



MORPHOMETRY AND SEXUAL DIMORPHISM IN THE YELLOW-LEGGED TINAMOU *Crypturellus noctivagus noctivagus* IN A RELICTUAL POPULATION FROM SOUTHERN BRAZIL

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Abstract: The Yellow-legged Tinamou (*Crypturellus noctivagus*) is a terrestrial forest bird endemic to Brazil. Females and males of the subspecies *Crypturellus noctivagus noctivagus* are indistinguishable at the naked eye and there is lack of knowledge regarding means to discriminate sexes in the field. We investigated morphometric differences between sexes of *C. n. noctivagus* to determine which measures should be used to distinguish sexes. We compared eight morphometric measurements and total body mass of adult birds from a relictual population living in the wild, in the state of Rio Grande do Sul. Birds were captured during the breeding seasons of 2015 and 2016. Sex identification of individuals (four females and seven males) was obtained by molecular analysis. Differences between sexes were tested through Student's t and Mann-Whitney's tests and a through Principal Component Analysis. On average, females were 9.9% heavier and had bills 11.8% longer than males. Males had middle toes 8.7% larger and tails 19.0% larger than females, the latter presenting the highest degree of dimorphism among all measurements. Our results show that, when molecular sexing is not feasible, morphometric variables may be used to determine the sex of *C. n. noctivagus* individuals.

Keywords: Intersexual differences; Molecular sexing; Tinamidae; Tinamiformes.

INTRODUCTION

Sexual dimorphism in size and shape is a widely distributed trait in invertebrate and vertebrate species (Fairbairn 1997). In birds, dimorphism is usually moderate (Amadon 1959), but extreme cases, such as the extravagant plumage of male birds of paradise and pheasants can also occur. Variations among sexes are mostly related to the type of social mating system and respective roles in parental care (Owens & Hartley 1998, Dunn *et*

al. 2001), display agility (Szekely *et al.* 2007) and fecundity (Tubaro & Bertelli 2003). Sexual size dimorphism in birds is usually male-biased, with males being larger than females in total body size (Amadon 1959, Szekely *et al.* 2007). However, although much less common, the reverse (female-biased: females larger than males) also occurs in several taxa, such as raptors (Falconiformes), owls (Strigiformes), shorebirds (Charadriidae), seabirds (Procellariiformes), tinamous (Tinamiformes) and rails (Rallidae) (*e.g.*, Handford & Mares 1985, Jehl & Murray 1986, Fairbairn & Shine 1993, Szekely *et al.*

2007, Choi *et al.* 2013, Marcondes & Silveira 2015).

The Tinamous correspond to a ground dweller avian group endemic to the Neotropical region. Species belonging to this taxon are predominantly polygamous, displaying both the polyandrous and the polygynous system (Cabot 1992, Sick 1997). However, monogamy can arise in some species depending on constraints in the breeding season, such as the lack of capability of a male to protect the chicks against predators just by himself or due to nutritional stress (Handford & Mares 1985). Males, regardless of the mating system, usually perform parental care, while females delimit and defend the territory (Magalhães 1994). Most Tinamous are dimorphic, particularly species of the genera *Crypturellus* and *Tinamous* (Sick 1997, Tubaro & Bertelli 2003). Variation can be found in both morphology (Cabot 1992) and plumage (Tomotani & Silveira 2016). Due to the prevalence of a polygamous breeding system, there is a tendency for females to be slightly larger than males (Magalhães 1994, Bertelli *et al.* 2002, Tubaro & Bertelli 2003, Laverde-R & Cadena 2014).

The Yellow-legged Tinamou *Crypturellus noctivagus* (Wied, 1820) is a forest bird endemic to the Atlantic Forest in Brazil. Two subspecies are recognized for this taxon: *Crypturellus noctivagus zabele* (Spix, 1825), found in the northeast region of Brazil; and *Crypturellus noctivagus noctivagus* (Wied, 1820), which inhabits remnant lowland forests in southern Brazil (Sick 1997, Piacentini *et al.* 2015, Birdlife International 2016), reaching the state of Rio Grande do Sul (Corrêa *et al.* 2010, Corrêa & Petry 2018, Corrêa & Petry 2019). Due to consistent differences in diagnostic characters among subspecies, such as tarsus color and breast plumage of females and non-overlapping distribution, Tomotani & Silveira (2016) suggested that they should be recognized as distinct species. However, there is still a lack of molecular-based evidences in literature to support this distinction.

