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MORPHOMETRIC TRAITS AND THEIR ASSOCIATION WITH SEX IN THE KEEL-BILLED TOUCAN *RAMPHASTOS SULFURATUS*

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Abstract · Sexing monomorphic birds is essential for reproduction in conservation programs. In ramphastids (toucans) species, males have longer beaks than females, making beak morphometry potentially useful for sexing. In this study, we measured the following traits: curved beak length, total culmen length, straight beak length, curved and straight length of the ventral line of the gnathotheca, beak width, lateral area of the rhinotheca, and cloacal opening in 44 Keel-billed Toucans, as well as previously reported morphometric data from 17 additional individuals. Measurements were obtained using two methods (manual and image processing software), and the reliability between the two methods was assessed using the intraclass correlation coefficient (ICC). Individuals of unknown sex were sexed via PCR tests. The ICC showed poor reliability between the two measurement methods, so we only used manual measurements for comparisons between the sexes. We used GLMMs to determine whether the morphometric traits we measured could be used to determine sex in keel-billed toucans. In addition, we evaluated GLMMs to determine which morphometric trait was the strongest predictor of sex in this species. Beak length was longer in adult males than in females. The GLMMs indicated that beak traits associated with sex in this species are beak curved length, beak straight length, straight length of the ventral line of the gnathotheca, and total culmen length. The straight length of the ventral line of the gnathotheca was the strongest predictor of sex in the Keel-billed Toucan. Unlike previous research, the development of GLMMs allows us to determine the probability of a toucan's sex based on continuous variables, facilitating sexing and allowing us to evaluate the reliability of the estimation of the sexing of each toucan. This method is an alternative for situations where other sexing methodologies cannot be performed.

Resumen · Rasgos morfométricos y su asociación con el sexo en el tucán pico canoa *Ramphastos sulfuratus*

El sexado en aves monomórficas es fundamental para la reproducción en los programas de conservación. En especies de ranfástidos (e.g., tucanes) los machos tienen un pico más largo que las hembras, por lo que su morfometría podría ser potencialmente útil para el sexado. En este estudio, medimos los siguientes rasgos: largo curvo del pico, largo del culmen total, largo recto del pico, largo curvo y recto de la línea ventral de la gnanoteca, ancho del pico, área lateral de la rinoteca, y apertura cloacal, en 44 tucanes pico canoa. Adicionalmente, utilizamos medidas morfométricas de 17 individuos reportadas previamente. Estos datos fueron obtenidos utilizando dos métodos de medición (manual y usando un software de procesamiento de imágenes). Determinamos la fiabilidad entre ambos métodos de medición usando el coeficiente de correlación intraclase (ICC). Los individuos de los cuales no se conocía el sexo fueron sexados mediante pruebas moleculares (PCR). El ICC mostró poca fiabilidad entre ambos métodos, por lo que solamente utilizamos las medidas obtenidas manualmente para las comparaciones entre sexos. Utilizamos GLMMs para determinar si los rasgos fenotípicos que medimos pueden ser usados para determinar el sexo en el tucán pico canoa. Adicionalmente, evaluamos los GLMMs para determinar que variable morfométrica es el predictor más fuerte del sexo en esta especie. El pico de los machos del tucán pico canoa, fue más largo que el de las hembras. Los GLMMS mostraron que la probabilidad de que un tucán sea hembra disminuye en función del incremento en la longitud del largo recto de la línea ventral de la gnanoteca, el largo recto, el largo curvo y el largo total del culmen. El largo recto de la línea ventral de la gnanoteca fue la variable más fuerte para determinar el sexo en el tucán pico canoa. A diferencia de investigaciones previas, los GLMMS permiten determinar la probabilidad del sexo en un tucán en función de variables continuas, facilitando el sexado y permitiendo evaluar la fiabilidad de la estimación. Este método es una alternativa en situaciones en las que no se pueden realizar otros métodos de sexado.

Key words: *Beak · dimorphism · morphometry · sexing · Ramphastidae*

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INTRODUCTION

The Keel-billed Toucan *Ramphastos sulfuratus,* (Piciformes, Ramphastidae; ITIS 2020) is distributed from southern Mexico to northern Colombia and northwestern Venezuela (Skutch 1971, Gual Sill et al*.* 1996). This species is characterized by having a long pale green beak with a maroon tip, an orange patch that extends from the maroon tip to the posterior end of the rhinotheca, and turquoise blue markings mainly on the gnathotheca. The body is black, except for the upper tail coverts, which are white and the under tail coverts, which are red (Howell & Webb 1995). The plumage of this species is monochromatic; therefore, its sex cannot be determined based on plumage aspect (Howell & Webb 1995, Jones & Griffiths 2020).

