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Sexing Cape Vulture *Gyps coprotheres* based on head morphometrics

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The Cape Vulture *Gyps coprotheres* is considered sexually monomorphic in the literature, but visual differences in head shape between the sexes have been observed. Furthermore, head morphometrics of other *Gyps* species show statistically significant variation between the sexes. We show that head morphometrics can be used to determine the sex of Cape Vultures. Males generally have wider and shorter heads, and larger bill depths than females. Discriminant function analysis with data from 63 individuals identified the three most predictive variables in sex determination to be head width, head length and bill depth. We also provide an equation that can be used in conjunction with head measurements as a method to determine the sex of Cape Vultures in the field with an overall accuracy of 84% (92% accuracy for females and 72% for males).

**Keywords:** Cape Vulture, head morphometrics, sexual dimorphism

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Sexing of individuals in wild or captive populations is important in studies of avian biology (Xirouchakis and Poulakakis 2008). Furthermore, sexual determination has serious conservation implications as it is fundamental in calculating effective population size, as well as playing a role in understanding population dynamics, population structure, habitat use, behaviour and mating systems (Hughes 1998). A wide range of ecological or behavioural studies have used data obtained from body characteristics to determine gender (Hughes 1998). However, vultures of the genus *Gyps* appear to lack plumage characteristics or external features to distinguish between the sexes (Xirouchakis and Poulakakis 2008), with Cape Vultures *Gyps coprotheres* having been reported to be sexually monomorphic in plumage (Mundy et al. 1992).

Several sex determination approaches in birds, including vultures, have been used, including observations of copulation and courtship behaviour (Catry et al. 1999), cloacal examination (Boersma and Davies 1987; Gray and Hamer 2001), brood patches during the breeding season (Cowley 1999), or the analysis of steroid hormones and DNA analysis (Fry 1983; Richner 1989; Griffiths et al. 1998; Wink et al. 1998; Ito et al. 2003). However, such approaches are only limited to narrow time periods (e.g. the breeding season), require trained researchers and specialised equipment, or are expensive and time consuming (Jodice et al. 2000; Palma et al. 2001). Some authors have suggested the use of morphometric traits in determining sex by either measuring a single feature (Coulson et al. 1983) or a discriminant multivariate analysis (van Franeker...
Multivariate analysis of morphological measurements has been regarded as a successful approach in sexing many bird species (Donohue and Dufty 2006; Svagelj and Quintana 2007; Reynolds et al. 2008), including vultures of the genus Gyps (Xirouchakis and Poulakakis 2008). Other studies in southern Africa have discussed sex differentiation in Cape Vultures according to general morphological characteristics of the head, highlighting that females have a narrow head (as seen from the lateral canthus of the eyes) with an egg-shaped dome on top, while the males have a more triangular, flattened head with more prominent eye sockets (Mundy et al. 1992; Naidoo et al. 2011; Nsikani et al. 2015; Hirschauer 2016). As this method has not been statistically validated, it therefore remains important to test the applicability of such morphological characteristics of the head as a method to determine sex in this species. The main objective of this study was to determine if head shape of Cape Vultures can be used as a sexing tool.

The study was conducted over the course of 18 months at VulPro, a vulture rehabilitation and breeding centre located outside Hartbeespoort in North West province, South Africa (25°42′7″ S, 27°57′2″ E). Measurements were taken opportunistically from 63 Cape Vultures of all ages (6 months and older) and backgrounds, i.e. birds kept for rehabilitation, captive bred individuals or dead vultures. Biometric data were taken using Vernier calipers following the standard measurements as described for vultures (Mendelssohn et al. 1989; Mundy et al. 1992): head length (HL), from the supraoccipital bone to the tip of the bill (Figure 1a); head width (HW), distance between the widest points in the auricular patches behind the eyes (Figure 1b); bill length (BL), from the tip of the culmen to its junction with the cere; bill plus cere length (BC), from the bill tip to the edge of implantation of feathers; and bill depth (BD), dorso-ventral distance at the nostrils (Figure 1c). However, some parameters were not measured for all subjects (HL \( n = 38 \), BD \( n = 44 \), the remaining features had \( n = 63 \)). Morphometric measurements were made on Cape Vultures while being handled according to VulPro’s protocols without any anaesthesia (see Wolter et al. 2014). A small blood sample was collected from each vulture with a 23-gauge needle and syringe from the tarsal vein. DNA sexing analysis was conducted by the National Zoological Gardens of South Africa following the methods described by Griffiths et al. (1998) based on two conserved CHD (chromohelicase-DNA-binding) genes located on the sex chromosomes of all birds.

