

## **Morphometrics and Sex Determination in the Endangered Bearded Vulture (*Gypaetus barbatus*)**

Authors: López-López, Pascual, Gil, Juan Antonio, and Alcántara, Manuel

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MORPHOMETRICS AND SEX DETERMINATION IN THE ENDANGERED BEARDED VULTURE  
(*GYPÆTUS BARBATUS*)

PASCUAL LÓPEZ-LÓPEZ<sup>1</sup>

*Cavanilles Institute of Biodiversity and Evolutionary Biology, Terrestrial Vertebrates Group, University of Valencia,  
C/Catedrático José Beltrán 2, 46980 Paterna, Valencia, Spain*

and

*Vertebrates Zoology Research Group, Instituto Universitario de Investigación CIBIO, University of Alicante, Apdo. 99,  
E-03080 Alicante, Spain*

JUAN ANTONIO GIL

*Fundación para la Conservación del Quebrantahuesos (FCQ), Plaza San Pedro Nolasco 1, 4-F,  
50.001 Zaragoza, Spain*

MANUEL ALCÁNTARA

*Dirección General de Desarrollo Sostenible y Biodiversidad, Departamento de Medio Ambiente, Gobierno de Aragón,  
Paseo María Agustín 36, 50.071 Zaragoza, Spain*

**KEY WORDS:** *Bearded Vulture*; *Gypaetus barbatus*; *molecular sexing*; *morphometric*; *Pyrenees*; *sexual size dimorphism*; *Spain*.

Sex determination is important for studying many aspects of avian biology, including behavior, evolutionary ecology, conservation genetics, population dynamics, dispersal, and demography (e.g., Clutton-Brock 1986, Dunn et al. 2001, Newton 1998, 2003). Moreover, sex identification is useful for conservation programs, including both wild and captive populations, and particularly for reintroduction programs of endangered species when a fixed sex ratio is preferred (Balbontín et al. 2001).

Although most species of raptors have reversed sexual size dimorphism (i.e., females are larger than males), there are some species for which sex determination by morphometric data is particularly difficult because they are apparently monomorphic (Krüger 2005). This is particularly the case of vultures, which lack obvious external morphometric differences. Although several studies have used external morphometrics for sex determination in some species of raptors (Ferrer and De Le Court 1992, Balbontín et al. 2001, Palma et al. 2001, Martínez et al. 2002, Delgado and Penteriani 2004, Zuberogoitia et al. 2005, 2011), including some vulture species (De la Puente and Elorriaga 2004, Xirouchakis and Poulakakis 2008), there is a dearth of data for many species of conservation concern, such as the en-

<sup>1</sup> Email address: Pascual.Lopez@uv.es

dangered Bearded Vulture (*Cybaetus barbatus*). For this species, sex determination by molecular methods has been recently optimized (García et al. 2009). However, sexing by molecular methods is usually expensive and time-consuming, and therefore it would be particularly useful to have an easy and reliable method for sex identification in the field.

We used a unique database of wild Bearded Vultures sexed by molecular methods to: (1) assess external morphometric differences between sexes, and (2) develop an equation to allow sex determination in the field.

#### METHODS

**Study Species and Field Sampling.** The Bearded Vulture is a cliff-nesting raptor that ranges throughout the mountains of Europe, Asia, and Africa. Although the Bearded Vulture is not globally threatened (BirdLife International 2011), the species declined substantially during the twentieth century and currently has a fragmented distribution in Europe, where populations are restricted to the Pyrenees, Alps (following a successful reintroduction program), Corsica, Crete, and the Balkans. The Pyrenean population is the only viable wild population in Europe but consists of fewer than 100 breeding pairs (Margalida and Heredia 2005, J. Gil and M. Alcántara unpubl. data) and therefore the species is classified as endangered in Spain (Spanish Royal-decree 139/2011, which establishes the National Catalogue of Endangered Species).

