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Author(s): Guilherme Tavares Nunes , Gustavo Da Rosa Leal , Cynthia Campolina , Thales Renato Ochotorena De Freitas , Márcio Amorim Efe and Leandro Bugoni

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Sex Determination and Sexual Size Dimorphism in the Red-billed Tropicbird (*Phaethon aethereus*) and White-tailed Tropicbird (*P. lepturus*)

GUILHERME TAVARES NUNES^{1,5,*}, GUSTAVO DA ROSA LEAL², CYNTHIA CAMPOLINA³,
THALES RENATO OCHOTORENA DE FREITAS⁴, MÁRCIO AMORIM EFE² AND LEANDRO BUGONI^{1,5}

¹Graduate Program in Biological Oceanography, Federal University of Rio Grande - FURG, CP 474,
CEP 96201-900, Rio Grande, RS, Brazil

²Institute of Biological and Health Sciences, Federal University of Alagoas - UFAL, Av. Lourival Melo Mota, s/n,
Cidade Universitária, CEP 57072-900, Maceió, AL, Brazil

³Conservation International's Marine Program, CP 52, CEP 45900-000, Caravelas, BA, Brazil

⁴Department of Genetics, Federal University of Rio Grande do Sul - UFRGS, CP 15053, CEP 91501-970,
Porto Alegre, RS, Brazil

⁵Institute of Biological Sciences, Federal University of Rio Grande - FURG, CP 474, CEP 96201-900,
Rio Grande, RS, Brazil

*Corresponding author; Email: gtn.biomar@yahoo.com.br

Abstract.—Tropicbirds (order Phaethontiformes) are a well-studied seabird taxon, but there have been no published studies on sexual size dimorphism. Here, we investigated sexual size dimorphism in the Red-billed Tropicbird (*Phaethon aethereus*) and the White-tailed Tropicbird (*P. lepturus*), and evaluated the applicability of using morphometrics and discriminant functions to sex birds in the field. We sampled Red-billed Tropicbirds ($n = 160$) and White-tailed Tropicbirds ($n = 57$) in the Atlantic Ocean off the coast of Brazil. Each individual had seven morphometric traits measured and blood sampled for molecular sex determination. Discriminant functions were generated using generalized linear models (GLM). Red-billed Tropicbirds had significant male-biased intersexual differences in bill length, wing chord, nostril-to-bill-tip, and head-plus-bill, and the best GLM included bill length and wing chord. White-tailed Tropicbirds were characterized by significant intersexual differences for wing chord, with females larger than males, and the best GLM for this species included wing chord, bill width, and bill depth. Discriminant functions had accuracy similar to those used for other seabird species with similar body sizes. *Received 16 January 2013, accepted 5 February 2013.*

Key words.—discriminant function, intersexual difference, *Phaethon aethereus*, *Phaethon lepturus*, Red-billed Tropicbird, White-tailed Tropicbird.

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Tropicbirds (Aves: Phaethontiformes) are monomorphic seabirds and are therefore thought to have limited sexual size dimorphism (SSD) (Orta 1992). The order comprises three species distributed in tropical and subtropical warm waters, breeding in colonies on oceanic islands, and catching their prey by plunge-diving in pelagic zones (Orta 1992). In Brazil, there are colonies of a few hundred Red-billed Tropicbirds (*Phaethon aethereus*) and White-tailed Tropicbirds (*P. lepturus*) at Abrolhos and Fernando de Noronha archipelagos, respectively (Sick 1997). Both species are listed as threatened in the Brazilian Red List, mainly due to the introduction of *Rattus rattus*, *Tupinambis merianae*, and *Felis catus* (Efe 2008).

In birds with sexually monomorphic plumage and soft-tissue coloration, small but significant intersexual morphometric differences could potentially allow gender discrimination in the field to enhance the interpretation of behavioral and ecological studies, allowing the optimization of conservation initiatives. One of the most successful strategies for gender discrimination is through fitting linear models with binomial function, a procedure that has been successfully applied to waterbirds (e.g., Ura *et al.* 2005; Hallgrimsson *et al.* 2008).

An increasing number of studies have identified some degree of SSD in seabirds, usually in the form of subtle morphometric differences inconspicuous to the human eye. Such differences have been reported in

various orders of seabirds: Charadriiformes (Devlin *et al.* 2004), Pelecaniformes (Dorr *et al.* 2005), Sphenisciformes (Bertellotti *et al.* 2002), Procellariiformes (Copello *et al.* 2006), and Suliformes (Quintana *et al.* 2003). However, information on SSD in the order Phaethontiformes is lacking.

