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Morphometric Differences Between Sexes of Two Subspecies of Black-crowned Night-Heron (*Nycticorax nycticorax*) Using Discriminant Function Analysis

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Abstract.—The objective of this study was to develop tools for distinguishing between sexes of the two Black-crowned Night-Heron (*Nycticorax nycticorax*) subspecies (*N. n. obscurus* and *N. n. hoactli*) using discriminant function analysis. Significant differences were found in length of the culmen, length of the bill from the gape, and length of the wing chord between the sexes of each subspecies, with males being larger than females. The resulting discriminant functions were able to differentiate between the sexes of each studied subspecies and between subspecies after determining the sex of the individuals (with a correct classification of 77.8% for females and 97.8% for males). In females, all morphometrics were greater for *N. n. obscurus* than *N. n. hoactli*; this was also the case for males, except for bill width, which was greater in *N. n. hoactli*. Wing chord length was the most useful measurement for constructing the discriminant functions. External morphometrics are a valuable tool not only for discriminating between *N. n. hoactli* and *N. n. obscurus* but also for sexing these subspecies. *Received 16 August 2017, accepted 30 September 2017.*

Key words.—Ardeidae, Black-crowned Night-Heron, discriminant function analysis, morphometrics, *Nycticorax nycticorax*, sexual dimorphism, subspecies dimorphism.

In many avian species, sex can be determined by observing plumage or sex-specific structural characteristics (such as colored soft tissue), sex-specific behavior or by measuring morphological characteristics (Jodice *et al.* 2000). However, this method cannot be applied to birds that are sexually monomorphic in both size and plumage (Coulson *et al.* 1983), making it necessary to use other methods such as anatomical examination (Richter *et al.* 1991) and genetic analysis (Childress *et al.* 2005). The use of external morphometrics to sex birds is of great value because it is inexpensive and allows a rapid and effective determination (Montalti *et al.* 2012). External morphometric indexes have been widely used to assist in the sexing of birds, but there is little information on the morphometrics of most Neotropical birds (Oniki 1986; Torlaschi *et al.* 2000; Montalti *et al.* 2004; Fuchs and Montalti 2016). On the other hand, discriminant function analysis (DFA; Sokal and Rohlf 1995) has been successfully applied in studies involving diverse bird species of different groups (Her-ring *et al.* 2010; López-López *et al.* 2011; Riordan and Johnston 2013; Baladrón *et al.* 2015). Two subspecies of Black-crowned Night-Heron (*Nycticorax nycticorax*) occur in southern South America: *N. n. hoactli* is distributed from South America to northern Chile, east of the Andes to Río Negro, Argentina; *N. n. obscurus* can be found from northern Chile (north to Atacama) and central Argentina (from Neuquén and southern Río Negro) to south, Tierra del Fuego. *N. n. hoactli* differs from *N. n. obscurus*, having a smaller average size and generally a paler plumage (Blake 1977).

Based on the morphological similarities between *N. n. hoactli* and *N. n. obscurus*, our goal was to distinguish between these two subspecies and between the males and females using DFA of body measurements.

Methods

We examined adults of *N. n. hoactli* (40 males and 20 females) and *N. n. obscurus* (9 males and 11 females). The examined material belonged to the Ornithological

Collection of the Museo de La Plata ($n = 48$), the Ornithological Collection of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" ($n = 27$), and the Collection of the Instituto Argentino de Investigación de Zonas Áridas ($n = 5$). We only worked with bird skins in a good state of preservation; all specimens had labels with complete information of sex and subspecies identification. A taxidermist determined the sex of specimens through direct gonadal examination when they were entered into the ornithological collections. We did not use skins with doubtful subspecies assignment. Measurements of external characters followed the methods of Baldwin *et al.* (1931) and Winker (1998) and included: length of exposed culmen (from the anterior margin of the nares to the tip of the bill), bill width (at the base of the bill), length of bill from gape (straight-line distance from the tip of the maxilla to the corner of the mouth), and tarsus length (from the notch on the back of the intertarsal joint to the ventral surface of the foot with toes extended). These characters were measured using a Vernier caliper (accuracy ± 0.01 mm). In addition, we measured the length of the wing chord, which is defined as the distance from the distal portion of the carpus to the tip of the longest primary feather without attempting to flatten out the curve of this feather (Baldwin *et al.* 1931). For this measure, we used a zero-stop metal ruler (accuracy ± 1 mm) (Winker 1998). Since some species are bilaterally asymmetric with one side larger than the other, we took all measurements from the right side of each bird (McNeil and Martínez 1967).