Data were collected as a part of a long-term monitoring program on the Bearded Vulture population in the Spanish and French Pyrenees (Gil et al. 2010). Juveniles were sampled primarily in the Spanish regions of Aragón, Navarra, and Catalonia, with one in Aquitania (France), during the period 1992–2010. Juveniles were sampled in their nests as a part of a banding program and/or telemetry projects (for further details, see Gil et al. 2010). Free-flying birds were captured at vulture restaurants located in the Spanish Pyrenees using a cannon-projected net trap, which consisted of three cannons and a nylon net of 12 × 10 m (Gil et al. 2010). Bearded Vultures were classified into four age classes according to plumage characteristics (Llopis and Adam 2003): juvenile (<120 d), immature (from ca. 4 mo–3 yr), subadult (3–6 yr), and adult (>6 yr).

All birds were sexed using three different molecular methods based on polymerase chain reaction analysis of the chromobox-helicase-DNA binding protein gene. For further details of procedures used for sex identification in this study, see García et al. (2009).

**Morphometric Measurements.** We sampled 96 Bearded Vultures: 40 juveniles, 25 immatures, 14 subadults, and 17 adults. The following morphometric data were taken using ten standard measurements as described for vultures in Mundy et al. (1992): (1) wing chord, from the carpal joint of the bent wing to the tip of the longest straightened primary; (2) wing width, from the carpal joint of the bent wing to the tip of the first secondary (s1); (3) wing width, from the carpal joint of the bent wing to the tip of the

second primary (p2); (4) bill length, from the tip of the culmen to the distal dorsal border of the cere; (5) bill width, at the level of the nostrils; (6) bill depth, dorsoventral distance at the distal edge of the cere; (7) tarsus length, the distance from the tarsometatarsal joint to the articulation of the middle toe; (8) forearm length, the distance from the extremity of the cubit to the extremity of the ulna; (9) tail length, from the insertion of the central rectrices to their tips; and (10) body mass. Measurements were taken with calipers and a tape measure to the nearest 0.1 mm. Body mass was measured with a dynamometer and a digital precision balance to the nearest 1 g. To minimize measurement errors, each bird was measured twice and by the same person.

**Statistical Analyses.** Descriptive statistics were calculated for all variables and are presented as mean ± standard deviation. After a preliminary visual inspection of data, we divided the database into two age groups: one including only juveniles (which showed clear morphometric differences with the rest of age-classes) and the second including immature, subadult, and adult birds. For this second age group, a preliminary multivariate analysis of the variance (MANOVA) was done to test for differences in morphological measurements between the three age groups, sexes, and the interaction between sex and age. This analysis showed a high degree of cohesion within the group, with significant differences found between the sexes ( $F = 5.36$ ,  $P = 0.005$ ), but not among age classes ( $F = 0.65$ ,  $P = 0.748$ ), nor in the interaction between sex and age-class ( $F = 0.70$ ,  $P = 0.706$ ). Therefore, individuals from this second group (hereafter “non-juveniles”) were pooled for subsequent analyses.

The analyses for juveniles and non-juveniles were conducted separately, following similar studies of sex determination (e.g., Delgado and Penteriani 2004, Xirouchakis and Poulakakis 2008), by means of a two-step procedure as follows. First, normality tests were run for all variables by means of Kolmogorov-Smirnov (K-S) and Shapiro-Wilk’s tests (Zar 1996). Then, univariate tests for differences between sexes were computed using  $t$ -tests. When the assumption of normality was violated, comparisons were made with Mann-Whitney’s test (Zar 1996). Significance level was set at  $P < 0.05$ . The percentage of sexual dimorphism in each measurement was calculated as  $100 * [(x_m/x_f) - 1]$ , where  $x_m$  and  $x_f$  were the mean values of males and females respectively (Krüger 2005, Zuberogoitia et al. 2011).