In this study we aim to: 1) identify the degree of SSD in Red-billed Tropicbirds and White-tailed Tropicbirds and determine in which traits it is expressed; 2) generate linear models from morphometric measurements to discriminate sex of both species; and 3) assess the degree of confidence in sex discrimination by the fitted models.

METHODS

White-tailed Tropicbirds ($n = 57$) breeding on Fernando de Noronha archipelago ($3^{\circ} 51' \text{ S}$, $32^{\circ} 25' \text{ W}$) and Red-billed Tropicbirds ($n = 160$) breeding on Abrolhos archipelago ($17^{\circ} 58' \text{ S}$, $38^{\circ} 42' \text{ W}$) were captured on nest burrows in 2011 and 2012. Eight body measurements were taken: bill length (exposed culmen), nostril-to-bill-tip (measured from the distal end of the nostril to the bill tip), bill width (measured at the nostrils), bill depth (measured at the nostrils), head-plus-bill (bill tip to the posterior ridge formed by the parietal-supraoccipital junction), tarsus length (from middle of midtarsal joint to distal end of tarsometatarsus), wing chord (carpal joint to tip of the longest primary, with flattened wing), and body mass.

Wing chord was measured using a metal rule with stop ($\pm 1 \text{ mm}$), while all other linear measurements were taken using digital Vernier calipers ($\pm 0.01 \text{ mm}$). Body mass was measured to the nearest 5 g using Pesola spring scales, but excluded from the analysis due to large variations during the breeding season (Croxall 1995). All samples were collected by the authors of this study using standardized techniques. In order to prevent resampling, all sampled birds were banded with metal rings.

Blood samples of each individual were collected and stored on FTA Classical cards for molecular sex determination, and DNA was extracted using the protocol of Boyce *et al.* (1989). Molecular sexing was performed using P2-P8 primers (Griffiths *et al.* 1998). The final volume of amplification reactions was 10 μl containing 10 ng of DNA, 0.2 μM of each primer, 0.2 μM of dNTP, Polymerase Chain Reaction (PCR) Buffer 1x, 1.5 mM of MgCl_2 e 1.0 unit of Taq DNA polymerase (GIBCO-BRL Life Sciences/Invitrogen). Amplifications were performed as follows: denaturation at 94°C for 5 min, 30 cycles at 94°C for 1 min, annealing at 46°C for 1 min, and final elongation at 72°C for 5 min. PCR products were separated by electrophoresis in 8% denaturing polyacrylamide gel and revealed using the methodology

of staining with silver nitrate (Bassam *et al.* 1991). Males were characterized by the presence of only one band on the gel, while females were characterized by two bands.

Heterozygosity for the intron CHD-Z was tested with P0 accessory primer (Han *et al.* 2009). We randomly selected 10 individuals of each sex, which had sex determined by P2-P8 universal primers, and multiplex reactions with P2-P8 plus P0. As a result, polymorphism was not detected in any of the tested individuals, rejecting heterozygosity on CHD-Z intron for tropicbirds.

Intersexual differences from morphometric measurements were assessed through univariate *t*-test. For this analysis, outliers were identified and removed by the probability test based on standard deviation present in BioEstat 5.0 (Ayres *et al.* 2007). Therefore, all individuals of both species had equality of group covariance matrices, and all data were normally distributed, after Bartlett and Shapiro-Wilk's tests, respectively. Furthermore, *P*-value was adjusted with Bonferroni correction for multiple comparisons (Zar 2010). Sexual size dimorphism index was calculated as the ratio between the average values for females and males.

The ability of the morphometric analysis to discriminate among males and females was examined using generalized linear models (GLMs) with binomial errors and a logit link function, where sex was treated as a binary response variable. Outliers previously identified were maintained in the total sample set, because the analysis is robust to violations of normality and homoscedasticity (Hair *et al.* 2009). Pearson's correlation was used to check the interaction between variables, excluding those variables correlated amongst themselves ($P < 0.05$). To fit the GLMs, significant variables were selected by the model through a stepwise procedure, testing the deviance of the likelihoods of full and reduced models (Burnham and Anderson 2002). The best models for each species were selected using Akaike's Information Criterion (AIC), assuming that smaller values indicate a better fit of the model.

