The reliability of morphometric discriminant functions in determining the sex of Chilean flamingos *Phoenicopterus chilensis*

Diego MONTALTI[1,2*], Maricel GRAÑA GRILLI[2], René E. MARAGLIANO[3] and Guillermo CASSINI[4]

[1] Cátedra de Ornitología, Facultad de Ciencias Naturales y Museo, Paseo del Bosque, B1900FWA-La Plata, Argentina

[2] CONICET, Cerrito 1248, C1010AAZ-Buenos Aires, Argentina

[3] Jardín Zoológico de La Plata, Paseo del Bosque, B1900-La Plata, Argentina

[4] Departamento de Ciencias Básicas, Universidad Nacional de Luján, Ruta 5 y Av. Constitución, B6700-Luján, Buenos Aires, Argentina

Abstract   Monomorphic birds cannot be sexed visually and discriminant functions on the basis of external morphological variations are frequently used. Our objective was to evaluate the reliability of sex classification functions created from structural measurements of Chilean flamingos *Phoenicopterus chilensis* museum skins for the gender assignment of live birds. Five measurements were used to develop four discriminant functions: culmen, bill height and width, tarsus length and middle toe claw. The functions were tested on a sample of live flamingos from a zoo. The best classification for museum flamingos was given by a function using tarsus length, bill width and middle toe claw (97%). However, this function did not give the best classification for the zoo-based flamingos (81%) which had the best sex assignment by a function including measurements of tarsus, culmen and bill height and width (85%). This shows that a function giving good results in the sample from which it originated may not be as good when applied to another group of animals. Our study emphasizes the need for assessing the accuracy of a function by testing it with other methods to ensure its suitability when being applied [*Current Zoology* 59 (6): 851–855, 2012].

Keywords   Morphometrics, Discriminant function, Sexual dimorphism, Sex classification

Behavioural interpretation and ecological data are often improved when an individual's sex is known. Accurate sex identification is important for sex-specific studies, such as when studying breeding behaviour, ecology and energetics. For many avian species, sex can be determined without internal examination, by observing plumage or sex-specific structural characteristics (such as colored soft-tissue), measuring morphological characteristics, or the observation of sex-specific behavior (Jodice et al., 2000). This is not possible in many species because of the lack of external characteristics that allow for sex determination. Two methods are used to identify the sex of birds in such cases: (1) laparotomy, a procedure in which the gonadal tissue of an anesthetized bird is surgically examined (Risser, 1971; Richter and Bourne, 1990; Richter et al., 1991); and (2) collection of blood or feather samples for genetic analysis (Griffiths et al., 1998; Bertault et al., 1999; Fridolfsson and Ellegren, 1999; Childress et al., 2005). However,

both methods are invasive and expensive procedures that may alter metabolic rates, and result in infection or even death.

To avoid destructive or invasive techniques the use of external morphometrics to reliably sex birds is of great value. From known correlations between sex and measurements in a sample of sexed birds, a Discriminant Function Analysis (DFA) (Sokal and Rohlf, 1981) can weight characters to distinguish groups (sexes) of unknown individuals of monomorphic species. Therefore, DFA can use morphometric differences between known sexed-birds to predict the sex of animals in the field (Dechaume-Moncharmont et al., 2011).

DFA has been successfully applied to a wide variety of bird species from different groups including penguins (Scolaro et al., 1983; Gales, 1988), divers (Okill et al., 1989), petrels (Albores-Barajas et al., 2010), cormorants (Casaux and Baroni, 2000), vultures (López-López et al., 2011), gulls (Herring et al., 2010), skuas (Hamer and

Furness, 1991; Montalti, 2005), moorhens (Anderson, 1975), rooks (Green, 1982), and flamingos (van Couteren and Verheyen, 1988; Childress et al., 2005). The method for discrimination constructs a formula that calculates a discriminant score for each individual on the basis of its measurement. The point that scores males and females into groups is taken as the midpoint of the interval between the group means of sexed males and females. However, age-dependent variation in size or shape need to be considered (Nugent, 1982; Scolaro et al., 1983; Hamer and Furness, 1991) and differences among populations, related to geographical variation or differences in growth and development, may restrict the use of DFA to the same population from which it was conducted.

External morphometric indices have been widely used to assist in the sexing of birds but little is known about the morphometrics of most Neotropical birds (Oniki, 1986; Montalti et al., 2004). The Chilean flamingo *Phoenicopterus chilensis* is an example of a bird that lacks sex-specific plumage. Differences in measurable characteristics, however, may reveal dimorphisms sufficient to distinguish sexes. van Couteren and Verheyen (1988) presented a discriminant function to sex Chilean Flamingos using body mass and wing length, two variables that change with the condition of birds. They also propose the use of just tarsus length as a classificatory variable, obtaining a good reliability for classification of the sample that originated the function but without testing it in another group of flamingos.