Some ramphastids, including those of the genus *Selenidera* (toucanets) and a few aracari species, such as *Pteroglossus viridis* and *Pteroglossus inscriptus,* show marked sexual dimorphism. However, the remaining toucans have a monochromatic plumage. In species with monomorphic plumage, accurate sexing is critical to ensure that suitable partners are available in captive breeding programs (Worell 1988). Sexing methods include cytogenetic studies (Takagi et al*.* 1972), laparoscopy, fecal steroid analysis, and molecular techniques such as polymerase chain reaction (Miyaki et al*.* 1998). Nevertheless, these methods involve surgical risks, high monetary costs or take time to process the samples and obtain the results. An alternative is the development of probabilistic sexing based on statistical models of morphometric traits that allow us to reliably infer the sex of an individual based on inexpensive and relatively less invasive external measurements.

Studies carried out on some species, such as *Aulacorhynchus prasinus, Ramphastos toco, Ramphastus dicolorus, R. sulfuratus,* and *Pteroglossus torquatus*, have shown that males have longer beaks than females (Gual Sill et al*.* 1996, Castro et al*.* 2003, Peralta-Moreno et al*.* 2017, Porras-Morfin et al*.* 2018, Quinto et al*.* 2018). Castro et al*.* (2003) reported that the morphometric traits useful to distinguish the sex in *R. toco* and *R. dicolorus* are the culmen, tomium, length of the lower horny beak, and length of the cloacal opening. Gual Sill et al*.* (1996) determined through beak measurements of 17 anesthetized adult individuals (nine males and eight females) that four traits can be used to distinguish between male and female Keel-billed toucans (curved length of the rinotheca, lower margin of the rinotheca, curved length of the ventral line of the gnathotheca and curved length of the ventral line of the gnathotheca without including the beak branches). Porras-Morfin et al*.* (2018) measured the beak length and width of 24 Keel-billed toucans (six males and 18 females) and concluded that specimens in which the length of the beak measures 12.65 cm or more should be considered males and those with a length of 12.34 cm or less may be considered females. However, if the beak length is within the range of 12.34 to 12.65 cm, the individual cannot be reliably sexed (Porras-Morfin et al*.* 2018). These previous studies concluded that different beak traits may be useful for determining sex in keel-billed toucans (Gual Sill et al*.* 1996, Porras-Morfin et al. 2018). However, these results present a range of uncertainties in sex determination. In addition, the sample size used to determine the relationship between beak length and sex was small (especially for males, N = 6; Porras-Morfin et al. 2018). Additionally, it has not been evaluated which of these measures is the most important for determining sex in keel-billed toucans.

Historically, morphometric measurements have been performed manually using rulers, measuring tapes or digital calipers. For morphometric measurements, it is necessary to immobilize the individual to be measured, either through physical (Porras-Morfin et al*.* 2018) or chemical restraint (e.g., anesthesia, Gual Sill et al. 1996). This handling could generate stress in the measured individuals and bias in taking the measurements when the restraint is physical. An alternative to reduce handling is to take digital photographs of the structures that one wishes to measure and later analyze them through image processing software (e.g., ImageJ, Schneider et al. 2012). The use of photographs and their subsequent analysis through image processing software to obtain precise measurements has been implemented in birds to analyze the morphology of body parts, such as the beak or tarsus (Ryeland et al*.* 2017, Williams et al*.* 2020). Additionally, photographs can be stored and used in later research (Williams et al*.* 2020).

In this context, we made seven measures of the beak and cloacal opening width of male and female keel-billed toucans, taking both manual measurements and digital photography-based techniques. Additionally, we determined the sex of the toucans through blood sample analysis using molecular PCR tests when the sex of the individual was unknown. Finally, we compared the variability and repeatability measured by the two measurement methods (manual measures and digital photography followed by software analysis) and determined whether the probability of Keel-billed Toucan being female decreases with increasing measurements of the beak and cloacal opening length. We hypothesized that all measures related to beak length would be significantly associated with sex (i.e., indicators of sex). We expected to detect which of the seven measures of the beak as well as the width of the cloacal opening were the strongest predictors of sex and therefore most useful for sexing keel-billed toucans. In addition, we expected a strong reliability between the two measurement methods. To test these hypotheses, we compared the variability and repeatability of the measures obtained using the two measurement methods (manual measurements and digital photography followed by software analysis) using the intraclass correlation coefficient (ICC). We performed generalized linear mixed models (GLMMs) on seven measures of the beak, as well as the width of the cloacal opening of Keel-billed Toucan males and females.