Second, we used a Discriminant Function Analysis (DFA; McLachlan 2004) to assess which variables discriminated between males and females, generating an equation (i.e., the discriminant function) that included a linear combination of the variables that provided the maximum separation between sexes. Only variables with significant differences in the univariate tests were used in the DFA. Sex was set as the categorical dependent variable. A backward removal procedure was used for selection of the independent variables (morphometric measurements) that best

Table 1. Sexual size dimorphism and univariate comparisons of morphometric measurements of juvenile male and female Bearded Vultures from the Pyrenees (Spain-France; 1992–2010) sexed by molecular techniques. Measurements are given in cm and body mass in kg. Significant differences between sexes are marked in bold. Note that the negative values of the % of dimorphism mean that, for the corresponding variable, females are larger/heavier than males.

VARIABLE	JUVENILE BEARDED VULTURES													
	MALES					FEMALES					% DIMOR-			
	MEAN	SD	MIN	MAX	<i>n</i>	MEAN	SD	MIN	MAX	<i>n</i>	PHISM	<i>T</i>	<i>Z</i>	<i>P</i>
Wing chord	54.86	4.34	46.50	65.50	16	58.54	5.66	48.90	67.00	14	-6.30	-2.018		0.053
Wing width to s1	40.63	3.81	34.00	50.00	14	39.37	2.74	34.70	42.50	11	3.19	0.921		0.367
Wing width to p2	44.91	3.49	39.50	50.50	14	45.05	3.57	40.40	49.00	11	-0.31		-0.328	0.743
Bill length	4.54	0.17	4.30	4.80	19	4.66	0.18	4.40	5.20	17	-2.63		-1.822	0.068
Bill width	2.16	0.16	1.90	2.50	19	2.15	0.25	1.70	2.50	17	0.50	0.157		0.876
Bill depth	2.87	0.26	2.20	3.50	19	2.98	0.30	2.10	3.50	17	-3.64		-1.759	0.079
Tarsus length	10.47	0.91	8.40	11.70	19	10.34	0.82	8.90	11.40	16	1.26	0.439		0.663
<b>Forearm length</b>	27.78	1.01	26.00	30.00	18	28.57	0.84	27.50	31.00	15	-2.76		-2.278	<b>0.023</b>
Tail length	33.00	3.44	27.00	38.50	12	34.05	4.29	28.00	42.00	11	-3.10	-0.653		0.521
<b>Body mass</b>	4.89	0.40	3.95	5.45	20	5.47	0.43	4.71	6.25	18	-10.48	-4.291		<b>&lt;0.001</b>

discriminated between sexes, using the Wilk’s lambda statistic (McLachlan 2004). All assumptions of the DFA were met, namely: (1) normal distribution of the variables included in the model; (2) absence of multi-collinearity (all Pearson and Spearman correlations coefficients  $r < 0.35$ ); and (3) homoscedasticity (i.e., homogeneity of the variance/covariance matrices among groups; Box’s M statistic = 9.238;  $\chi^2 = 8.550$ ;  $df = 6$ ;  $P = 0.20$ ). *A priori* classification probabilities were set to  $P = 0.5$  for being male or female. Finally, we used a jackknife procedure to assess the

predictive power of the discriminant function, in which each individual was classified using a function derived from the total sample minus the one individual (McLachlan 2004). This cross-validation technique provided a good indicator of DFA accuracy and has been regularly employed in similar analyses when an external independent dataset is not readily available (e.g., Ballbontín et al. 2001, Palma et al. 2001, Xirouchakis and Poulakakis 2008). All statistical analyses were conducted with Statistica v. 7.0 (StatSoft Inc. 2004).

Table 2. Sexual size dimorphism and univariate comparisons of morphometric measurements of non-juvenile male and female Bearded Vultures from the Pyrenees (Spain-France; 1992–2010) sexed by molecular techniques. Measurements are given in cm and body mass in kg. Significant differences between sexes are marked in bold. Note that the negative values of the % of dimorphism mean that, for the corresponding variable, females are larger/heavier than males.