Females and males of the subspecies *C. n. noctivagus* are indistinguishable at sight, since there is no plumage dimorphism or conspicuous body size differences (Sick 1997, Tomotani & Silveira 2016). However, previous studies found morphological dimorphism in a few measures, such as wing and tarsus length (Tomotani & Silveira 2016) and bill length (Tubaro & Bertelli 2003). Nonetheless, these studies investigated intersexual variations either

based on a single measure (Tubaro & Bertelli 2003), or based on a few measures that were available in embalmed specimens (Cabot 1992, Tomotani & Silveira 2016). It has been suggested that the species has a polyandric breeding system (Cabot 1992).

Sampling wild birds, on the other hand, allows taking measures of total body mass and total length, thus improving intersexual comparisons (Berkunsky *et al.* 2009, Choi *et al.* 2013, Scherer *et al.* 2014). In this study, we evaluate morphometric variation and investigate intersexual dimorphism in wild *C. n. noctivagus* from a relictual population in the state of Rio Grande do Sul, Brazil, as well as indicate appropriate measures to distinguish males from females in the field.

MATERIAL AND METHODS

Study region and population

We collected morphometric data in a relictual population of *C. n. noctivagus*, which inhabits a 450 ha forest remnant completely isolated from other forest patches (30°05'35.3" S, 53°36'22.9" W) (Figure 1). The forest remnant is located between the municipalities of São Sepé and Formigueiro in the state of Rio Grande do Sul (Corrêa *et al.* 2010, Corrêa & Petry 2018, Corrêa & Petry 2019). The region is within the Pampa Biome, and is covered by Seasonal Deciduous Forest (IBGE 2004). The climate is temperate, with an average annual temperature of 19°C, and average annual precipitation of 1.750 mm (Alvares *et al.* 2013). At the national level, the Yellow-legged Tinamou is considered vulnerable to extinction (ICMBio 2016). In the state of Rio Grande do Sul, since only a small population is known (Corrêa *et al.* 2010, Corrêa & Petry 2018), the species is considered Critically Endangered (FBZ 2014). Luiz C. C. Corrêa (unpublished data) estimated the population at 18 adult individuals.

Sampling and morphometric measurements

Between October and December of 2015 and 2016, we captured *C. n. noctivagus* using bell-traps. Three bell-traps were adapted using a nylon cast net (detailed description in Corrêa & Petry 2018). From each captured bird we measured Bill height (BH), Bill length (BiL) (exposed culmen), Head length (HL), Tarsus length (TaL) and Middle toe length (MTL) to the nearest mm using a digital caliper; with an aluminum ruler we measured Tail