METHODS

The study subjects were 44 adult keel-billed toucans over three years old (the age criterion was based on the records of each of the institutions) belonging to four institutions: “El Nido” Sanctuary (Ixtapaluca, Mexico), XCARET Eco-Archaeological Park (Playa del Carmen, Mexico), ZOOFARI (Morelos, Mexico) and Chapultepec Zoo (Mexico City, Mexico). The specimens originated from confiscations, donations, or individuals born in captivity. The specimens’ diet consisted of seasonal fruits such as papaya, banana, apple, and melon; additionally, at XCARET and Chapultepec Zoo, Mazuri® pelleted food is offered. All the Keel-billed Toucans were measured between December 2021 and January 2022. The project and methodology were approved by the Internal Committee for the Care and Use of Animals (CICUA, Comité Interno para el Cuidado y Uso de los Animales) of the FMVZ-UNAM and by each of the participating institutions.

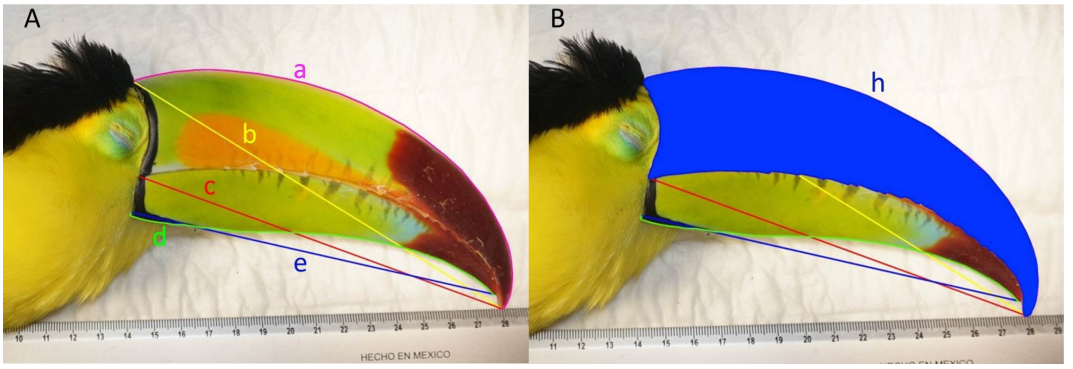

Figure 1. Measurements taken of Keel-billed Toucans' beaks *Ramphastos sulphuratus*. In "A" the following measurements are shown: curved length (a), total culmen length (b), straight length (c), curved length of the ventral line of the gnathoteca (d), straight length of the ventral line of the gnathoteca (e). In "B" the estimation of the lateral area of the rhinotheca (h) is shown.

Toucan measurements. Measurements were performed using two methods: manual measurements and image processing using ImageJ software (Schneider et al. 2012), the variability and repeatability between the measurement methods were calculated. For manual measurements, the specimens were captured using towels and nets and held according to the technique suggested by Porras-Morfin et al. (2018). This technique involves holding the toucan's beak with one hand and the tarsi, the distal part of the folded wings, and the body with the other hand, while a second person takes measurements. We measured all toucans using this holding technique, except for the specimens at XCARET, which were measured while they were under general anesthesia with isoflurane for their annual physical evaluation. All beak measurements were taken by the same person (DELS) using a measuring tape and a STEREN® brand digital caliper with an accuracy of 0.1 mm. Additionally, each individual was weighed using a portable digital scale (CGOLDENWALL®, 0.01 g accuracy). We took the following measurements: curved beak length (Figure 1A, a), total culmen length (Figure 1A, b), straight beak length (Figure 1A, c), curved length of the ventral line of the gnathotheca (Figure 1A, d), straight length of the ventral line of the gnathotheca (Figure 1A, e), cloacal opening (Figure 2A, g), and beak width (Figure 2B, f).