VARIABLE	IMMATURE, SUBADULT, AND ADULT BEARDED VULTURES (POOLED DATA)													
	MALES					FEMALES					% DIMOR-			
	MEAN	SD	MIN	MAX	<i>n</i>	MEAN	SD	MIN	MAX	<i>n</i>	PHISM	<i>T</i>	<i>Z</i>	<i>P</i>
<b>Wing chord</b>	76.22	4.75	57.00	80.50	22	79.48	2.42	75.5	84.00	33	-4.09		3.112	<b>0.002</b>
<b>Wing width to s1</b>	43.63	2.25	39.50	47.50	22	45.64	2.76	40.0	50.50	32	-4.40	2.818		<b>0.007</b>
<b>Wing width to p2</b>	49.28	1.94	46.00	53.00	20	51.43	2.96	43.5	56.00	31	-4.19	2.662		<b>0.010</b>
<b>Bill length</b>	4.87	0.23	4.40	5.50	22	5.02	0.20	4.7	5.40	33	-2.84	2.431		<b>0.018</b>
Bill width	2.23	0.49	1.60	3.40	22	2.10	0.29	1.7	2.90	33	5.91		-0.688	0.492
<b>Bill depth</b>	3.00	0.14	2.70	3.30	22	3.14	0.14	2.9	3.50	33	-4.35		3.221	<b>0.001</b>
Tarsus length	10.49	0.85	8.80	12.00	21	10.83	0.73	8.8	11.60	33	-3.14		1.620	0.105
Forearm length	28.22	0.97	26.50	30.00	22	28.45	0.81	27.0	30.00	30	-0.79	0.787		0.435
<b>Tail length</b>	49.66	1.93	47.00	53.10	22	51.90	2.22	48.0	57.00	31	-4.31	3.253		<b>0.002</b>
<b>Body mass</b>	5.18	0.55	3.63	6.05	21	5.85	0.50	5.0	7.15	33	-11.48	3.948		<b>&lt;0.001</b>

## RESULTS AND DISCUSSION

Bearded Vultures' sexes according to our molecular methods were: juveniles (20 males, 20 females), immatures (10 males, 15 females), subadults (4 males, 10 females), and adults (8 males, 9 females). Juvenile males and females differed only in forearm length ( $Z = -2.278$ ;  $P = 0.023$ ) and body mass ( $t = -4.291$ ;  $P < 0.001$ ), the latter showing the greatest percentage of dimorphism ( $-10.48\%$ ; Table 1). Sex differences were not found in plumage morphometrics, probably because juveniles had not attained full plumage development at the time when the measurements were taken (Table 1). When both biomass and forearm length were included in the DFA, only body mass was retained as a significant predictor of the sex (Wilks' lambda = 0.857;  $F_{1,29} = 9.454$ ;  $P < 0.001$ ). However, the accuracy of the discriminant function was relatively low, with 75.0% of all cases being correctly classified (72.2% of males and 78.6% of females). Consequently, it was not possible to accurately predict the sex of juvenile Bearded Vulture by means of the DFA.

For non-juveniles, males and females differed significantly for seven variables (all  $P < 0.05$ ), but not for bill width, tarsus length, and forearm length ( $P > 0.10$ ; Table 2). Females tended to have longer feathers and to be heavier than males, with the percentage of dimorphism ranging from  $-2.84\%$  to  $-11.48\%$  for significant variables. The DFA (eigenvalue = 1.212;  $\chi^2 = 35.325$ ;  $df = 3$ ;  $P < 0.001$ ) showed that the best predictors of the sex were: (1) wing width to p2 (Wilks' lambda = 0.845;  $F_{1,44} = 8.068$ ;  $P < 0.01$ ); (2) tail length (Wilks' lambda = 0.790;  $F_{1,44} = 11.710$ ;  $P < 0.005$ ); and (3) body mass (Wilks' lambda = 0.772;  $F_{1,44} = 13.004$ ;  $P < 0.001$ ). The discriminant function equation was:  $D = 34.280 - 0.222 * (\text{wing width to p2}) - 0.315 * (\text{tail length}) - 1.222 * (\text{body mass})$ .