We checked data distributions for normality and homoscedasticity using the Shapiro-Wilks' test and Levene's test, respectively, before parametric statistical analysis. The Student's t-test was used to test for statistically significant differences in the variables between sexes within each subspecies and between subspecies within each sex using Infostat software (Di Rienzo *et al.* 2016). Cohen's *d* and its confidence interval (CI) was calculated with the Effect Size Calculator (Centre for Evaluation and Monitoring 2000; Nakagawa and Cuthill 2007) when we evaluated sexual dimorphism. There were significant differences in morphometric characters between the sexes of each subspecies. Therefore, the studied subspecies were compared for each sex separately. We performed a forward stepwise DFA using the SPSS statistical program (SPSS, Inc. 2010) to distinguish between sexes within each subspecies and between subspecies for each sex separately. We established the validity of the discriminant function by the proportion of specimens from each group that was correctly classified and by the Jackknife procedure (Krebs 1989). We obtained cut-off values by fitting data (each discriminant score and the associated posterior probability) to a sigmoid-type function using program OriginPro (OriginLab 2016).

RESULTS

In *N. n. hoactli*, all morphometrics were significantly greater for males than females (Table 1). In *N. n. obscurus*, the culmen

Table 1. Morphometric measurements (mm) from males and females of Black-crowned Night-Heron subspecies: *N. n. hoactli* and *N. n. obscurus* (mean ± SD, minimum-maximum); P-values from the Student's t-test; Cohen's *d* with 95% CI for each variable.

Measurements	*N. n. hoactli*				*N. n. obscurus*			
	Males $n = 40$	Females $n = 20$	P	Cohen's *d* (95% CI)	Males $n = 9$	Females $n = 11$	P	Cohen's *d* (95% CI)
Culmen	74.30 ± 3.48 66.02-81.80	70.93 ± 3.32 65.05-76.15	0.0007	0.97 (0.41-1.54)	78.27 ± 5.41 69.68-83.44	73.35 ± 3.72 64.94-78.16	0.030	1.04 (0.10-1.97)
Bill width	19.86 ± 1.53 17.15-25.40	18.78 ± 1.60 15.45-23.82	0.010	0.68 (0.13-1.23)	18.57 ± 0.91 17.22-19.66	18.18 ± 0.65 17.35-19.48	0.200	0.49 (-0.41-1.38)
Length of bill from gape	98.69 ± 5.94 80.66-108.20	94.98 ± 6.80 80.00-104.80	0.030	0.59 (0.04-1.14)	106.58 ± 3.27 99.84-110.60	99.54 ± 6.78 88.32-111.30	0.008	1.22 (0.27-2.18)
Tarsus	79.92 ± 4.49 73.77-97.46	77.14 ± 4.86 66.51-89.71	0.030	0.60 (0.05-1.14)	81.74 ± 6.85 69.50-91.29	78.98 ± 5.07 67.11-85.59	0.300	0.45 (-0.45-1.34)
Wing Chord	303.51 ± 9.22 282-319	292.19 ± 9.06 278-308	0.0001	1.22 (0.59-1.85)	326.56 ± 12.50 302-345	311.82 ± 8.91 300-327	0.006	1.32 (0.35-2.30)

length, length of bill from gape and wing chord length were significantly greater for males than for females (Table 1). We performed DFA to distinguish males from females of the same subspecies. The discriminant function obtained for *N. n. hoactli* was

$$D_{nnh\ (m\ vs.\ f)} = 0.162 \times \text{Culmen length} + 0.077 \times \text{Wing Chord length} - 596.607$$

