wyoming. We attempted to correlate 13 external measurements to sex using discriminant function analyses. Sex was verified with a DNA test that identified females with 2 PCR-amplified gene copies (1 each from the W and Z chromosomes) and males with 1 gene copy (only Z chromosome). We created a predictive model of sex of ravens for easy field use. We found that by using 2 separate discriminant functions with footpad length and body mass measurements simultaneously, we were able to correctly classify 97% of female samples, 91% of male samples, and had an unknown category that included 15% of samples.

Key words: Common Raven, *Corvus corax,* discriminant function, DNA isolation, gender, sexing

Sex determination of individuals is crucial in many aspects of biological studies, yet can be impossible for many monomorphic species without the use of detailed morphometrics, molecular techniques, laparoscopy, or dissection. As such, it has become common to investigate the use of discriminant function analysis (DFA) on a variety of morphometrics to model sex within many species. In many cases, sexes are separated using 1 or 2 external characteristics with 100% certainty.

Making a field determination of sex in many corvid species (*Corvidae*) can be difficult, even when the bird is in hand. Because of this, several researchers have measured and compared morphological differences between sexes. For example, Baumel (1953, 1957) looked for correlations of many external and skeletal measurements of Chihuahuan Ravens (*Corvus cryptoleucus*) and Fish Crows (*C. ossifragus*) and found that while males were generally larger than females no measurements could accurately and consistently predict sex for either species. Likewise, Clark and others (1991) found that American Crows (*C. brachyrhynchos*) could only be accurately classified as males or females 91.9% of the time (based on 3 measurements), and considered this estimation to be too low for most studies. Conversely, Caffrey (1992) was able to use measurements and discriminant function analysis (DFA) to determine the sex of American Crows 96% of the time, and considered this as an acceptable measure. Reese and Kadlec (1982) were able to accurately predict the sex of 95% of Black-billed Magpies (*Pica pica*) using DFA and 3 measurements, while Brown (1957) was able to correctly classify 96% of magpies using footpad length alone. Blanco and others (1996) were able to correctly determine the sex 100% of the time in Choughs (*Pyryhocorax rhyocorax*) using DFA. All studies involving DFA assigned sex using dissection, laparoscopy and/or laboratory techniques.

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Many DFAs of corvids could predict sex within a local population with greater than 95% accuracy using external measurements. Further, sex identification by polymerase chain reaction (PCR) of sex linked genes can determine true sex with 100% certainty (Norris-Caneda and Elliot 1998). Use of PCR with chromohelicase-DNA (CHD) binding protein genes to accurately determine sex of birds has become a common and widely accepted practice (such as Palma and others 2001; South and Wright 2002; Delven and others 2004; Vegara and Aguirre 2006). Because of this, we tested DFA in combination with molecular techniques to determine if we could create a field method for assigning sex using morphometrics in a population of Common Ravens (*Corvus corax*) in northwestern Wyoming.

METHODS

Locality and Sub-species

All ravens were captured within the Jackson Hole valley of northwestern Wyoming (43° 27′–50′N, 110° 50′–27′W). The valley is home to one of the densest recorded populations of breeding Common Ravens in North America (Bedrosian 2005) and also has a very large population of non-breeders (unpubl. data). Presumably, all of the ravens sampled were *C. corax sinuatus* based on range (Rea 1986) although our body mass measurements (725–1250 g) were slightly lower than the published range for this subspecies (Boarman and Heinrich 1999).

Captures and Morphometrics

We captured ravens using a variety of different methods and baits. We primarily used a net launcher (CODA Ent., Mesa, AZ) with carrion or molasses rolled corn as bait. We also used an enlarged drop-in trap (a.k.a. Z-trap or Australian crow trap) with a live raven lure and molasses rolled corn. We also used noose carpets over carrion, a mechanical Great Horned Owl lure (Jacobs 1996) with a mist net at nest sites, an individual-sized drop-in trap, and a bow net to capture individuals. Most birds were trapped during the fall and winter months. Each raven captured was aged as an adult or juvenile (hatch-year or 2nd-year) based on plumage characteristics (Heinrich and Marzluff 1992; Heinrich 1994; Restani and others 1996; Pyle 1997).