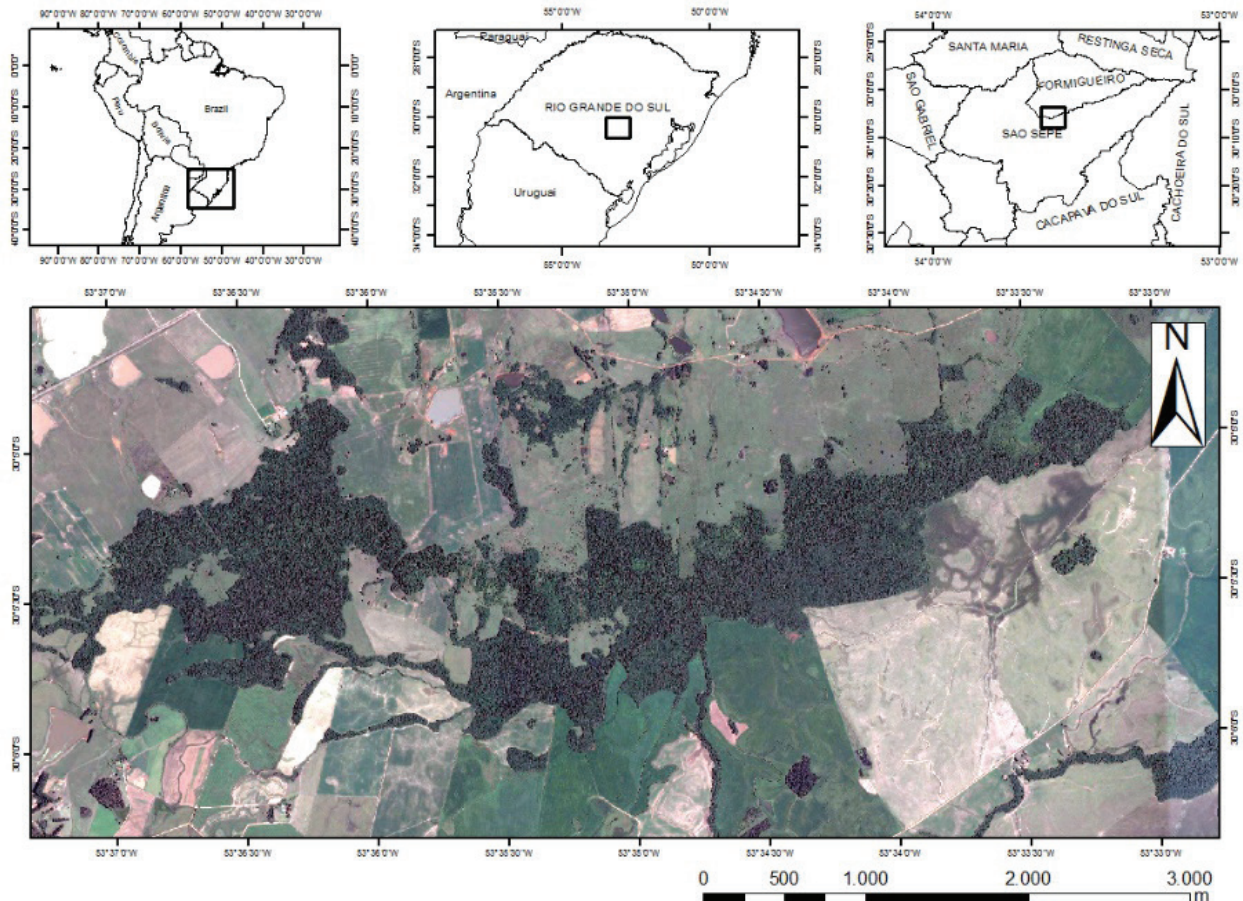


Figure 1. Study Area: Seasonal Deciduous Forest remnant in a Pampa Biome matrix in Rio Grande do Sul State, Brazil. Source: Correa & Petry (2018).

length (TL), Left wing length (WL) and Body length (BL). Total body mass (BM, in grams) was sampled using a 1,000 g Pesola® scale. All morphometric measurements were sampled by L. L. C. Corrêa, according to procedures detailed in Roos (2010). A blood sample was collected using a 1 ml disposable syringe. Blood samples were stored on FTA® paper (Whatman International) and sent to the specialized laboratory São Camilo Biotecnologia (www.scbiotec.com.br) for molecular sexing. Samples were analyzed through Polymerase Chain Reaction – PCR (Griffiths *et al.* 1998, Griffiths 2000). The capture technique was approved by the System of Authorization and Information of Biodiversity (SISBIO, Nº 47126-3) and the Ethics Committee on the Use of Animals in Research from the Universidade do Vale do Rio dos Sinos (CEUA, Nº PPECEUAI0.2014). Captured individuals were marked with numbered metal rings, provided by the National Center of Research and Conservation of Wild Birds (CEMAVE), and then were released in the same location where captured.

Sexual dimorphism analysis

Student's t-tests and Mann-Whitney's tests were used to test for morphometric and mass differences between sexed males and females, using significance level at $p < 0.05$. Normality in the distribution of data was tested through a Shapiro-Wilk test. In order to detect which measures were responsible for the distinction of sexes, we performed a Principal Component Analysis (PCA) with a correlation matrix. All statistical tests were performed using the software Past 3.14 (Hammer *et al.* 2001). We also calculated the percent difference of measurements that were statistically different between sexes using the following formula:

$$\frac{|m - f|}{\left[\frac{m + f}{2}\right]} \times 100$$

Where, m = mean value of a male measurement, and f = mean value of the female measurement. The percent difference equals the absolute value of

the change in value, divided by the average of the 2 numbers, all multiplied by 100 (Bennett *et al.* 2008).

RESULTS

In a total of 240 h of sampling effort, we captured 11 adult individuals of *C. n. noctivagus* (four females and seven males). We found significant differences between sexes in measures of bill length ($t = -4.06$, $p = 0.002$), tail length ($U = 0$, $p = 0.009$), middle toe length ($U = 0$, $p = 0.01$), and total body mass ($t = 3.65$, $p = 0.005$). The other morphometric measurements overlapped between sexes. On average, females were 9.9% heavier and had bills 11.8% longer than males. Females weighted at least 660 g, while the heaviest male weighted 640 g. The smallest bill length recorded in females was 27.8 mm, while the longest bill in males measured 27.4 mm (Table 1). However, males had, on average, middle toes and tails 8.7% and 19.5%, respectively, larger than females, the latter measure presenting the highest degree of dimorphism among all measures assessed.

