A method for identifying the sex of lesser adjutant storks *Leptoptilos javanicus* using digital photographs

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មូលន័យសង្ខេប

ចំពោះសត្វស្លាបជាច្រើន ការកំណត់អត្តសញ្ញាណភេទទាមទារការធ្វើតេស្តDNA ឬការសង្កេតពីឥរិយាបថ។ ឯកសារស្រាវជ្រាវនេះពិពណ៌នាពីវិធីសាស្ត្រថ្មីសម្រាប់កំណត់អត្តសញ្ញាណភេទនៃសត្វត្រដក់តូច (*Leptoptilos javanicus*) ដោយប្រើរូបថតឌីជីថល។ រូបថតត្រូវបានថតសត្វត្រដក់តូចចំនួន២០ (ឈ្មោល១១ ញី៩) នៅមជ្ឈមណ្ឌលអភិរក្សជីវៈចម្រុះអង្គរនៅប្រទេសកម្ពុជា និងវិភាគលក្ខណៈក្បាលពីចំហៀងដោយប្រើកម្មវិធី Adobe Photoshop CS2 ។ លក្ខណៈខុសគ្នារវាងភេទត្រូវបានគេរកឃើញដោយកំរិតនៃការវាស់ក្បាលតាមអក្ស័ឈររបស់វា ដែលត្រូវបានប្រើដើម្បីបង្កើតមុខងារខុសៗគ្នា។ មុខងារនេះចែកជាភេទត្រឹមត្រូវបាន៩០ភាគរយនៃក្រុមសិក្សា។ យើងសន្និដ្ឋានថា រូបភាពឌីជីថលអាចជួយកំណត់អត្តសញ្ញាណភេទរបស់ត្រដក់តូចបាន។

Abstract

For many birds, gender identification requires DNA tests or behavioural observations. This paper describes a new method of identifying the sex of lesser adjutant storks *Leptoptilos javanicus* using digital photographs. Photographs were taken of 20 captive lesser adjutant storks (11 males, nine females) at the Angkor Centre for Conservation of Biodiversity in Cambodia, and lateral head features analysed using Adobe Photoshop CS2. Differences between the sexes were found in their vertical head measurement ratios which were used to generate a discriminant function. This function correctly classified the genders of 90% of the study group. We conclude that digital photographs can aid the gender identification of lesser adjutant storks.

Keywords

Cambodia, discriminant function, gender identification.

Introduction

The lesser adjutant stork *Leptoptilos javanicus* is currently classified as globally Vulnerable (BirdLife International, 2011). Current conservation efforts for the species include, amongst others, nest protection measures (Visal & Clements, 2008; Clements *et al*., 2009; BirdLife International, 2011) as well as small-scale captive breeding programmes (Salakij *et al*., 2004; Maust *et al*., 2007; ACCB, unpubl. data). Such programmes may benefit from researchers being able to identify the gender of the individuals involved without having to capture the birds or do extensive behavioural observations, which might not be reliable (Dorr *et al*., 2005; Cwiertnia *et al*., 2006; Cheong *et al*., 2007; ACCB, unpubl. data). Reliable sex identification in this species is currently possible only through DNA analysis of blood or feathers, or laparoscopy. These are techniques that usually involve handling or even anaesthetizing the birds (Greenwood, 1983; Harvey *et al*., 2006; Maust *et al*., 2007).

For other waterbird species, sex identification using morphometrics has been reported (e.g. Cwiertnia *et al*., 2006; Cheong *et al*., 2007; Dorr *et al*., 2005). Cheong *et al*. (2007) showed that in the Oriental white stork *Ciconia boyciana*, lateral head features derived from digital photographs could reliably distinguish the sexes. Following this method, we investigated whether or not the same method could be applied to lesser adjutant storks.

Methods

Study Species

The lesser adjutant stork belongs to the order Ciconiiformes, suborder Ciconiae, family Ciconiidae, and tribe Leptoptilini (giant storks) (Elliot *et al.*, 1994). This large stork (122-129 cm tall) is primarily dark grey-black above with a white underside, a largely naked head and neck, and a large, horn-coloured bill (Wells, 1999; BirdLife International, 2011). The global population is currently estimated at 6,500–8,000 individuals, but suspected to be declining rapidly. Cambodia holds 2,500–4,000 individuals (BirdLife International, 2011). This species inhabits both open and forested wetlands, where it nests colonially in large trees. Major threats include habitat loss through the felling of nest trees and the conversion of wetlands, agricultural intensification and increased pesticide use, collection of eggs and chicks, hunting of adults and, especially in Cambodia and Nepal, incidental mortality from the practice of poisoning pools to catch fish (BirdLife International, 2011).