Morphometric measurements taken were unflattened wing chord length, tail length, body mass, culmen length (nares to tip), bill depth, tarsus length, and tarsus width based on Pyle (1997). We measured footpad and hallux nail length based on Bortolotti (1984). We also measured cranium length (tip of culmen to the back of the skull; Caffrey 1992), culmen width (taken perpendicular to the nares), fore nail length, and throat feather length. Throat feather length was measured on the longest central throat feather held at a 90° angle to the body. We did not measure potential asymmetry of measurements for each bird [such as Flemming and others 1991 found slight differences between right and left footpad lengths in Spotted Owls (*Strix occidentalis*)]. We also obtained a blood sample intravenously from the brachial vein and placed it in 0.5 ml of Longmire's cell lysis solution (100 mM Tris, pH 8.0, 100 mM EDTA, 10 mM NaCl and 0.5% SDS; Longmire and others 1988). We used whole blood rather than feather samples to extract DNA because we found feathers were more difficult to digest with Proteinase K than whole blood and feathers did not always yield DNA, whereas whole blood did (Unpubl. data).

Molecular Sex Determination

We isolated DNA from each blood sample using a DNeasy Tissue Kit (QIAgen, Valencia, CA) following the protocol for whole nucleated blood with the following modifications. We placed approximately 100 μl of blood from the Longmire's solution in a tube containing 20 μl of Proteinase K and 200 μl of AL buffer without the addition of phosphate buffered saline and then incubated the sample at 70°C for 1 h or overnight. Then, we determined the concentration of DNA using $OD_{260/280}$ measurements and then diluted the sample to 100 ng/μl using deionized water.

We determined the sex of each raven by 1st using PCR to isolate fragments of the sex chromosomes. We conducted the amplification of the CHD gene on the W and Z chromosomes (CHD-W and CHD-Z, respectively) using an iCycler iQ thermocycler (BioRad, Hercules, CA) following the protocol of Fridolfsson and Ellegren (1999). Fridolfsson and Ellegren (1999) specifically and successfully tested Common Ravens for the CHD genes. Briefly, reactions were performed in a 20 μl reaction containing 1X buffer [Promega, Madison,WI; 10 mM Tris-

HCl (pH 9.0)], 50 mM KCl and 0.1% Triton® X-100, 2 pmol of primers 2550F (forward, 5′-GTTACTGATTCGTCTACGAGA-3′) and 2718R (reverse, 5′-ATTGAAATGATCCA GTGCTTG-3′), 200 μM dNTPs (Promega), 1.75 mM $MgCl_2$ and 0.05 U *Taq* DNA polymerase (Promega). The thermal profile followed the protocols of Don and others (1991) with an additional 35 cycles at a constant annealing temperature at 50°C. PCR products were then separated using electrophoresis in 3% agarose standard tris-acetate-EDTA buffer followed by ethidium bromide staining.

We determined sex for each sample by examining the agarose gel. Male ravens have only 1 amplified band because they are homogametic (2 copies of the CHD-Z gene) while females have 2 amplified bands; 1 each for the CHD-W and CHD-Z genes. Our results were consistent with Fridolfsson and Ellegren (1991) in finding that the amplification of the CHD-W gene has a shorter PCR product (484 basepairs) and the CHD-Z gene has a longer product (684 basepairs). Length was determined with the aid of a 100 basepair ladder. Occasionally in some female raven samples, the CHD-Z gene does not amplify as intensely as the W chromosome. We checked these samples at least 1 additional time to verify that they were indeed females.

Statistical Analysis

After testing each morphometric variable for normality, we performed general multivariate analysis of variance (MANOVA) to determine if there were differences between hatch-year (HY) and after-hatch-year (AHY) age classes and sexes. Because it is often difficult to determine the correct age of ravens and we were interested in obtaining an easy field method of determining sex using morphometrics for this species, we targeted variables to statistically determine sex regardless of age. We tested variables that were not different between sexes for a correlation with time (month of capture) using correlation tests. We excluded variables from the DFA that were determined to be different between age classes from individual 1-way ANOVA tests. If the MANOVA tests detected differences between the subsequent variables that were not significantly different between age classes, we proceeded with individual 1-way ANOVA tests on each variable to determine which variable(s) to include for the DFA of sex. If a variable was found to be different between sexes, it was then included in a best subsets regression to find the most predictive variables. The individual or group of variables with the highest predictive power from the best subsets regression were then used in a DFA using cross-validation (Jackknife procedure) to assess the predictive power of the variable or group of variables in determining sex. We also calculated a sexual dimorphism coefficient between the males and females by finding the difference in the mean values of each variable tested.