(Wilks' Lambda = 0.685; $P < 0.0001$). The function correctly classified 81.1% (95% CI: 77.3-84.6%) of the analyzed specimens, and the Jackknife procedure yielded the same percentage. The cut-off point for the function $D_{nnh\ (m\ vs.\ f)}$ was -0.866, above and below which specimens were classified as males and females, respectively (Fig. 1A). The discriminant function for *N. n. obscurus* was
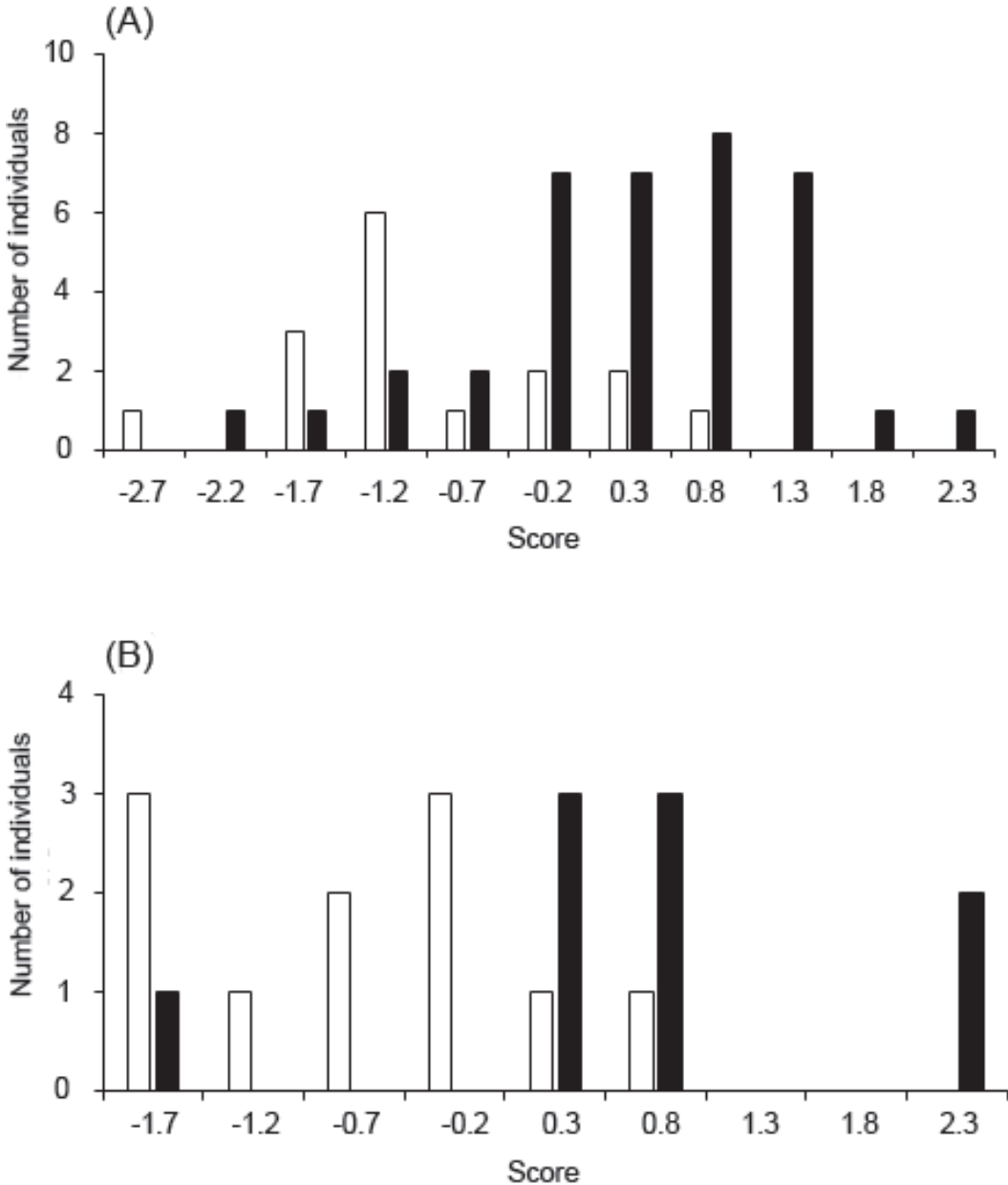