After the manual measurements, we took three photographs with a digital camera (SONY, model α6000 with 24.3 megapixel APS-C sensor), from a distance of approximately 40 cm between the specimen and the camera: a) side view of the peak, b) ventral view of the beak, and c) ventral view of the pubic area (Figure 1 and 2) for later analysis using ImageJ software. These images included the desired area, a ruler (to set a scale in the software), and a specimen identifier. In addition to the manual measurements , we measured the lateral area of the rhinotheca (cm^2; Figure 1B, h) using ImageJ software. We measured 44 keel-billed toucans using both measuring methods.

Sexing of toucans. Of the 44 Keel-billed toucans included in the present study, 17 were previously sexed through molecular PCR or laparoscopy tests. The remaining individuals (N = 27) were sexed using a PCR protocol (Lee et al. 2010). For this test, a sample of 0.5 ml of blood was extracted from the metatarsal vein using an insulin syringe and stored in a tube with EDTA (BD Microtainer®) under refrigeration. DNA was extracted from a 50 µL subsample of the stored blood using the Gentra Puregene (Qiagen GmbH, Hilden, Germany) commercial kit (following the manufacturer's instructions). For PCR, we used the primers described by Lee et al. (2010), which amplified fragments of the CHD1-W and CHD1-Z gene between 320bp and 690bp, respectively. Females carry both the W and Z chromosomes, and males carry two copies of the Z chromosome (Lee et al. 2010). The reaction mix consisted of 1µL of each primer at a concentration of 10 micromoles (µMol)/µL, 2µL of DNA at a concentration of 100 nanograms (ng), and 8.5µL of nuclease-free water. The PCR cycle consisted of an initial period of 94°C for 3:00 min; 40 cycles of 94°C for 30s, 63°C for 1:30 min, and 72°C for 2:00 min; and a final period of 72°C for 7 min.

Statistical analysis. In addition to the 44 keel-billed toucans (males = 25 and females = 19) that we measured in this study, for some measurements we included the morphometric data of 17 keel-billed toucans (males = 9 and females = 8) reported by Gual Sill et al. (1997). The total sample included in the analyses was 61 toucans (males = 34 and females = 27) for straight length, curved length, weight, and curved length of

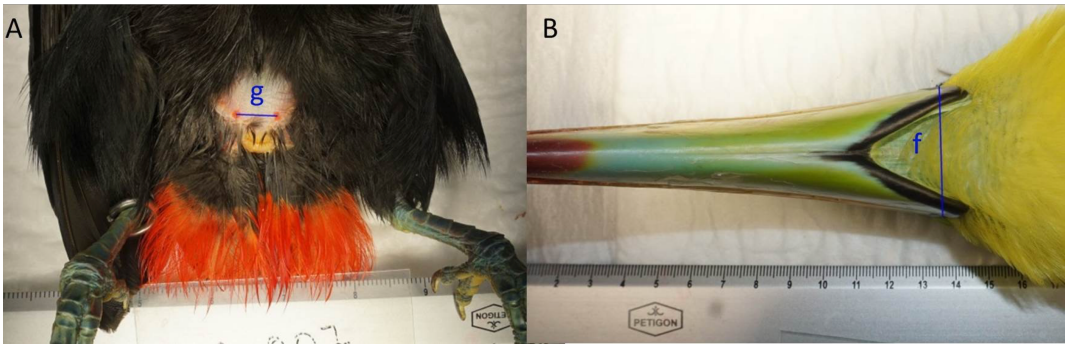

Figure 2. Measurements taken of Keel-billed Toucans *Ramphastos sulphuratus*). In "A" the estimation of the cloacal opening (g) is shown. In "B" the width of the beak is shown (f).

Table 1. Linear correlations between the morphometric measurements of 44 individuals of the Keel-billed Toucan *Ramphastos sulphuratus*. Statistical significance between sexes is indicated at $P < 0.05$ (*) and $P < 0.01$ (**). CL = Curved Length, TCL = Total Culmen Length, SL = Straight Length, CLVG = Curved Length of the Ventral line of the Gnathoteca, SLVG = Straight Length of the Ventral line of the Gnathoteca, CO = Cloacal Opening, LAR = Lateral Area of the Rhinotheca.