The PCA resulted in two relevant components (eigenvalue > 2) that accounted for 63% of data variance (Figure 2). PC1 alone accounted for 40% of the variance and showed positive correlation to the female-biased measures: body mass and bill length; and negative correlation to the male-biased measures: middle toe and tail length. PC2 was positively correlated with total length and

negatively correlated with tarsus length (Table 2). This shows that longer bodies usually have smaller tarsus. The correlations between axes and characters were moderate (all coefficients ranged between 0.4 and 0.6).

DISCUSSION

In general, tinamous present female-biased sexual dimorphism (*e.g.*, Handford & Mares 1985, Cabot 1992, Tubaro & Bertelli 2003), especially regarding body mass and bill length (Tubaro & Bertelli 2003). In our study, females of *C. n. noctivagus* confirmed this pattern for some measures, differing significantly from males not only in total body mass and bill length, but also in middle toe length and tail length. However, since this study was conducted during the breeding period (*i.e.*, between the austral spring and summer), it is important to consider that females could be developing eggs. Thus, differences in total body mass between males and females may be attributed to extra egg weight in females (Szekely *et al.* 2007), as well as to energy reserves required for the production of those eggs (Mueller 1986). Therefore, the females body weight measurements we observed might be biased. Such difference between males and females body mass is expected to be lower during the non-breeding period, as reported for other taxa (Sick 1997, Hipkiss 2002). Notwithstanding Belton (1994) reported a

Table 1. Comparisons of the nine morphometric variables assessed between females and males of the Yellow-legged Tinamou (*Crypturellus noctivagus noctivagus*) inhabiting a relict forest in the Brazilian federal state of Rio Grande do Sul, Brazil, by means of Student's t-test (parametric data, t) and Mann-Whitney's test (non-parametric data, U). Average values and Standard Deviation (SD) of each variable, maximum and minimum values, mean difference between sexes in percentage (D%), t and U values and its respective significance values (p) are presented. Significant differences are depicted in bold ($p < 0.05$). Measurements are in mm except for body mass (g).

Variable*	Males (N = 7)		Females (N = 4)		D%	t or U	p
	Mean ± SD	Range	Mean ± SD	Range			
Bill height	6.04 ± 0.45	5.48 - 6.55	6.06 ± 0.38	5.77 - 6.72	-	0.07	0.938
Bill length	26.13 ± 0.89	25.34 - 27.4	29.40 ± 1.31	27.83 - 30.52	11.8	5.33	0.004
Middle toe length	30.80 ± 0.45	30.10 - 31.31	28.22 ± 1.64	25.86 - 29.50	8.7	0	0.01
Tarsus length	58.73 ± 2.90	56.44 - 63.61	58 ± 3.67	52.95 - 61.66	-	-0.37	0.717
Head length	66.72 ± 2.01	64.23 - 70.34	67.22 ± 2.64	64.49 - 70	-	0.36	0.729
Tail length	73.14 ± 4.86	70 - 80.50	60.12 ± 0.25	60.00 - 60.50	19.5	0	0.009
Wing length	173.14 ± 7.32	160.50 - 180	177.75 ± 4.84	170.50 - 180.50	-	7.5	0.222
Body length	334.64 ± 6.83	331 - 350	331.50 ± 8.35	320 - 340	-	13	0.923
Body mass	604.28 ± 33.60	540 - 640	667.50 ± 5	660 - 670	9.9	-3.65	0.005