The cutting score, calculated as the arithmetic mean of group centroids (males = 1.331; females =  $-0.872$ ) was 0.23. Therefore, values of  $D >$  cutting score represent males and values of  $D <$  cutting score represent females.

The application of the DFA exhibited a general success rate of 89.58% (94.74% and 86.21% of males and females were correctly classified, respectively). The equation discriminated males from females when the three variables were analyzed separately (Fig. 1) or jointly (Fig. 2). When the jackknife cross-validation procedure was performed, the accuracy of sex determination was 86.8%, with 85.7% and 87.5% of males and females being correctly classified, respectively, which indicated that the results obtained with the discriminant equation were satisfactory.

In conclusion, we found that differences between sexes were not apparent until just after fledging, which takes place around  $123 \pm 10$  d (Margarida et al. 2003). The observed lack of morphometric differences in juveniles suggests that predicting sex accurately when Bearded Vultures are still in the nest may not be feasible. In contrast, sex determination of immature, subadult and adult Bearded Vultures can be easily implemented in the field, using

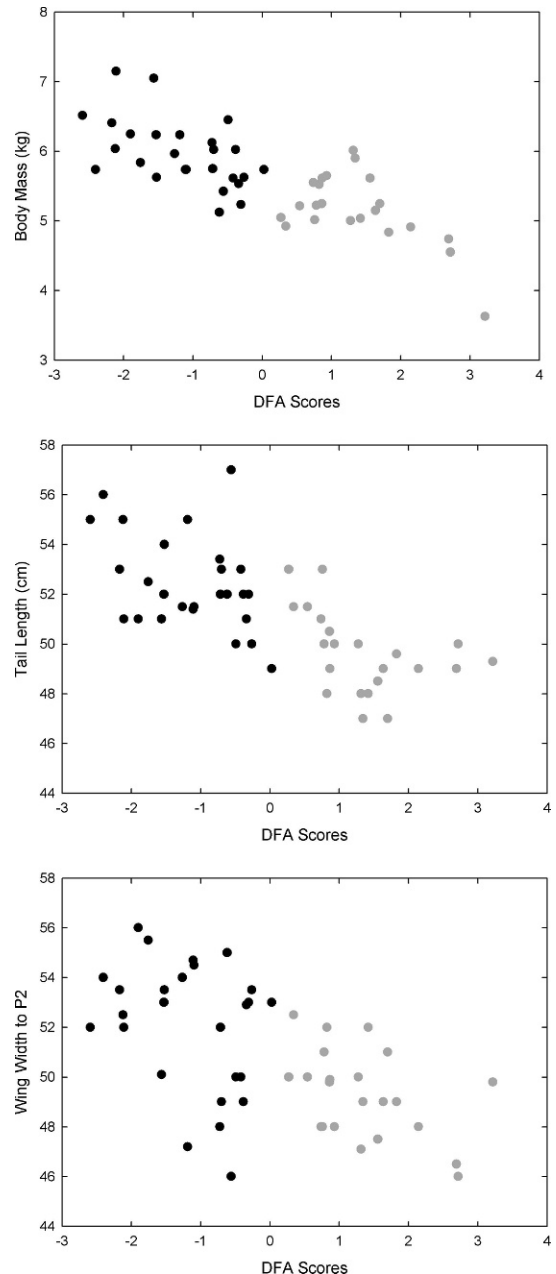


Figure 1. Discriminant function scores of non-juvenile male (grey) and female (black) Bearded Vultures from the Pyrenees (Spain-France; 1992–2010) in relation to the three variables included in the best discriminant equation used for sex determination.