Figure 1. Classification of female (white bars) and male (black bars) Black-crowned Night-Heron (*Nycticorax nycticorax*) subspecies using discriminant function analysis. (A) *N. n. hoactli* applying $D_{nnh\ (m\ vs.\ f)}$. (B) *N. n. obscurus* applying $D_{nno\ (m\ vs.\ f)}$.
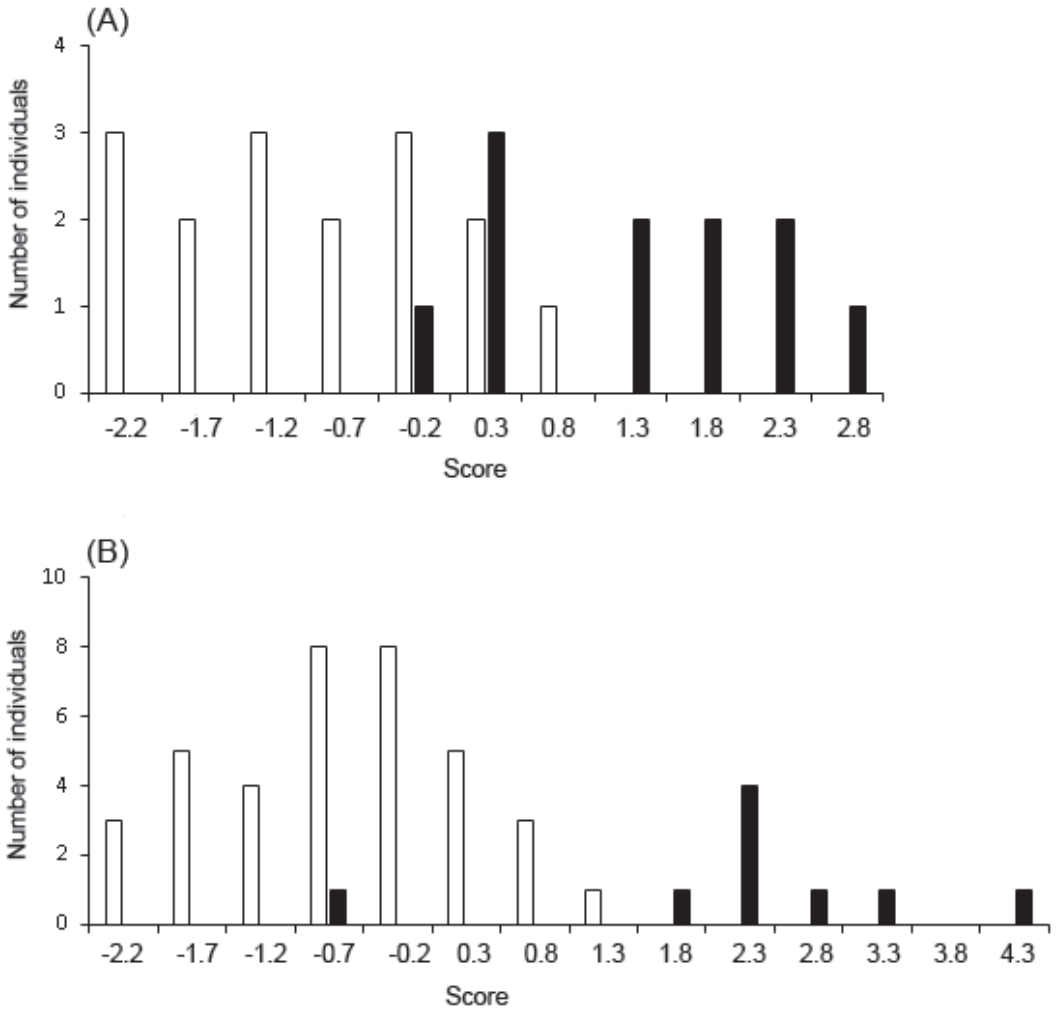
Figure 2. Classification of Black-crowned Night-Heron subspecies: *N. n. hoactli* (white bars) and *N. n. obscurus* (black bars) using discriminant function analysis. (A) Females applying $D_{nn\ (fh\ vs.\ fo)}$ discriminant function. (B) Males applying $D_{nn\ (mh\ vs.\ mo)}$ discriminant function.

$$D_{nno\ (m\ vs.\ f)} = 0.094 \text{ x Wing Chord length} - 29.891$$

(Wilks' Lambda = 0.65; $P = 0.007$). The function correctly classified 80% (95% CI: 76.1-83.6%) of the analyzed specimens, and the same value was obtained with the Jackknife procedure. The cut-off point for the function $D_{nno\ (m\ vs.\ f)}$ was 0.069; specimens with scores above it were predicted to be males and those below it to be females (Fig. 1B).

The comparison of females between subspecies indicated that the tarsus of *N. n. obscurus* was significantly longer than that of *N. n. hoactli* ($P < 0.0001$) (Table 1). Although we did not find significant differences for culmen length, length of bill from gape, bill width and wing chord length ($P > 0.05$), the females of *N. n. obscurus* showed a trend toward higher values, except for bill width (Table 1).