Results

We captured 271 Common Ravens from 9 July 2004 through 30 March 2006. We determined that 194 were adults, 69 1st-year birds, and were unable to determine age for 8 ravens. Of the total captured, we documented that 56% ($N = 151$) were female and 44% ($N = 120$) were male by PCR of the CHD genes on the W and Z chromosome(s).

Using a MANOVA test, we found that there was a difference of morphometrics between age classes ($P < 0.01$) which were manifested by a shorter wing chord, tail, and throat feather lengths in hatch-year birds (ANOVA tests; all $P < 0.01$; Fig. 1). Since we found no differences in age for any other measurement and the feather measurements were not significantly different between sexes of either age class, the 3 feather measurements were excluded and the remaining measurements were pooled for all other analyses. Based on our sample, we found no measurement correlated with time (month; all $P > 0.05$). However, we found that body mass was approaching significance ($P = 0.08$), but our sample size (as it relates to month) may not be adequate to address this question because most of our captures took place in the fall and winter.

We determined that footpad length and body mass were the only variables that were significantly different between the sexes, with males being, on average, 16% heavier and their footpad length 9% longer than females (Table 1). Using these 2 measurements together and separately in DFAs with cross-validation, we found that footpad length alone proved to be the best predictor of sex. Footpad length was able to accurately classify 85% of males and 91% of fe-