	Weight (g)	CL (cm)	TCL (cm)	SL (cm)	CLVG (cm)	SLVG (cm)	CO (cm)	Width (cm)	LAR (cm^2)
Weight (g)	—	0.371*	0.360*	0.164	0.299*	0.119	0.428*	0.122	0.626**
CL (cm)	.	—	0.899**	0.828**	0.890**	0.782**	0.128	0.303	0.776**
TCL (cm)	.	.	—	0.797**	0.868**	0.761**	0.226	0.26	0.775**
SL (cm)	.	.	.	—	0.843**	0.945**	0.06	0.356*	0.791**
CLVG (cm)	.	.	.	.	—	0.881**	0.097	0.278	0.76**
SLVG (cm)	.	.	.	.	.	—	-0.00007	0.317*	0.770**
CO (cm)	.	.	.	.	.	.	—	0.083	0.308
Width (cm)	.	.	.	.	.	.	.	—	0.749**
LAR (cm^2)	.	.	.	.	.	.	.	.	—

Table 2. Means and standard deviations by sex of the studied variables were obtained through two measurement methods (manual and image processing software). Standard Deviation (±SD). Asterisks (*) indicate significant differences between sexes.

Variable	Manual (female)		Manual (male)		Software (female)		Software (male)	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Weight (g)	456.96	78.83	483.31	74.4	.	.	.	.
Curved length (cm)*	15.37	0.93	17.57	1.01	17.98	2.17	20.3	2.65
Total culmen length (cm)*	13.42	0.78	15.55	1.06	15.39	1.71	17.46	2
Straight length (cm)*	12.43	0.63	14.16	0.8	14.41	1.82	16.48	2.04
Curved length of the ventral line of the gnathotheca (cm)*	11.97	0.65	13.88	0.62	14	1.79	16.01	1.81
Straight length of the ventral line of the gnathotheca (cm)*	11.85	0.65	13.43	0.71	13.85	1.84	15.8	1.85
Cloacal opening (cm)	1.59	0.44	1.51	0.4	2.01	0.81	1.64	0.51
Lateral area of the rhinotheca (cm^2)	.	.	.	.	49.65	17.17	54.38	13.84
Width (cm)	3.26	0.16	3.34	0.23	3.93	0.46	3.93	0.23

the ventral line of the gnathotheca; 44 toucans (25 males and 19 females) for a straight length of the ventral line of the gnathotheca, total culmen length, cloacal opening, beak width, and 41 toucans for the lateral area of the rhinotheca (males = 23 and females = 18). We calculated descriptive statistics and performed a comparison of means (Student's t-test) between the sexes for each morphometric variable. We calculated the intraclass correlation coefficient (ICC) to estimate the reliability of the measures obtained through two measurement methods (manual and image processing software). ICC values range between 0 and 1; values closer to 0 indicate poor reliability and values close to 1 indicate strong reliability (Koo & Li 2016) between measurement methods. The ICC was calculated for each of the seven morphometric variables obtained by the two measurement methods: curved beak length, total culmen length, straight beak length, curved beak ventral length, straight beak ventral length, beak width, and cloacal opening. In addition, we estimated the coefficient of variation for each morphometric variable obtained using each measurement method. ICC was calculated using the R package 'irr' (Gamer et al. 2019).

We used GLMMs (binomial distribution and logit link function) to estimate the probability that a given individual would be female given its morphometric measurements. In these GLMMs, we included each of the morphometric variables (straight length of the ventral line of the gnathotheca, straight length, curved length, total culmen length, weight, curved length of the ventral line of the gnathotheca, cloacal opening, beak width, and lateral area of the rhinotheca) as explanatory variables, sex of each toucan as a response variable, and toucan identity as a random factor. Female toucans were categorized as "1" and males as "0." Due to the strong collinearity between morphometric variables (Table 1), we tested each explanatory variable in the independent models. To detect the most important variable to predict sex in keel-billed toucans, we estimated the R^2 of the fixed variable in significant models (i.e., including the random effect and the predictor variable). The GLMMs were constructed using the package lme4 version 1.1-27.1 (Bates et al. 2015). We estimated R^2 using the package "partR2" version 0.9.1 (Stoffel et al. 2021). The statistical analyses were performed in R 3.5.3 (R Core Team 2019).