When males of the two subspecies were compared, *N. n. obscurus* had a significantly larger culmen length ($P = 0.008$), length of bill from gape ($P = 0.0004$) and wing chord length ($P < 0.0001$), while bill width ($P = 0.02$) was significantly larger for males of *N. n. hoactli* (Table 1).

A DFA to distinguish between subspecies was performed for each sex separately. The discriminant function for females was

$$D_{nn\,(fh\,vs.\,fo)} = 0.111 \times \text{Wing Chord length} - 33.347$$

(Wilks' Lambda = 0.446; $P < 0.0001$). The function correctly classified 77.8% (95% CI: 73.8-82.1%) of the specimens, and the same percentage was obtained using the Jackknife procedure. The cut-off point for the function $D_{nn\,(fh\,vs.\,fo)}$ was 0.540; females with scores below this cut-off point would belong to *N. n. hoactli* while those with scores above it would belong to *N. n. obscurus* (Fig. 2A).

In the case of males, the discriminant function to distinguish between subspecies was

$$D_{nn\,(mh\,vs.\,mo)} = -0.440 \times \text{Bill width} + 0.101 \times \text{Wing Chord length} - 22.453$$

(Wilks' Lambda = 0.416; $P < 0.0001$). This function correctly classified 97.8% (95% CI: 92.8-99.3%) of the analyzed individuals, and the Jackknife procedure yielded the same percentage. The cut-off point for the function $D_{nn\,(mh\,vs.\,mo)}$ was 1.355, below which males would belong to *N. n. hoactli* and above which they would belong to *N. n. obscurus* (Fig. 2B).

DISCUSSION

Our results indicate that males are larger than females in *N. n. hoactli* and *N. n. obscurus*, with significant differences in culmen length, length of bill from gape and wing chord length. Larger measurements for males of both subspecies have also been found by Blake (1977), Bó and Darrieu (1993), and Bó (1955).

We compared our results with those obtained by Bó and Darrieu (1993). For females of *N. n. hoactli*, we found a similar mean culmen length, a similar mean tarsus length with considerable overlap of ranges and a slightly higher mean value of wing chord length in our sample. For males of *N. n. hoactli*, these authors reported a higher mean value for culmen length, a similar mean tarsus length and a higher mean value for wing chord length. For both females and males of *N. n. obscurus*, we recorded a similar mean culmen length, a slightly lower mean tarsus length and a slightly higher mean wing chord length.

In the case of *N. n. hoactli*, Blake (1977) reported that males show higher mean values of culmen length and wing chord length than females, which is in agreement with our data; however, in contrast to our results, this author found higher values of mean tarsus length for females. For *N. n. obscurus*, Blake (1977) recorded that the culmen length and wing chord length of males was longer than that of females, which is consistent with our results. When we compared the two subspecies, we found that males and females of *N. n. obscurus* showed larger body measurements than males and females of *N. n. hoactli*, except for bill width, which was higher in *N. n. hoactli* males. Similar results were reported by Blake (1977), Bó and Darrieu (1993), and Bó (1955).

Wing chord length was the variable that most contributed to the discrimination between sexes and subspecies. This character was included in all the discriminant functions derived from our analysis; it was the only measurement present in two discriminant functions, whereas it was accompanied by culmen length or bill width in two other discriminant functions. These measurements are advantageous in that they are easy to obtain (Montalti *et al.* 2012).

External morphometrics appeared to be a reliable tool for differentiating between the sexes of each studied subspecies and between subspecies after determining the sex of the individuals. The use of external morphometrics to sex birds is less expensive and invasive than laparotomy and blood sample collection (Montalti *et al.* 2012), and a morphometric approach may provide useful information for taxonomic, ecological, behavioral, physiological, and evolutionary purposes (Jodice *et al.* 2000; Møller 2000; Ruckstuhl and Clutton-Brock 2005). In addition, DFA has proven to be useful for sexing not only the Black-crowned Night-Heron but also other bird species such as the Chilean Flamingo (*Phoenicopterus chilensis*; Montalti *et al.* 2012) and the White-faced Ibis (*Plegadis chihi*; Fuchs *et al.* 2017). Since there are studies that found effective the use of DFA

(made with museum specimens) in living birds (Montalti *et al.* 2012), the present work would be useful for sexing birds in the field.

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