The aim of this work was to develop sex discriminant functions from preserved Chilean flamingo museum skins using only structural measurements that do not vary with bird condition and apply those functions to a group of living birds in order to assess their reliability for application to wild flamingos.

1 Materials and Methods

1.1 Sampling

This study was based on 72 adult Chilean flamingos divided in two groups, one group of museum skins and one group of birds living in captivity. The group of museum birds consisted of 38 birds (24 males and 14 females) coming from different localities that were obtained from the following museum collections: Museo de La Plata (MLP), La Plata; Museo Argentino de Ciencias Naturales Bernardino Rivadavia (MBR), Buenos Aires; Fundación Miguel Lillo (FML), Tucumán, Argentina; Museu Nacional Río de Janeiro (MNRJ), Río

de Janeiro, Brazil; American Museum of Natural History (AMNH), New York, and Academia of Natural Sciences of Philadelphia (ANSP), Philadelphia, United States of America.

The group of live birds consisted of 34 flamingos (16 males, 18 females) from the La Plata Zoological Garden, La Plata, Argentina caught as adults from wild populations and living in captivity for several years. All zoo birds were already banded and sexed.

1.2 Morphometric analysis

Morphometric variables measured were culmen length (CU) from the anterior end of the nostril to the tip of the bill; bill height (BH) and width (BW) measured in the middle of the bill; tarsus length (TL) from the notch on the back of the intertarsal joint to the ventral surface of the foot with toes extended; and the middle toe claw length (MC). TL and MC were taken from the right side of each individual because of the possibility of bilateral asymmetry as suggested by McNeil and Martínez (1967) and McNeil et al. (1971). For bill and claw measurements we used a metal vernier caliper (± 0.01 mm), and for tarsus length a metal ruler with a perpendicular stop at zero (± 1 mm). In order to avoid observer bias all measurements from museum specimens and living flamingos were taken by the same person.

The sex of the museum specimens was taken from the museum database.

1.3 Statistical analysis

Comparisons of morphometric data between sexes were done using a two factor analysis of variance (ANOVA) with sex and origin as factors. Normality and homogeneity of variance were tested using Kolmogorov-Smirnov and Levene tests. Several Discriminant Function Analyses (DFA) were performed to develop classification functions to assign the sex of flamingos using the museum flamingos as the learning sample. First we performed a stepwise DFA which included the measurements of tarsus, bill width and middle toe claw, and then three other DFA including in each one all the measured variables, the variables that showed significant differences, and only the tarsus length, respectively. All DFA were done using the jackknife procedure. The cutting point for scores of males and females was calculated as the mean point between the weighted mean of the canonical variables for each group. Later, the functions where applied to sex the sample of live flamingos in order to look for differences in classification accuracy.

2   Results

Males flamingos were bigger than females in all the measurements taken. Significant differences between the males and females of both groups were found for tarsus, bill height and width and culmen; only middle toe claw showed significant differences between zoo and museum birds (Table 1).

The function obtained by stepwise DFA included the variables tarsus, bill width and middle toe claw and was the one that best classified the museum birds (Table 2). However, we found different relationships in the accuracy of classification among the different functions when applied to the museum or zoo flamingos, so the function obtained by stepwise DFA was not the best at classifing the zoo sample. Instead, the function incorporating the variables that showed significant differences (tarsus, bill width and height and culmen) performed best on zoo birds (Table 2).

Table 1   Comparison of body measurements (mm) of Chilean flamingos *Phoenicopterus chilensis* by sex and origin (museum and zoo)

	Male (n = 33)	Female (n = 22)	F	P	Museum (n = 29)	Zoo (n = 26)	F	P
Culmen length	109.2 ±0.8 (107.7–110.7)	105.2±0.9 (103.4–107.1)	10.73	0.001	107.8± 0.9 (106.0–109.5)	106.7±0.8 (105.0–108.4)	0.75	0.389
Bill height	36.6±0.3 (36.1–37.2)	35.0 ±0.3 (34.3–35.7)	13.14	< 0.001	35.5 ±0.3 (34.8–36.1)	36.2 ±0.3 (35.6–36.8)	2.79	0.101
Bill width	24.9 ±0.2 (24.5–25.3)	23.9 ±0.3(23.4–24.4)	9.38	0.003	24.1±0.2 (23.6–24.6)	24.7 ±0.2 (24.2–25.2)	3.46	0.068
Tarsus length	258±2.6 (253–263)	227±3.2(221–234)	55.38	< 0.001	243±3.0 (237–249)	242±2.9 (236–248)	0.05	0.817
Middle toe claw	10.7±0.1(10.4–11.0)	10.3±0.2(9.9–10.7)	2.44	0.125	10.1 ±0.2 (9.7–10.4)	10.9±0.2 (10.6–11.3)	12.12	0.001

Data are presented as mean ± standard errors (SE) and with 95% confidence intervals.