RESULTS

The PCR tests determined that of the 26 unsexed keel-billed toucans, 16 were male and 10 were female. All beak measurements analyzed in the present study were on average larger in males than in females (Table 1). On average, the cloacal opening was smaller in males (1.51 ± 0.40 cm) than in females (1.59 ± 0.44 cm; Table 2). We found significant differences between the sexes in the following morphometric measurements: curved length (t: –9.27, $P < 0.0001$), total culmen length (t: –8.20, $P < 0.0001$), straight length (t: –9.85, $P < 0.0001$), curved length of the ventral line of the gnathotheca (t: –12.73, $P < 0.0001$), and straight length of the ventral line of the gnathotheca (t: –8.62, $P < 0.0001$). The intraclass correlation coefficient indicated a poor reliability between the two measurement methods (Table 3). The highest reliability value (ICC = 0.71) was obtained for the cloacal opening. However, most of the morphometric variables (curved length, straight length, curved length of the ventral line of the gnathotheca, and straight length of the ventral line of the gnathotheca) had poor reliability ($P < 0.1$) between the measurement methods (Table 3). The morphometric data of the beak obtained through manual measurements presented lower coefficients of variation than the measurements obtained through the ImageJ software; therefore, we used the measurements obtained manually to perform the GLMMs.

The GLMMs indicated that the probability of a toucan being a female decreased with increasing curved length, straight length, straight length of the ventral line of the gnathotheca, and total culmen length (Table 4, Figures 3 and 4). According to the R^2, the strongest predictor of sex in the Keel-billed Toucan was the straight length of the ventral line of the gnathotheca (R^2 = 0.77, IC 95% = 0.21 – 0.94; Table 4).

DISCUSSION

Contrary to our expectations, not all beak morphometric traits were significantly associated with sex in Keel-billed toucans.

Table 3. Coefficient of variation and reliability between measurement methods (manual and image processing software) for each morphometric variable of *Ramphastos sulphuratus*. Reliability was quantified using the intraclass correlation coefficient (ICC). CL = Curved Length; TCL = Total Culmen Length; SL = Straight Length; CLVG = Curved Length of the Ventral line of the Gnathotheca; SLVG = Straight Length of the Ventral line of the Gnathotheca; CO = Cloacal Opening; BW = Beak Width.

Morphometric traits	Coefficient of variation		Reliability between methods
	Manual	Software	
CL	9.07	13.83	0.05
TCL	9.68	12.77	0.16
SL	8.09	13.96	0.03
CLVG	8.66	13.46	0.01
SLVG	8.12	13.75	0.04
CO	27.82	38.31	0.71
BW	6.39	8.64	0.45

Table 4. GLMMs evaluating whether morphometric traits are predictors of sex in the Keel-billed Toucan *Ramphastos sulphuratus*. R^2 is reported for the significant predictor models. The sample size for each measurement is given in parentheses beside the variable abbreviation. SLVG = Straight Length of the Ventral line of the Gnathotheca; SL = Straight Length; CL = Curved Length; TCL = Total Culmen Length; W = Weight; CLVG = Curved Length of the Ventral line of the Gnathotheca; CO = Cloacal Opening; BW = Beak Width; LAR = Lateral area of the Rhinotheca.

Morphometric traits	Estimate	ES	Z	P	R^2	R^2 IC 95%
SLVG (41)	-3.596	1.055	-3.408	**0.0006**	0.77	0.21-0.94
Intercept	45.031	13.276	3.392	**0.0007**	.	.
Random effect	Variance	SD				
Toucan id	<0.0001	<0.0001	.	.	.	.
SL (61)	-3.001	0.733	-4.091	**<0.0001**	0.7	0.11-0.79
Intercept	39.585	9.741	4.064	**<0.0001**	.	.
Random effect	Variance	SD				
Toucan id	<0.0001	<0.0001	.	.	.	.
CL (61)	-2.389	0.602	-3.963	**<0.0001**	0.69	0.37-0.83
Intercept	38.865	9.828	3.955	**<0.0001**		
Random effect	Variance	SD				
Toucan id	<0.0001	<0.0001	.	.	.	.
TCL (44)	-26.86	11.18	-2.404	**0.017**	0.64	0.17-0.43
Intercept	383.51	160.51	2.389	**0.016**	.	.
Random effect	Variance	SD	.	.	.	.
Toucan id	811.2	28.48	.	.	.	.
W (61)	-0.004	0.003	-1.138	0.255	.	.
Intercept	1.673	1.68	0.996	0.319	.	.
Random effect	Variance	SD				
Toucan id	0.002	0.047	.	.	.	.
CLVG (61)	-21.73	26.22	-0.829	0.407	.	.
Intercept	279.24	336.89	0.829	0.407	.	.
Random effect	Variance	SD				
Toucan id	0.557	0.746	.	.	.	.
CO (44)	0.844	0.79	1.068	0.285	.	.
Intercept	-1.587	1.288	-1.232	0.218	.	.
Random effect	Variance	SD				
Toucan id	0.006	0.08	.	.	.	.
BW (44)	-2.749	2.189	-1.256	0.209	.	.
Intercept	8.768	7.172	1.223	0.222	.	.
Random effect	Variance	SD				
Toucan id	0.024	0.156	.	.	.	.
LAR (41)	-0.024	0.021	-1.101	0.271	.	.
Intercept	1.015	1.179	0.861	0.389	.	.
Random effect	Variance	SD				
Toucan id	<0.0001	<0.0001	.	.	.	.