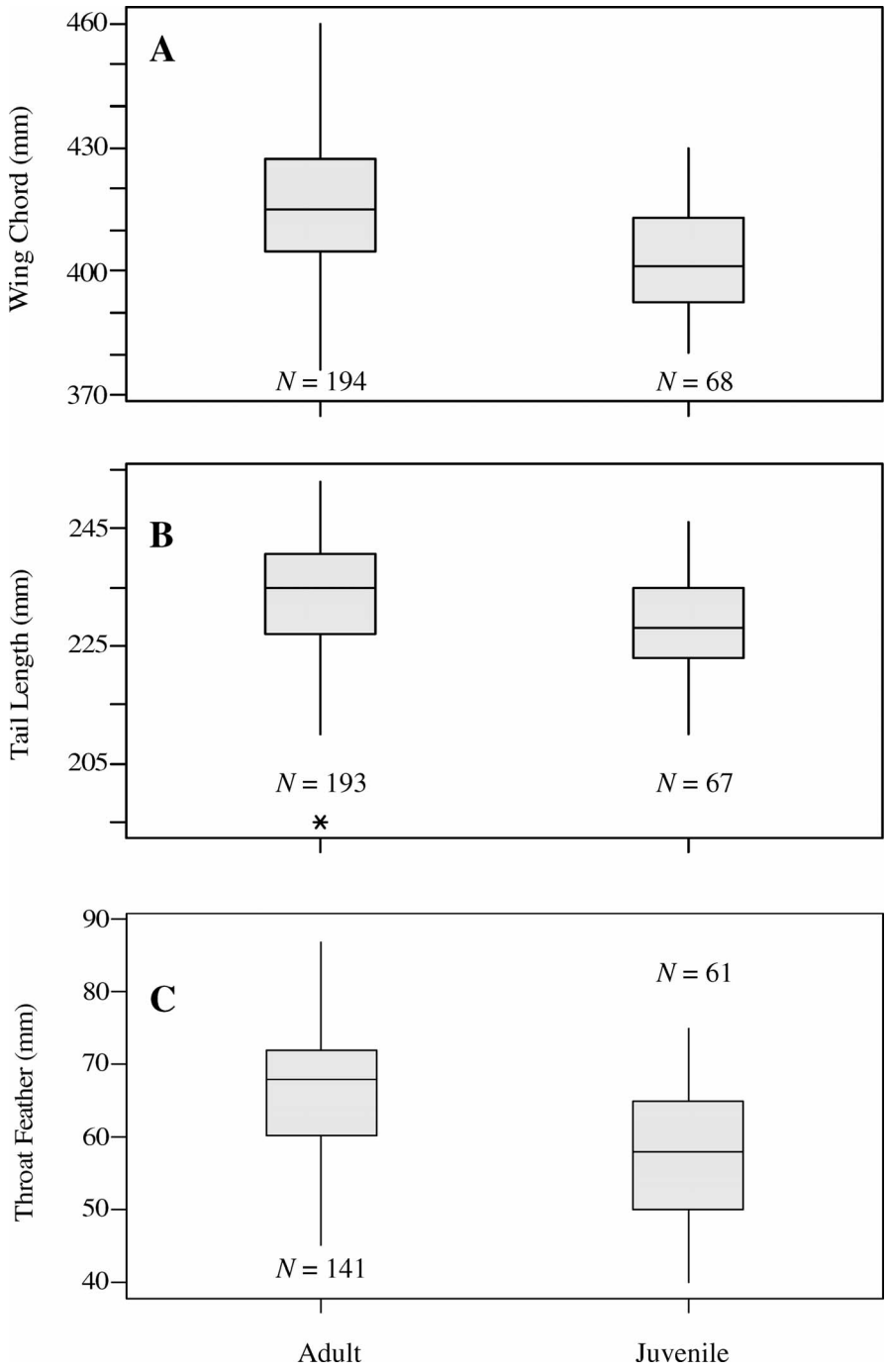

FIGURE 1. Boxplots of feather length differences between adult and juvenile (hatch-year and 2nd-year) Common Ravens in NW Wyoming. Age classes were statistically different for each feather length measurement: Wing Chord (A; $P < 0.001$; $F = 54.93$), Tail Feather (B; $P < 0.001$; $F = 41.92$), and Throat Feather (C; $P = 0.001$; $F = 10.94$). Asterisk identifies outlier.

TABLE 1. Sex differences in 10 morphological measurements in Common Ravens in NW Wyoming. All measurements except mass are reported as means in mm ± *s*. Mass is reported as means in g ± *s*.

	Females	Males	Dimorphism Coefficient
Culmen Length	47.3 ± 2.1 (147)	50.0 ± 2.1 (118)	1.06
Culmen Width	20.5 ± 1.9 (147)	21.6 ± 1.7 (116)	1.05
Bill Depth	23.7 ± 1.2 (148)	25.2 ± 1.0 (120)	1.06
Cranium	119.8 ± 3.7 (130)	125.7 ± 2.9 (110)	1.05
Tarsus Length	66.2 ± 2.0 (148)	69.1 ± 2.7 (118)	1.04
Tarsus Width	5.6 ± 0.4 (146)	6.0 ± 0.4 (120)	1.07
Hallux	22.9 ± 1.2 (150)	24.2 ± 1.0 (120)	1.06
Fore Nail	18.3 ± 1.4 (150)	19.6 ± 1.3 (120)	1.07
Footpad Length	73.9 ± 2.7 (149)*	79.6 ± 3.3 (121)	1.08
Body Mass	936.2 ± 75.9 (149)*	1090.2 ± 76.5 (119)	1.16

* Significant difference at $\alpha = 0.05$

males, and body mass correctly classified 82% of males and 91% of females.

Using linear regression to obtain equations for the relationships between footpad length and sex and body mass and sex, we obtained the following results:

$$Z_1 = -6.26 + 0.0825(\text{footpad length}) \quad (1)$$

$$Z_2 = -3.24 + 0.00327(\text{body mass}). \quad (2)$$

Negative *Z*-values from each equation predict females and positive values predict males. However, because the predictive power of each DFA was <100%, we found the range for males was (−0.49 to 0.99) and females was (−0.70 to 0.92) using the footpad length DFA ($P < 0.01$). Using body mass, the range for males and females were (−0.70 to 0.92) and (−0.87 to 0.55), respectively ($P < 0.01$). Misclassified birds were not systematically predicted (hence the large magnitude *Z*-value ranges for either sex). We found that when both equations are used concurrently, the predictive power increases and also adds an unknown category. If a bird's measurements are used in both equations and Z_1 and Z_2 have the same sign, then sex should be assigned accordingly. If Z_1 and Z_2 have opposing signs, then sex should not be predicted. Confidence can be assigned to a sample by adding Z_1 and Z_2 and examining the magnitude of the sum, where samples with a large magnitude sum would be more likely to be correctly classified than samples with a small magnitude.

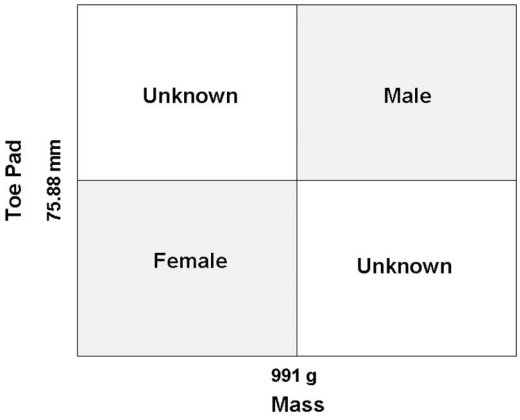

FIGURE 2. Graphical depiction of sex classification based on discriminant function analysis (DFA) of 271 Common Ravens (*Corvus corax*) from NW Wyoming. The DFA correctly classified 97% of females and 91% of males sampled. For example, if footpad length <75.88 mm and body mass <991 g, then the bird should be classified as a female. If footpad length <75.88 mm and body mass >991 g, then sex should not be assigned.