From selected models for Red-billed Tropicbirds, two validation methods were applied: 1) with the full sample set, which was used to fit the models; and 2) a sample obtained from random selection without replacement with 50% of the full data set (jackknife method), which was used to validate the model fitted with the full set (Dechaume-Moncharmont *et al.* 2011). Due to small sample size, for White-tailed Tropicbirds best models were only validated using the full sample. Cutpoint (*C*) for discriminant scores to each model was calculated according to Hair *et al.* (2009), considering groups with different sizes, as follows:

$$C = \frac{(Nf * Zm) + (Nm * Zf)}{Nf * Nm}$$

in which *C* is the cutpoint, *N* is the sample size for males (*m*) and females (*f*), and *Z* is the centroid (mean of discriminant scores) for each sex. All statistical analyses were carried out using the software R (R Development Core Team 2011).

RESULTS

Red-billed Tropicbird

Males had bill length, nostril-to-bill-tip, head-plus-bill, and wing chord larger than females (Table 1). Bill length, nostril-to-bill-tip, and head-plus-bill were correlated ($P < 0.001$), and the latter two were excluded from the data set to fit GLMs. Thus, the model that used bill length and wing chord showed discriminatory power of 67.5% ($n = 108$), while the model that used only bill length correctly classified 62.5% of the individuals (Table 2).

$$D = (\text{bill length} * 0.33) + (\text{wing chord} * 0.11) - 17.76$$

Individuals with discriminant scores $D < -0.01$ were sexed as females, whereas those with $D \geq -0.01$ were males, and the jackknife validation method correctly classified 65% ($n = 104$), which were randomly selected from the original data set.

White-tailed Tropicbird

In White-tailed Tropicbirds, significant SSD was detected for wing chord only. However, SSD values indicated reverse sexual dimorphism (RSD) for this trait. As for Red-billed Tropicbird, bill length, nostril-to-bill-tip, and head-plus-bill were correlated ($P < 0.001$), and the latter two were excluded from the data set to fit GLMs. In this case, in the GLM, four variables (wing chord, bill length, bill depth, and bill width) contributed to the best fit model (lowest AIC in Table 2). This model with the four variables above was the best in classifying birds, and was able to correctly sex 39 out of 54 birds: 20 females (80.0%) and 19 males (65.5%). Nevertheless, discrepancy on classification rates between males and females was higher in the model with better fit than the second best model. Thus, the most parsimonious model is the one that employs only wing chord, bill depth, and bill width, which despite correctly classifying a lower number of birds (38 out of 54), includes fewer variables and more similar classification rates between males and females. This model is as follows:

$$D = (\text{wing chord} * -0.15) + (\text{bill width} * 1.38) + (\text{bill depth} * 0.78) + 41.92$$

Table 1. Body measurements of Red-billed Tropicbirds (*Phaethon aethereus*) and White-tailed Tropicbirds (*Phaethon lepturus*) sampled on Abrolhos and Fernando de Noronha archipelagos (Brazil), respectively. Measurements of each sex are in mm, with mean $\pm$ SD (sample size); SSD = male:female (means). *P*-values of *t*-tests were adjusted with Bonferroni correction.

Variables	Red-billed Tropicbirds					White-tailed Tropicbirds				
	δ	$\varnothing$	SSD	<i>t</i>	<i>P</i>	δ	$\varnothing$	SSD	<i>t</i>	<i>P</i>
Bill length	63.20 $\pm$ 2.26 ($n = 71$)	61.06 $\pm$ 2.15 ($n = 68$)	1.03	-5.69	<0.001	47.64 $\pm$ 2.21 ($n = 31$)	48.11 $\pm$ 2.11 ($n = 26$)	0.99	0.81	0.42
Nostril-to-bill-tip	55.93 $\pm$ 1.99 ($n = 71$)	54.88 $\pm$ 1.97 ($n = 68$)	1.01	-3.13	0.002	37.16 $\pm$ 2.04 ($n = 32$)	37.83 $\pm$ 2.11 ($n = 26$)	0.98	1.21	0.23
Wing chord	324.92 $\pm$ 6.60 ($n = 39$)	320.62 $\pm$ 5.39 ($n = 40$)	1.01	-3.16	0.002	271.84 $\pm$ 6.33 ($n = 32$)	276.56 $\pm$ 6.10 ($n = 25$)	0.98	2.84	0.006
Tarsus length	29.68 $\pm$ 1.53 ($n = 32$)	29.27 $\pm$ 1.89 ($n = 26$)	1.01	-0.89	0.36	23.12 $\pm$ 1.33 ($n = 32$)	22.95 $\pm$ 1.33 ($n = 26$)	1.00	-0.48	0.63
Bill width	11.44 $\pm$ 0.92 ($n = 32$)	11.71 $\pm$ 1.20 ($n = 28$)	0.97	0.96	0.33	8.01 $\pm$ 0.52 ($n = 31$)	8.30 $\pm$ 0.77 ($n = 26$)	0.96	1.63	0.098
Bill depth	21.46 $\pm$ 1.10 ($n = 32$)	21.11 $\pm$ 1.10 ($n = 28$)	1.01	-1.44	0.16	16.13 $\pm$ 0.76 ($n = 29$)	15.93 $\pm$ 0.85 ($n = 25$)	1.01	-0.89	0.37
Head-plus-bill	121.65 $\pm$ 3.00 ($n = 32$)	119.48 $\pm$ 3.23 ($n = 28$)	1.01	-2.67	0.009	97.52 $\pm$ 2.08 ($n = 31$)	98.29 $\pm$ 2.53 ($n = 26$)	0.99	1.23	0.21