Study location

The study was carried out at the Angkor Centre for Conservation of Biodiversity (ACCB), a wildlife rescue and environmental education centre in Siem Reap Province, Cambodia. The lesser adjutant storks were housed in a 1.1 ha paddock that consists of a main enclosure (approximately 0.9 ha) with a pond and seven smaller compartments for separate individuals or pairs.

Study population

The sample group contained 20 DNA-sexed birds including 11 males (two adults, six subadults, three juveniles) and nine females (three adults, two subadults, four juveniles). These birds were considered to be juvenile up to two years old, subadult from two to five years old and adult when over five years old, based on the estimated age of first breeding (ACCB, unpubl. data). All of the birds originated from confiscations or donations and were not fit for release, so they are part of ACCB's breeding programme for selected globally threatened species.

Data collection and analysis

The study was conducted from August to December 2009. Digital photographs were taken of the sides of the storks' heads during daylight using a Canon PowerShot A610 digital camera. The photographs were taken from a distance of 5-15 metres with an image resolution of 180 dpi. Reference points were defined (Fig. 1), adapted from Cheong *et al*. (2007), and distances between reference points were measured using Adobe Photoshop CS2, with an accuracy of ± 1 mm. Because not all of the storks had been photographed from exactly the same distance and angle, the horizontal and vertical measurements were not absolute values, and therefore ratios were generated. In total, 28 horizontal and 55 vertical measurement ratios were calculated (Table 1).

Horizontal measurements

- H1 Tip of the bill to commissural point (corner of the gape where maxilla and mandible meet).
- H2 Tip of the bill to beginning of skin flap.
- H3 Tip of the bill to beginning of skull (i.e. exposed culmen).
- H4 Tip of the bill through commissural point to occiput.
- H5 Commissural point to occiput (= H4-H1).
- H6 Length of nostril.
- H7 Tip of bill to anterior edge of nostril.
- H8 Beginning of skull tangential to upper edge of eyeball to occiput.

Vertical measurements

- V1 Proximal bill height (beginning of skin flap to beginning of skull).
- V2 Proximal maxilla height (at beginning of skull).
- V3 Proximal mandible height (= V1-V2).
- V4 Bill height at distal edge of nostril, perpendicular to H1.
- V5 Maxilla height at distal edge of nostril, perpendicular to H1.
- V6 Mandible height at distal edge of nostril (= V4-V5).
- V7 Commissural point to crown, tangential to posterior baso-lateral edge of the eyeball.
- V8 Commissural point to crown, tangential to anterior edge of the eyeball.
- V9 Beginning of skin flap tangential to posterior edge of eyeball to crown.
- V10 Beginning of skin flap tangential to anterior edge of eyeball to crown.
- V11 Head height perpendicular to H5 and tangential to posterior edge of eyeball.