However, we detected four beak variables that were associated with sex in the species: curved beak length, straight beak length, straight length of the ventral line of the gnathotheca, and total culmen length. Of these variables, the straight length of the ventral line of the gnathotheca was the best predictor of sex in Keel-billed toucans. Finally, contrary to our expectations, we found poor reliability between the two measurement methods (manual versus image processing software).

In this study, all beak measurements were on average, larger in males than in females, while the cloacal opening had the opposite pattern. It has been reported that in the Toco Toucan (*R. toco*) and the Green-billed Toucan (*R. dicolorus*), the length of cloacal opening can be used as a parameter to distinguish the sex of these two species since their length tends to be larger in males than in females (Castro et al. 2003). However, in our study, the length of the cloacal opening was not significantly associated with sex. Therefore, it does not represent a useful measure for distinguishing between males and females in the Keel-billed Toucan. Numerically, we found that on average, males have a shorter cloacal opening length compared to females, indicating the opposite pattern reported for the Toco Toucan and the Green-billed Toucan (Castro et al. 2003).

In our study, the beaks of sexually mature Keel-billed Toucan males (> 3 years) were longer than those of the females. This pattern is consistent with what has been previously reported by other authors (Skutch 1971, Gual Sill et al. 1996, Porras-Morfin et al. 2018). Our results suggest that the straight length of the ventral line of the gnathotheca, straight beak length, curved beak length, and the length of the total culmen are the measures that allow reliable sex determination for the Keel-billed Toucan; an individual is more likely to be female the shorter this measurement. Unlike the ranges or specific measurements reported in previous studies (Gual Sill et al. 1996, Porras-Morfin et al. 2018), the use of logistic models allowed us to determine the probability of the toucan's sex based on continuous variables rather than a strict binary determination with no associated continuous probability. This facilitates the sexing of the toucans as well as an estimate of the reliability of that sex assignment. We also evaluated the importance of these morphometric variables. We determined that the most important variable for predicting sex in the Keel-billed Toucan is the straight length of the ventral line of the

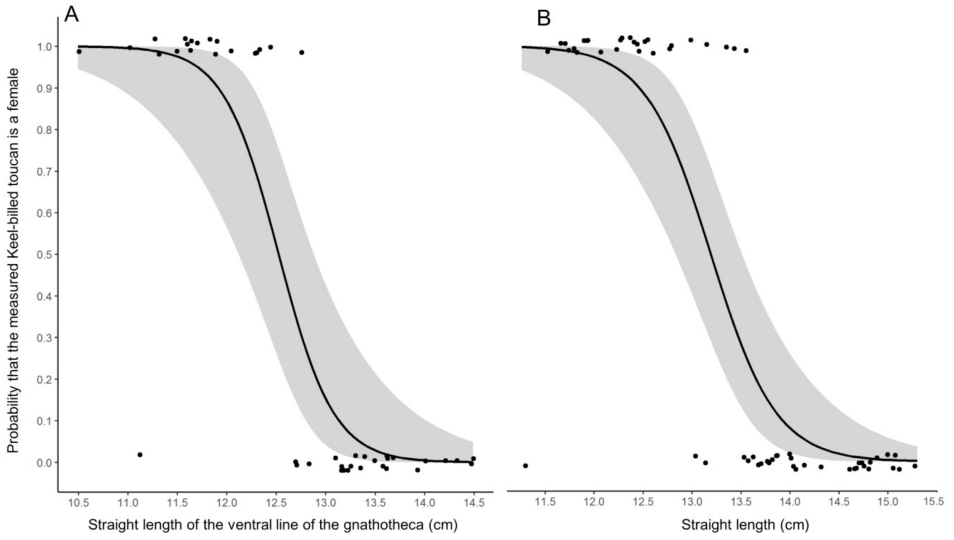

Figure 3. Predicted values from GLMMs indicating the probability that the measured Keel-billed Toucans *Ramphastos sulphuratus* is a female (black continuous lines) according to (A) the straight length of the ventral line of the gnathoteca and (B) the straight beak length. The black dots are the measures of each toucan, and the grey ribbons are the 95% confidence intervals.