Table 2. Generalized Linear Models with best accuracy to discriminate the sex of Red-billed Tropicbirds (RBT, *Phaethon aethereus*) and White-tailed Tropicbirds (WTT, *Phaethon lepturus*) on Abrolhos and Fernando de Noronha archipelagos (Brazil), respectively. AIC = Akaike Information Criterion.

Species	Model	Discriminatory Power (%)			
		♂	♀	Total	AIC
RBT	Sex = bill length	67.5	57.5	62.5	107.65
	Sex = bill length + wing chord	72.5	62.5	67.5	101.52
WTT	Sex = wing chord	65.5	64	64.8	71.95
	Sex = wing chord + bill width + bill depth	65.5	76	70.4	68.11
	Sex = bill length + wing chord + bill width + bill depth	65.5	80	72.2	67.87

Here, individuals with discriminant scores $D < 0.12$ were females, whereas those with $D \geq 0.12$ were males.

DISCUSSION

Sexual Size Dimorphism

In many bird species, males are typically larger than females (Székely *et al.* 2007), a pattern which has also been observed in most seabirds (Schreiber and Burger 2001). In the current study, Red-billed Tropicbirds displayed this typical pattern (i.e., males had bill length and wing chord larger than females). However, in White-tailed Tropicbirds females typically had greater wing lengths than males. Thus, based on these two species there is no consistent pattern of SSD in the order Phaethontiformes.

The causes of SSD have long been discussed. Darwin (1871) suggested that larger body size in males is largely determined by selection during male-male disputes, where males with a larger body size have more mating opportunities. The evolution of SSD has subsequently been widely studied and, in most cases, sexual selection or niche segregation between sexes has been identified as key selective forces (reviewed in Shine 1989). Serrano-Meneses and Székely (2006) suggested that in seabirds the agility of males in sexual display may be an influential factor to the evolution of SSD. They also suggested that those species that display on the ground will have male-biased SSD, while females will be larger than males in species that perform aerial displays, such as tropicbirds.

The RSD found for White-tailed Tropicbird is uncommon within seabirds (Schreiber and Burger 2001). It has been recorded for wing chord in six *Puffinus* species (Bull *et al.* 2005), and has also been found in families that until recently were considered sisters of Phaethontidae, such as Sulidae and Fregatidae (Schreiber and Burger 2001). Tropicbirds perform aerial courtship displays (Orta 1992) and, thus, female-biased SSD was expected (Serrano-Meneses and Székely 2006). Therefore, from an adaptive perspective, aerial sexual display may explain the observed pattern of RSD in White-tailed Tropicbirds.

Sex Discrimination

This is the first time that discriminant functions have been applied to the study of morphometrics of Phaethontiformes. For White-tailed Tropicbird, although sexual dimorphism occurs only for the wing chord, the fitted model was able to identify sexes, confirming the relevance and intensity of RSD. For Red-billed Tropicbird, the application of jackknife subsampling technique as an alternative method for validating the model resulted in a similar discriminatory power compared to the validation performed with the total data set. This technique has been suggested by Dechaume-Moncharmont *et al.* (2011) precisely to avoid overestimated discriminant rates with intermediate sample sizes ($n < 200$).

Although there is SSD in both species, there is strong overlap in the range of traits that showed statistical differences between the sexes. Hence, even if the sample size in-

creases, it is possible that the discriminatory power of new discriminant functions will be similar to this study. In summary, we clearly demonstrate that discriminating functions are a plausible method for sex determination of the two tropicbirds in the field, with discriminatory power similar to other seabirds in which the same methods have been applied.