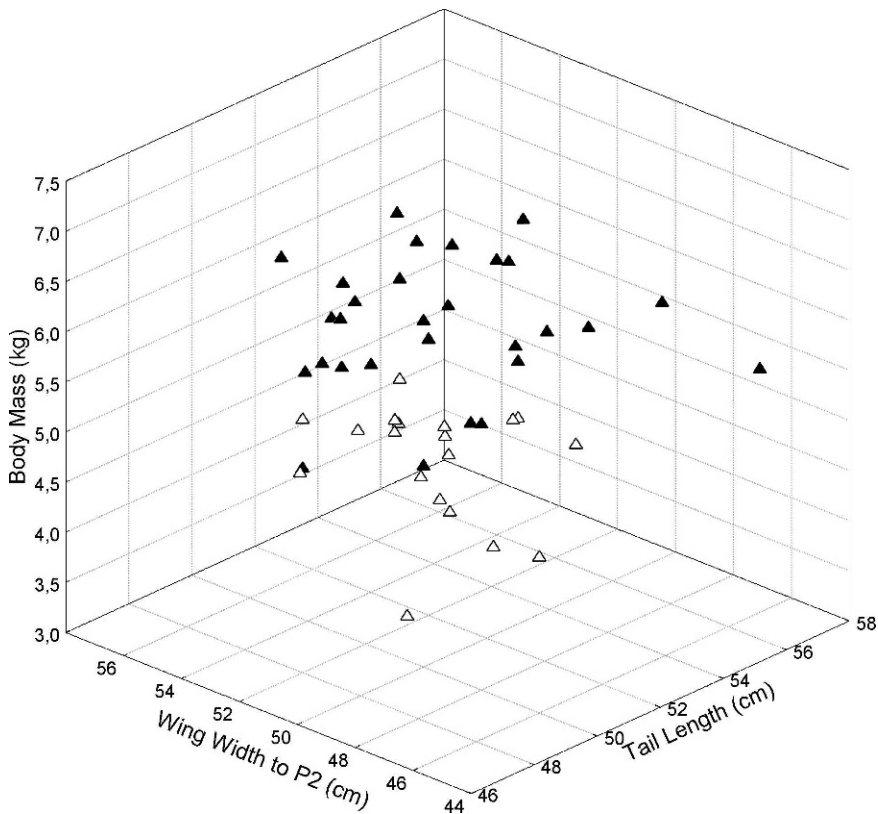


Figure 2. Wing width to p2, tail length, and body mass can be used for sex determination in Bearded Vultures older than approximately 4 mo (immature, subadult, and adult) according to our discriminant function analysis. Females (black triangles); males (open triangles).

morphological measurements that are routinely measured, as shown by the DFA.

#### MORFOMETRÍA Y DETERMINACIÓN DEL SEXO EN LA ESPECIE AMENAZADA *GYPÆTUS BARBATUS*

RESUMEN.—En este estudio evaluamos las diferencias morfológicas entre 42 machos y 54 hembras de una muestra de individuos de *Cypaetus barbatus* recopilada entre 1992 y 2010, proveniente de los Pirineos (España-Francia) y sexados mediante métodos moleculares. Mediante el uso de análisis multivariados, desarrollamos una función discriminante que permite la determinación del sexo de forma fácil en el campo. Nuestros resultados mostraron cómo las diferencias entre sexos aparecen tan pronto como los individuos jóvenes de *G. barbatus* abandonan el nido. Después del emplumamiento, la anchura del ala medida hasta la segunda primaria, la longitud de la cola y el peso pueden ser utilizados como medidas efectivas para la discriminación del sexo en individuos mayores de 4 meses, de acuerdo con los resultados de la mejor función discriminante, la cual

clasificó correctamente cerca del 87% de los casos, después de un proceso de validación cruzada.

[Traducción de los autores editada]

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