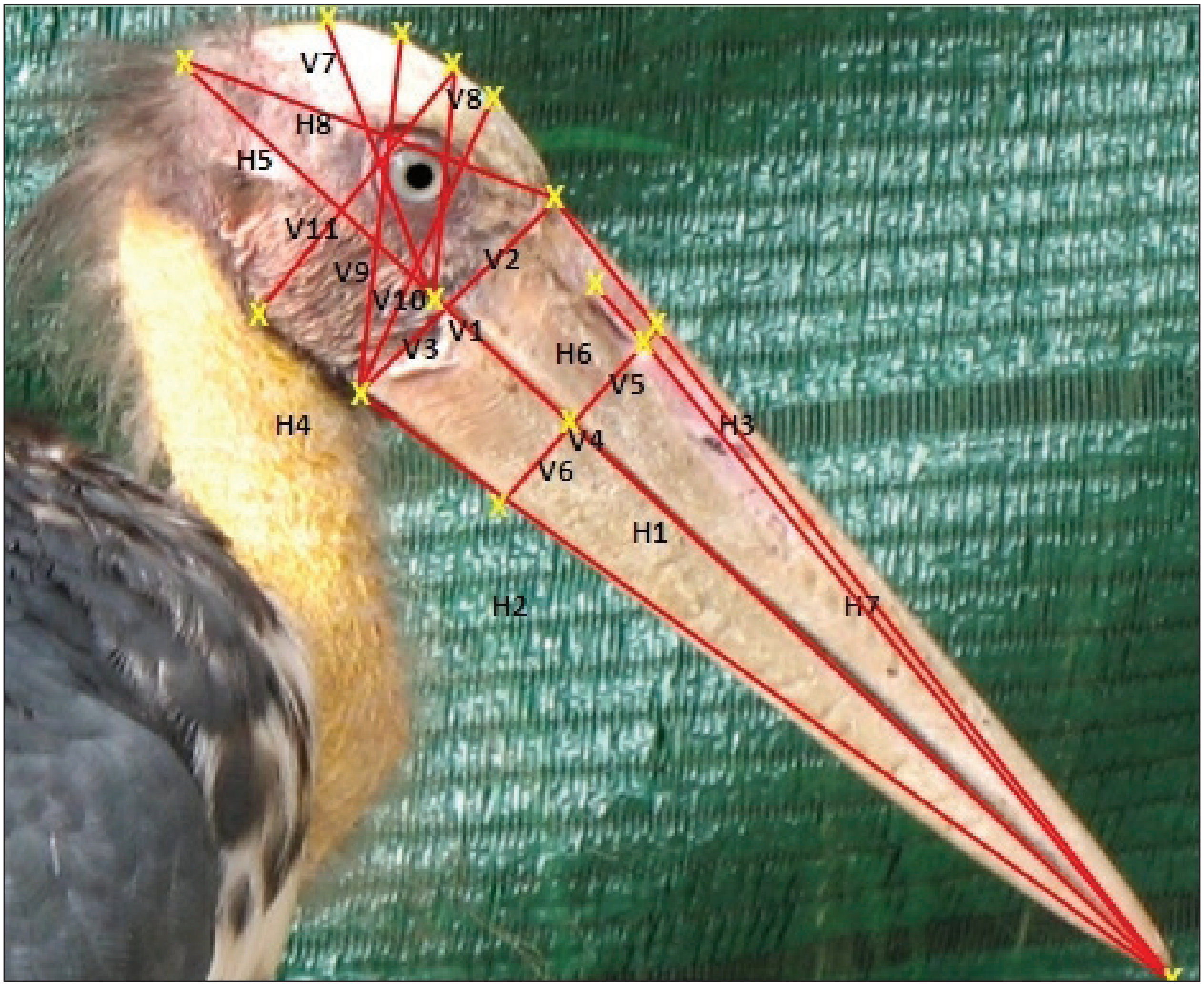

Fig. 1 Reference points (crosses) and measurements (lines) on the head of a lesser adjutant stork. H indicates a horizontal measurement, V indicates a vertical measurement.

Table 1 Horizontal and vertical measurement ratios used to compare the lateral head features of male (n = 11) and female (n = 9) lesser adjutant storks.

Horizontal measurement ratios									
H1/H2	H1/H3	H1/H4	H1/H5	H1/H6	H1/H7	H1/H8	H2/H3	H2/H4	H2/H5
H2/H6	H2/H7	H2/H8	H3/H4	H3/H5	H3/H6	H3/H7	H3/H8	H4/H5	H4/H6
H4/H7	H4/H8	H5/H6	H5/H7	H5/H8	H6/H7	H6/H8	H7/H8		
Vertical measurement ratios									
V1/V2	V1/V3	V1/V4	V1/V5	V1/V6	V1/V7	V1/V8	V1/V9	V1/V10	V1/V11
V2/V3	V2/V4	V2/V5	V2/V6	V2/V7	V2/V8	V2/V9	V2/V10	V2/V11	V3/V4
V3/V5	V3/V6	V3/V7	V3/V8	V3/V9	V3/V10	V3/V11	V4/V5	V4/V6	V4/V7
V4/V8	V4/V9	V4/V10	V4/V11	V5/V6	V5/V7	V5/V8	V5/V9	V5/V10	V5/V11
V6/V7	V6/V8	V6/V9	V6/V10	V6/V11	V7/V8	V7/V9	V7/V10	V7/V11	V8/V9
V8/V10	V8/V11	V9/V10	V9/V11	V10/V11					

Table 2 Vertical measurement ratios showing significant differences in lateral head features between male (n = 11) and female

	V1/V7	V1/V8	V1/V9	V1/V10	V1/V11	V3/V7	V3/V8	V3/V9
Males	0.95 ± 0.04	1.27 ± 0.04	0.77 ± 0.03	0.85 ± 0.03	0.87 ± 0.02	0.44 ± 0.02	0.59 ± 0.03	0.36 ± 0.02
Females	0.89 ± 0.05	1.18 ± 0.09	0.73 ± 0.04	0.81 ± 0.05	0.83 ± 0.04	0.41 ± 0.03	0.54 ± 0.06	0.33 ± 0.03
t	2.62	2.55	2.60	2.29	2.81	2.70	2.30	2.45
p	0.017*	0.020*	0.018*	0.034*	0.011*	0.015*	0.034*	0.025*