A one-way analysis of variance (ANOVA) was used to test whether measured head characteristics varied with sex in Cape Vultures, after testing for normality (Shapiro–Wilk test) and homogeneity of variance (Levene’s test). The sexual dimorphism index for each variable was also calculated following Weidinger and van Franeker (1998). The percentage of sexual dimorphism in each measurement was expressed as \( \frac{x_m - x_f}{x_m} \), where \( x_m \) and \( x_f \) are mean values for males and females, respectively. MANOVA analyses were conducted with head measurements as the dependent variables to identify and remove those features that may be influenced by either age or body condition. Subsequently, discriminant function analysis (DFA) was conducted to obtain canonical values that were then used to produce an equation to estimate the sex of any individual. All statistical analyses were conducted in R version 3.2.0 (R Core Team 2013).

The means and standard deviations of the morphometric measurements recorded for Cape Vultures in this study are summarised in Table 1. Only HW, HL, BC, and BD

Figure 1: Vernier calipers are used to measure Cape Vulture (a) head length, (b) head width and (c) bill depth.
differed significantly with sex ($p < 0.05$, test statistic values are presented in Table 1); BL did not differ significantly ($p > 0.05$). BC and HL for males were generally shorter than those of females, whereas BD and HW were larger for males. However, there was a large amount of overlap between the sexes in all of these features (Table 1).

MANOVA analyses using all four significant variables showed that age contributed a small but significant contribution to determining head morphometrics in Cape Vultures (Wilks’ lambda $= 0.255$, $F = 42.62$, $p = 0.012$). However, when BC was removed, age became non-significant (Wilks’ lambda $= 0.509$, $F = 41.52$, $p = 0.189$) with sex being highly significant (Wilks’ lambda $= 0.365$, $F = 17.00$, $p = 0.001$). Therefore, the three head measurements that provided the most predictive power in determining sex in DFA analyses were HW, BL and BD.

Canonical values produced from the DFA using the three variables that significantly differed by sex (HW, HL and BD) produced an equation that can be used to estimate the sex of any individual, based solely on these measurements. Values $\geq 24$ indicate a male and values $\leq 24$ a female.

$$X = (0.411 \times HW) - (0.354 \times HL) + (0.199 \times BD)$$

Cross-validation of the DFA resulted in the successful identification of 84% of individuals to the correct sex. By applying the equation given above, 92% of the females and 72% of the males were correctly assigned.

Although our sample sizes were sufficient to enable sexing of Cape Vultures, cross-validation in the discriminant function analysis can be influenced by small sample sizes (Dechaume-Moncharmont et al. 2011). We therefore recommend that this analysis be repeated with more birds, although we note that obtaining large samples of Cape Vultures for sexing and measuring may be challenging. Several other potentially influential variables were not measured in this study and may be worth investigating in the future; for example, our sample size was insufficient to consider the effect of age and we did not include body mass in our analyses. Based on the measurement of a large number of birds, Mundy (1982) concluded that there were no sexual differences in mass. However, it may be worth revisiting the relationship of both age and mass with sex. Within pair (i.e. a nesting male and female bird) comparisons were also not possible in our study. Such comparisons have been previously used in the field (Fletcher and Hamer 2003) and may be worth investigating in the future. Another worthwhile line of inquiry for future studies may be to investigate the adaptive significance of sexual differences in head shape and size that we have demonstrated in this study.

We have shown that morphological characteristics of the head can be successfully used to determine the sex of Cape Vultures, with head width, head length and bill depth being the most important features to measure. Males generally have shorter and wider heads, and larger bill depths than females. While our results are in agreement with those of Xiouchakis and Poulakakis (2008), who concluded that morphological differences in head width, head length and bill plus cere length are effective measurements for gender determination of Griffon Vultures ($Gyps fulvus$), some sex differences observed in Griffon Vultures show the reverse trend compared with Cape Vultures. Specifically, HL, BL and BC were all larger in male Griffon Vultures compared with females. In addition, BL was shown to be highly significant in analysis of Griffon Vulture morphometrics, but not Cape Vultures.

The method that we outline here can be readily and rapidly employed in the field to sex Cape Vultures and is cheaper than using molecular techniques.

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