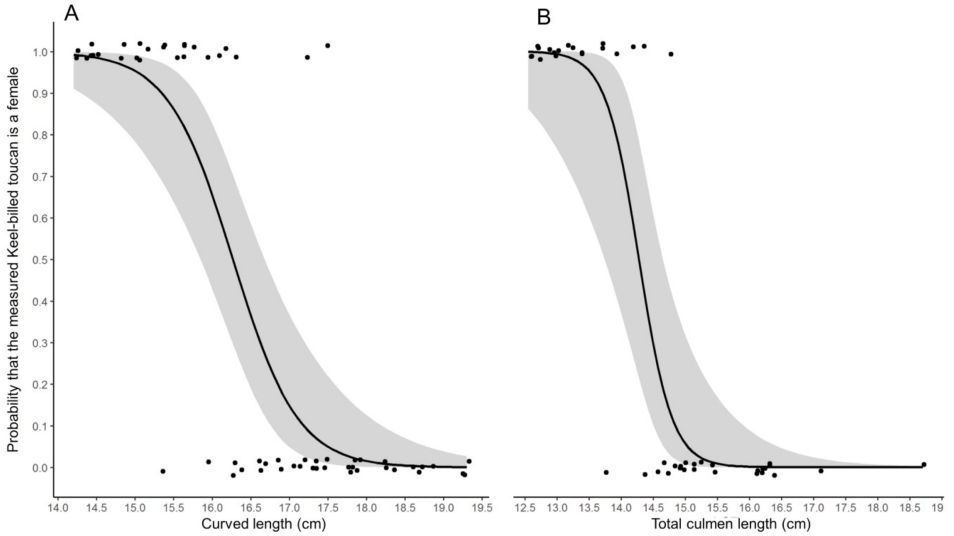

Figure 4. Predicted values of GLMMs indicating the probability that the measured toucan is a female (black continuous lines) according to (A) curved beak length and (B) total culmen length. The black dots are the measures of each toucan, and the grey ribbons are the 95% confidence intervals.

gnathotheca.

The implementation of probabilistic models based on measurements of the beak of the Keel-billed Toucan will allow the sex of an individual to be inferred quickly. This type of sexing could be a fast, inexpensive, and simple alternative in places where it is not possible to use another type of sexing methodology (e.g., institutions that confiscate wildlife). In this context, determining the sex of keel-billed toucans could strengthen breeding under human care and conservation programs of institutions such as zoos or sanctuaries, facilitating the establishment of reproductive pairs.

In our study, the measurements obtained through the analysis of photographs using ImageJ software presented an overestimation, higher coefficient of variation, and poor reliability compared with measurements obtained manually. This overestimation pattern was previously reported when comparing measurements made manually with those obtained using photographs and analyzing them through ImageJ (Ryeland et al. 2017, Williams et al. 2020). This poor reliability between the methods is because some of the regions of the toucan that were measured have a depth component (or width of the structure) that is lost in the photograph since the images are projected in two dimensions. This explains why the estimation of the cloacal opening has the highest reliability value (ICC = 0.71). In the photographs, the depth component was less important for estimating the length of the cloacal opening; therefore, there was less overestimation (Figure 2). To reduce this overestimation, the following considerations must be considered: 1) Standardize the distance at which the photographs are taken. This can be achieved using a tripod or by leaving the camera in a fixed place. 2) Ensure that the entire structure to be measured is in the same spatial plane, avoiding any region being rotated or tilted. This can be achieved by supporting the entire structure on a surface (e.g., a table). 3) Place the ruler used to calibrate each photograph in the software as close as possible to the structure to be measured and on the same spatial plane as that structure. Carrying out these considerations together avoids overestimating the measurements of the body parts generated by the difference in depth between the different planes in the photograph.

The results obtained in this study confirm that female keel-billed toucans have shorter beaks than males. Unlike previous works (Gual Sill et al. 1996, Porras-Morfin et al. 2018), we evaluated a greater number of morphometric measurements and quantified their importance as predictors of sex. The straight length of the ventral line of the gnathotheca is the most effective measure for inferring the sex of a Keel-billed Toucan. The development and implementation of GLMMs allows the determination of the probability of the sex of the toucan based on continuous variables, facilitating sexing and evaluating the reliability of each sex assignment.

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