To test for significant differences between the lateral head measurement ratios of males and females, independent samples t-tests were conducted using SPSS 16 (the data were normally distributed), and ratios that differed significantly were included in a discriminant function analysis. The same test was used to examine differences between measurement ratios of the age classes 'subadult' and 'adult'. Juveniles were not included into this test because they are often still growing and thus their features may differ naturally to a larger extent.

In addition, six individuals (one adult male, one adult female, one subadult male, one subadult female, one juvenile male and one juvenile female) from the study group of 20 birds were captured and horizontal measurements were taken directly using a calliper and a ruler. To test whether ratios obtained from digital measurements differ significantly from those taken from direct measurements, the measurements from every individual were compared using paired t-tests in Microsoft Office Excel 2007.

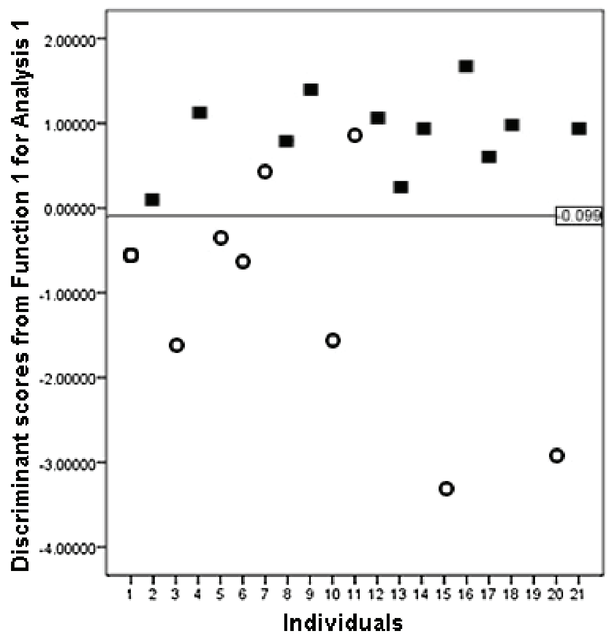

Fig. 2 The discriminant scores (D) obtained for 20 lesser adjutant storks predicting the sex of every individual. Males are indicated with a black rectangle, females with an open circle.

Results

Independent samples t-tests showed a significant difference between males and females in 17 of the 55 vertical measurement ratios calculated, but in none of the 28 horizontal measurement ratios (Table 2).

Using these 17 significant vertical measurement ratios, the discriminant function analysis yielded a Wilks' Lambda = 0.481. The discriminant function used to calculate the discriminant score (D) is:

D = (-0.942*V1/V7) + (0.324*V1/V8) + (-1.013*V1/V9) + (0.292*V1/V10) + (0.478*V1/V11) + (-1.824*V3/V7) + (1.277*V3/V9) + (2.797*V4/V7) + (-0.413*V5/V8) + (0.036*V6/V7)

This discriminant function correctly classified the gender of 90% (n = 18) of the individuals in the study group (n = 20). The group centroids were 0.892 for males and -1.090 for females, and thus the 'cut score' was (-1.090+0.892)/2 = -0.099. If a stork's score (D) is above -0.099, there is 90% certainty it is a male. If D is below -0.099, there is 90% certainty the bird is a female. The scatter plot in Fig. 2 illustrates the predicted sexes using the discriminant scores (D) obtained for every individual.

A stepwise discriminant function analysis showed that the ratio of the bill height (V4) and the distance from the commissural point to the crown (V7) was the best single predictor to distinguish between male and female, being larger in male storks than in female storks. This led to the simplified function D = (2.797*V4/V7). This function correctly classified the gender of 15 (75%) individuals in the study group (n = 20). Figure 3 illustrates this morphological difference with a stylised visualisation of (*n* = 9) lesser adjutant storks: * indicates a significant result (p <0.05); ** indicates a highly significant result (p <0.01).