Solving the regression equations for *Z* (sex), we determined that individuals with footpad lengths >75.88 mm and body mass >991 g are males. If an individual has a footpad length >75.88 mm and a body mass <991 g (or vice-versa), then the individual's sex should not be assigned (Fig. 2). By using both individual DFAs concurrently in this fashion, the predictive power increases substantially, with only 3% of females misclassified, 9% of males misclassified, and 15% unknown.

Discussion

We found ravens to be only slightly sexually dimorphic (Table 1). Only 2 morphometic measurements, body mass and footpad length, were statistically larger in males than females, and there was overlap between the sexes in both (Table 1). Footpad length has, similarly,

been found to correctly classify sex for a variety of other species (Flemming and others 1991). We were unable to determine a discriminant function that could separate the sexes with 100% certainty. When we used 2 separate discriminant models in conjunction, we were able to add an unknown category and correctly classify 91% of males and 97% of females. Unfortunately, there is no way to determine whether or not a bird has been misclassified, but this technique does provide a reasonable way to determine sex of live birds from external measurements.

The validity of this technique may also be hampered by the seasonality of the collected data. While body mass was not correlated with time using our sample, we may not have had large enough sample sizes during each month to adequately determine if there was a significant relationship between body mass and time of year. Additional data needs to be collected during the spring-summer period to validate the year-round accuracy of this technique.

We found that both sexes of hatch-year birds had significantly shorter feather lengths than adults (Fig. 1). This finding is consistent with other corvids. Fish Crow (Baumel 1957), American Crow (Verbeek and Caffrey 2002), and Black-billed Magpie (Trost 1999) juveniles all exhibited shorter wing and tail feathers. Lindsdale (1937) also found the shape of the terminal portion of the outer retrices differed with age in Black-billed Magpies; roundness indicated juvenile and squarness adult. Given that we found no significant differences in any morphological measurement other than feather length between hatch-year and after-hatch-year birds suggests that young ravens obtain their final body size shortly after fledging.

While we have not checked the validity of this method with other subspecies of live-captured ravens or museum specimens, our measurements are similar to other published reports for the Rocky Mountain subspecies of ravens. Willett (1941) reported bill depths of 24.3 and 23.4 mm for males and females, respectively (N = 14 and 10, respectively), while our means were 25.2 and 23.7 mm, respectively. Willett (1941) also reported mean wing chord lengths of 439 and 425 mm for adult males and females, respectively, which fall slightly above our mean for adults (416 mm), but within our range (376 to 460 mm). However, observed differences may be due to small sample sizes. Linz and others (1990) found a difference in mass between the sexes of *Corvus corax clarionensis,* but these were noticeably lighter than ours. Detailed morphometrics of sexes may be useful in reassessing the subspecies of ravens, as most avian subspecies are not rigorously defined (Zink 2004).

While we were unable to find a discriminant model that could predict sex with greater than 91% accuracy, we think that our discriminant functions are still a valuable field method. Studies that need more accurate estimates of sex may need to develop different techniques for determining sex in the field or use genetic techniques. In our study, genetic determination of sex added a cost of approximately $2.50/sample beyond costs associated with capture and this may be an acceptable cost if greater accuracy of sex determination is needed. There may be other morphological differences that we did not investigate that warrant further study. For example, there may be structural differences in the size of the pelvis due to the females need for egg laying (Baumel 1953). Ravens appear monochromic, but we hypothesize that differences in the amount of iridescence or the ultra-violet reflection from the feathers may exist. More detailed studies of the morphology of known sex birds will help elucidate these and other potential differences.

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LITERATURE CITED

BAUMEL JJ. 1953. Individual variation in the White-Necked Raven. Condor. 55:26–32.

———. 1957. Individual variation in the Fish Crow, Corvus ossifragus. Auk 74:73–78.

BEDROSIAN B. 2005. Nesting and post-fledging ecology of the Common Raven in Grand Teton National Park, Wyoming [thesis]. Joneboro, AR: Arkansas State University. 120 p.