Table 2   Classification functions generated by Discriminant Function Analysis from morphometric characters of Chilean flamingos *Phoenicopterus chilensis* and percentage classification of museum and zoo samples

Function	Wilks' λ	F	P	Cutting Point	Correct Classification %					
					Museum			Zoo		
					Total	Male	Female	Total	Male	Female
D = 0.175 TL + 0.846 BW − 1.058 MC − 51.370	0.4039	12.301	< 0.001	-0.05	97.0	100	88.9	80.8	92.3	69.2
D = -0.050 CU + 0.365 BH + 0.738 BW + 0.175 TL – 1.188 MC − 55.142	0.3968	6.994	< 0.001	0	92.3	94.1	88.9	80.8	92.3	69.2
D = -0.062 CU + 0.198 BH + 0.855 BW + 0.153 TL - 57.398	0.4112	10.737	< 0.001	-0.03	91.4	95.5	84.6	84.6	100	69.2
D = 0.129 TL – 30.567	0.4794	40.182	< 0.001	0	90.0	88.5	92.9	82.7	100	61.5

3   Discussion

The measurement of tarsus length is already used for sexing flamingos (Studer-Thiersch, 1986). van Couteren and Verheyen (1988) improved the accuracy of discrimination by developing a function that in addition to tarsus length used bill length, body weight and wing length. The latter measurements depend on the time elapsed from food intake, breeding stage and state of the wing feathers which is related to the molt cycle (Winker, 1998). Our intention was to develop functions that can be used regardless of the particular state of the flamingo, so we chose not to use body weight or measurements of wing feathers. The advantages of the method used here is that it is based on structural measurements that remain constant once a certain age has been reached (e.g. tarsus and bill) regardless of occasional conditions. Also, these kinds of measurements can easily be obtained from living and dead birds, as well as from museum collections, making sex determination possible for almost any bird of this species.

The absence of significant differences in most of the measurements between museum and zoo flamingos, suggests that no differential development patterns arose from the state of captivity. This was to be expected, as the zoo flamingos were caught as adults. That could have been a source of uncertainty as we used this group birds to test the validity of the discriminant function generated from the group of museum birds. Because we found differences only in the measurement of the middle toe claw, we consider that the functions generated by the measures taken from the museum birds were appropriately applied to the measurements obtained from zoo flamingos.

The measurements that showed significant differences between sexes for the museum group (bill height

and width, tarsus length and culmen) did not produce the best discriminant function for the sample of museum birds, but it was the one that best performed on the sample of zoo birds. Functions that included middle toe claw, the measurement that did not show significant differences when comparing sexes, improved the accuracy of discrimination of the museum sample but reduced the accuracy of the functions when applied to the zoo sample (Table 2). This result shows two things: (1) On one hand, when some measurements considered alone do not allow for classifying groups, they may improve the classification of individuals when considered jointly with other measurements; and (2) the accuracy of a classification function may change when it is applied to a group of individuals different from the one where it originated. In all cases the proportion of flamingos correctly sexed was lower in the test sample (zoo birds) than in the learning sample (museum birds). In this sense, van Couteren and Verheyen (1988) generated a sexing function for Chilean flamingos using tarsus length with only 4% misclassification, but when we tested it on our samples, males were correctly assigned only 18.8% of the time for the zoo sample and 45.8% for the museum one; all females were correctly classified as such. In our case, tarsus length did not provide the best function for classifying any of the two samples.

From this work it is apparent that even when functions give a good result in the sample from which they were created, these functions should be applied with caution to other groups of animals. The small data set from which these discriminant functions were developed could be an explanation for the low percent of correct discrimination in the test sample, as a high sensitivity of the discriminant rate to small sample sizes has been shown (Dechaume-Moncharmont et al., 2011). This work also highlights that it is essential to test the accuracy of the functions in a sample different from the one that generated it, as was done here, in order to generalize the function to the whole species.

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