V3/V11	V4/V7	V4/V10	V4/V11	V5/V8	V6/V7	V6/V8	V6/V9	V6/V10
0.40 ± 0.02	0.87 ± 0.03	0.78 ± 0.02	0.80 ± 0.02	0.62 ± 0.03	0.39 ± 0.02	0.52 ± 0.03	0.31 ± 0.02	0.35 ± 0.02
0.38 ± 0.03	0.82 ± 0.04	0.74 ± 0.04	0.76 ± 0.03	0.59 ± 0.04	0.36 ± 0.02	0.48 ± 0.04	0.30 ± 0.02	0.33 ± 0.02
2.17	3.32	2.89	3.31	2.27	2.97	2.70	2.30	2.45
0.044*	0.004**	0.010*	0.004**	0.036*	0.08*	0.015*	0.034*	0.025*

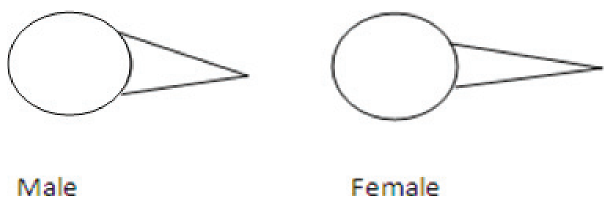

Fig. 3 Stylised head and bill proportions in male and female lesser adjutant storks as suggested by this study.

the proportions of the head and bill of male and female storks.

The comparisons of lateral head ratios of adults (*n* = 5) and subadults (*n* = 8) showed that seven of the 28 horizontal measurement ratios and eight of the 55 vertical measurement ratios differed significantly between age classes. When adults and subadults were tested separately for differences between the genders, only one vertical ratio (V4/V9) was found to significantly differ between subadult males (*n* = 6) and subadult females (*n* = 2). Of the 17 vertical measurement ratios that significantly differed between males and females in the entire sample group (*n* = 20), 15 were significantly different between the adult males (*n* = 2) and adult females (*n* = 3).

Overall, ratios taken from the eight direct horizontal measurements (taken with a calliper and ruler on six captured birds) did not differ significantly from those derived from photographs (*t*-test: paired two samples for means, average $p = 0.365$). The only exception was H6 (length of the nostrils), which differed in four of the six birds (one subadult male, one adult female, one juvenile male and one juvenile female).

Discussion

The results show that there is a significant difference between the lateral head features, derived from digital photographs, of male and female lesser adjutant storks. Previously, only behavioural differences had been reported as a noninvasive method of identifying the sexes in this species (Maust *et al*., 2007): a method that may be unreliable (ACCB, unpubl. data).

Our results suggest that the method we describe in this paper is suitable for identifying the genders of lesser adjutant storks with a high level of reliability. However, it must be noted that the study was carried out by a single observer, thus minimizing potential bias with regards to defining reference points and taking measurements. Although taking measurements in digital photographs on a computer screen using Adobe Photoshop CS2 was found to be reliable when tested against direct measurements, some of the reference points were hard to define due to variation between individuals' head features. It should also to be kept in mind that the study was carried out on a relatively small sample of 20 individuals, all of Cambodian origin, and the results might therefore have to be treated with caution when applied to populations from other geographical regions. Additional individuals from Cambodia and elsewhere need to be tested to determine whether head features are consistent within the study region and whether the species exhibits geographical variation.

None of the horizontal measurement ratios differed significantly between males and females. However, horizontal measurements were used to compare the ratios taken from direct measurements and indirect measurements (derived from photographs) because they were easier to measure and therefore required less time for handling the storks. This comparison showed that the ratios of measurements derived from photos accurately match the ratios derived from direct measurements.

Among the significantly different vertical measurement ratios, the ratio of the bill height to the distance from the commissural point to the crown (V4/V7) was found to be the best predictor of gender in a stepwise discriminant function analysis. The ratio is larger in male storks than in female storks: i.e. the difference between these two measurements is smaller in males.

Using the discriminant function obtained, the gender of 90% of individuals in the study group was classified correctly, with 75% of individuals sexed correctly using a simpler form of the function. Two female birds were classified incorrectly, but both were small and not much is known about their origin, i.e. there might have been food shortages or other problems during their growth and development at an early age. Furthermore, since both females were still subadult, repeating the measurements when they have reached adulthood might yield different results. Fewer measurement ratios differed between the sexes in the subadult age class than in the adults, and therefore sex identification using this method is not as reliable in the younger age classes.

Conclusions

1. We have demonstrated a new method for gender identification of lesser adjutant storks with a high level of accuracy (90%) using digital photographs.
2. There is a significant difference between the ratios of vertical lateral head measurements of male and female lesser adjutant storks.
3. Analogous horizontal head features are not suitable for gender identification in this species.

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