BLANCO G, TELLA JL, TORRE I. 1996. Age and sex determination of monomorphic non-breeding Choughs: A long-term study. Journal of Field Ornithology 67:428–433.

BOARMAN WI, HEINRICH B. 1999. Common Raven (Corvus corax), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/476

BORTOLOTTI GR. 1984. Sexual size dimorphism and age-related size variation in Bald Eagles. Journal of Wildlife Management 48:72–81.

BROWN RL. 1957. The population ecology of the magpie in western Montana [thesis]. Missoula, MT: Montana State University. 53 p.

CAFFREY C. 1992. Female-biased delayed dispersal and helping in American Crows. Auk 109:606–619.

CLARK RG, JAMES PC, MORARI JB. 1991. Sexing adult and yearling crows by external measurements and discriminant function analysis. Journal of Field Ornithology 62:132–138.

DEVLIN CM, DIAMOND AW, SAUNDERS GW. 2004. Sexing Artic Terns in the field and laboratory. Waterbirds 27:314–320.

DON RH, COX PT, WAINWRIGHT BJ, BAKER K, MATTICK JS. 1991. 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucleic Acids Research 19:4008.

FLEMMING TL, BUCHANAN JI, IRWIN LL. 1991. Footpad dimorphism as a possible means to sex of adult and juvenile Northern Spotted Owls (*Strix occidentalis caurina*). North American Bird Bander 16:1–3.

FRIDOLFSSON A-K, ELLEGREN H. 1999. A simple and universal method for molecular sexing of non-ratite birds. Journal of Avian Biology 30:116–121.

HEINRICH B. 1994. When is a Common Raven black? Wilson Bulletin 106:571–572.

HEINRICH B, MARZLUFF J. 1992. Age and mouth color in Common Ravens, *Corvus corax*. Condor 94:549–550.

JACOBS EA. 1996. A mechanical owl as a trapping lure for raptors. Journal of Raptor Research 30: 31–32.

LINSDALE JM. 1937. The natural history of magpies. Berkley, CA: Cooper Ornithological Club. 234 p.

LINZ GM, KNITTLE CE, JOHNSON RE. 1990. Activity of common ravens in relation to a California Least Tern colony on Camp Pendleton, California. 52nd Midwest Fish & Wildlife Conference, No. 300. p 323–324.

LONGMIRE JL, LEWIS AW, BROWN NC, CLARK JM, JONES MD, MEINKE LJ, MEYNE J, RATCLIFF RL, RAY FA, WAGNER RP, MOYZIS RK. 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. Genomic 2:14–24.

NORRIS-CANEDA KH, ELLIOT JR. JD. 1998. Sex identification of raptors using PCR. Journal of Raptor Research 32:278–280.

PALMA L, MIRA S, CARDIA P, BEJA P, GUILLEMAUD T, FERRAND N, CANCELA ML, DA FONSECA LC. 2001. Sexing Bonelli's Eagle nestlings: Morphometrics versus molecular techniques. Journal of Raptor Research 35:187–193.

PYLE P. 1997. Identification guide to North American birds. Part 1. Bolinas, CA: Slate Creek Press. 732 p.

REA AM. 1986. *Corvus corax,* geographic variation. In: Phillips AR. The known birds of North and Middle America. Part I. Denver, CO. p 65–66.

REESE KP, KADLEC JA. 1982. Determining the sex of Black-billed Magpies by external measurements. Journal of Field Ornithology 53:417–418.

RESTANI M, YATES RE, MARZLUFF JM. 1996. Capturing Common Ravens *Corvus corax* in Greenland. Dansk Ornitologisk Forenings Tidsskrifter 90: 153–158.

SOUTH JM, WRIGHT TF. 2002. Nestling sex ratios in the Yellow-naped Amazon: No evidence for adaptive modification. Condor 104:437–440.

TROST CH. 1999. Black-billed Magpie (Pica hudsonia), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/389

VERBEEK NA, CAFFREY C. 2002. American Crow (Corvus brachyrhynchos), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/647

VERGARA P, AGUIRRE JI. 2006. Age and breeding success related to nest position in a White Stork (Ciconia ciconia) colony. Acta Oecologica 30:414–418.

WILLETT G. 1941. Variation in North American ravens. Auk 58:246–249.

ZINK RM. 2004. The role of subspecies in obscuring biological diversity and misleading conservation policy. Proceedings of the Royal Society of London 271:561–564.

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