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LITERATURE CITED

- Ayres, M., M. Ayres, Jr., D. L. Ayres and A. A. Santos. 2007. Bioestat 5.0: aplicações estatísticas nas áreas das ciências biomédicas. Ong Mamirauá, Belém, Brazil.
- Bassam, B. J., G. Caetano-Anollés and P. M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry* 196: 80-83.
- Bertellotti, M., J. L. Tella, J. A. Godoy and G. Blanco. 2002. Determining sex of Magellanic Penguins using molecular procedures and discriminant functions. *Waterbirds* 25: 479-484.
- Boyce, T. M., M. E. Zwick and C. F. Aquadro. 1989. Mitochondrial DNA in the Bark Weevils: size, structure and heteroplasmy. *Genetics* 123: 825-836.
- Bull, L. S., B. D. Bell and S. Pledger. 2005. Patterns of size variation in the shearwater genus *Puffinus*. *Marine Ornithology* 33: 27-39.
- Burnham, K. P. and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information - theoretic approach. Springer-Verlag, New York.
- Copello, S., F. Quintana and G. Somoza. 2006. Sex determination and sexual size-dimorphism in Southern Giant-Petrels (*Macronectes giganteus*) from Patagonia, Argentina. *Emu* 106: 141-146.
- Croxall, J. P. 1995. Sexual size dimorphism in seabirds. *Oikos* 73: 399-403.
- Darwin, C. 1871. The descent of man, and selection in relation to sex. John Murray Publishers, London, U.K.
- Dechaume-Moncharmont, F. X., K. Monceau and F. Cezilly. 2011. Sexing birds using discriminant function analysis: a critical appraisal. *Auk* 128: 78-86.
- Devlin, C. M., A. W. Diamond and G. W. Saunders. 2004. Sexing Arctic Terns in the field and laboratory. *Waterbirds* 27: 314-320.
- Dorr, B., D. T. King, J. B. Harrel, P. Gerard and M. G. Spalding. 2005. The use of culmen length to determine sex of the American White Pelican. *Waterbirds* 28: 102-106.
- Efe, M. A. 2008. *Phaethon aethereus/Phaethon lepturus*. Pages 414-417 in *Livro Vermelho da Fauna Brasileira Ameaçada de Extinção*. Vol. II (A. B. Machado, G. M. Drummond and A. P. Paglia, Eds.). Fundação Biodiversitas, Brasília, Brazil.
- Griffiths, R., M. C. Double, K. Orr and R. J. Dawson. 1998. A DNA test to sex most birds. *Molecular Ecology* 7: 1071-1075.
- Hair, J. F., W. C. Black, B. J. Babin and R. E. Anderson. 2009. Multivariate data analysis 7th ed. Pearson Prentice-Hall, Upper Saddle River, New Jersey.
- Hallgrimsson, G. T., S. Pálsson and R. W. Summers. 2008. Bill length: a reliable method for sexing Purple Sandpipers. *Journal of Field Ornithology* 79: 87-92.
- Han, J.-I., J.-H. Kim, S. Kim, S.-R. Park and K.-J. Na. 2009. A simple and improved DNA test for avian sex determination. *Auk* 126: 779-783.
- Orta, J. 1992. Family Phaethontidae. Pages 280-289 in *Handbook of the Birds of the World*, vol. I: Ostrich to Ducks (J. Del Hoyo, A. Elliot and J. Sargatal, Eds.). Lynx Edicions, Barcelona, Spain.
- Quintana, F., G. Somoza, M. Uhart, C. Cassará, P. Gandini and E. Frere. 2003. Sex determination of adult Rock Shags by molecular sexing and morphometric parameters. *Journal of Field Ornithology* 74: 370-375.
- R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, v. 2.15.2. Vienna, Austria. <http://www.R-project.org/>, accessed 10 November 2012.
- Schreiber, E. A. and J. Burger (Eds.). 2001. *Biology of marine birds*. CRC Press, Boca Raton, Florida.
- Serrano-Meneses, M. A. and T. Székely. 2006. Sexual size dimorphism in seabirds: sexual selection, fecundity selection and differential niche-utilisation. *Oikos* 113: 385-394.
- Shine, R. 1989. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *Quarterly Review of Biology* 64: 419-461.
- Sick, H. 1997. *Ornitologia brasileira: uma introdução*. Editora Nova Fronteira, Rio de Janeiro, Brazil.
- Székely, T., T. Lislevand and J. Figuerola. 2007. Sexual size dimorphism in birds. Pages 27-37 in *Sex, Size and Gender Roles* (W. Blanckenhorn, D. Fairbairn and T. Székely, Eds.). Oxford University Press, Oxford, U.K.
- Ura, T., N. Azuma, S. Hayama and S. Higashi. 2005. Sexual dimorphism of Latham's Snipe *Gallinago hardwickii*. *Emu* 105: 259-262.
- Zar, J. H. 2010. *Biostatistical analysis*, 5th ed. Pearson Prentice-Hall, Upper Saddle River, New Jersey.