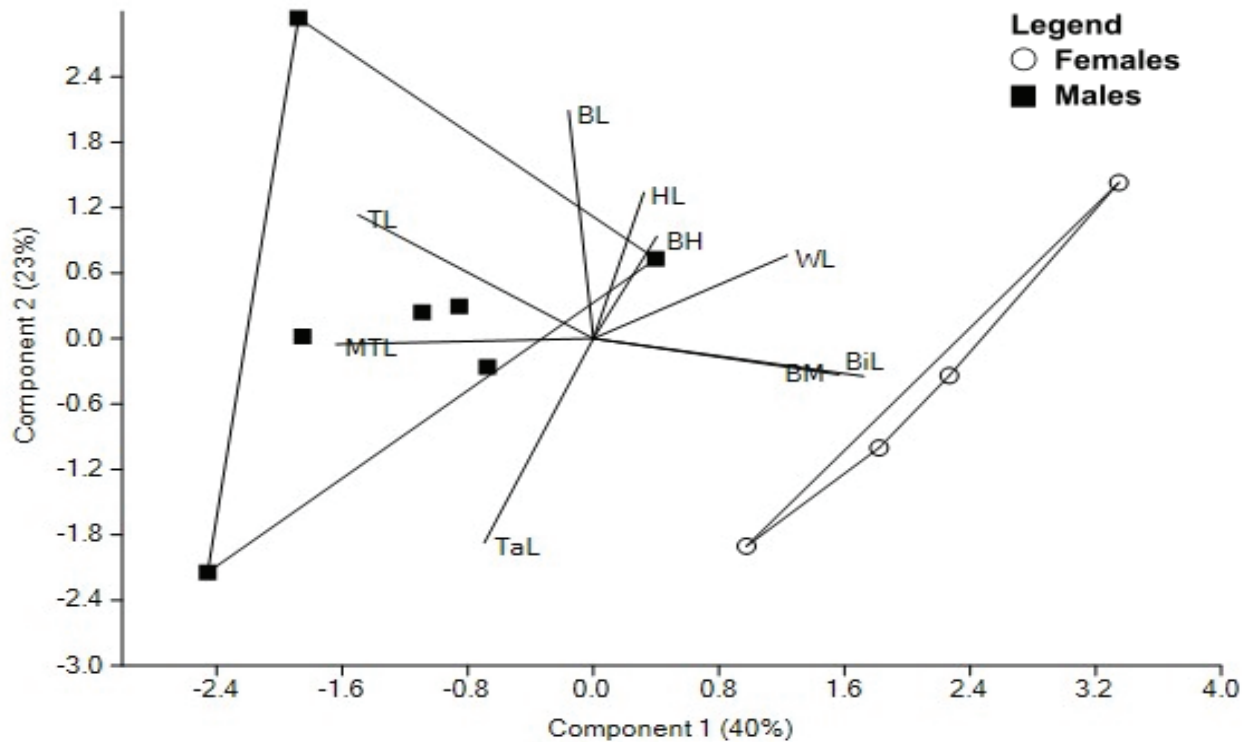


Figure 2. Principal Component Analysis results (Total eigenvalue 63%) expressing morphological differences between females and males of the Yellow-legged Tinamou (*Crypturellus noctivagus noctivagus*) in the Rio Grande do Sul state, Brazil. Morphometric variables used for the analysis: Bill height (BH), Bill length (BiL), Head length (HL), Tail length (TL), Wing length (WL), Tarsus length (TaL), Middle toe length (MTL), Body length (BL) and Body mass (BM).

historical record of a male Yellow-legged Tinamou weighing 800 g during the breeding season. This weight is 17% and 25% superior to those of females and males observed in this study, respectively.

On the other hand, tail length was the most representative measure in distinguishing males from females sampled in this study. Males had tails at least 9.5 mm longer than females. This male-biased sexual dimorphism may be related to the breeding behavior of the species, in which males are responsible for egg incubation and chick rearing (Sick 1997). Brennan (2009), while monitoring the incubating behavior of the Great Tinamou (*Tinamus major*), observed that under a potential predation pressure, males remained on the nest, vigorously raising tail feathers and the head to intimidate the predator. In this sense, a longer tail may benefit males against predation, although this hypothesis still needs to be tested. Tomotani & Silveira (2016) reported that *C. n. noctivagus* females had slightly shorter wings and shorter tarsus than males. We did not find differences regarding these measures in

Table 2. Correlation coefficients of morphometric variables of females and males of the Yellow-legged Tinamou (*Crypturellus noctivagus noctivagus*) inhabiting a relict forest in the Brazilian federal state of Rio Grande do Sul with PCA loadings (PC1 and PC2) obtained from the Principal Component Analysis. Significant correlations are in bold (> 0.4).

Variable	PC1	PC2
Bill height	0.1139	0.2636
Bill length	0.4844	-0.0972
Middle toe length	-0.4607	-0.0147
Tarsus length	-0.1952	-0.5267
Head length	0.0908	0.3768
Tail length	-0.4209	0.3186
Wing length	0.3475	0.2145
Body length	-0.0443	0.5876
Body mass	0.4404	-0.0933

our survey. This could be related to population differences or even the small sample size of this study.

On average, *C. n. noctivagus* of this relic population are smaller than those of other regions of Brazil, except for the tarsus length (Tomotani & Silveira 2016). Geographic variations in morphology and vocalization patterns are common in forest tinamous species (Laverde-R & Cadena 2014, Negret *et al.* 2015), especially among subspecies of the genera *Tinamus* and *Crypturellus* (Schelsky 2004). It is important to highlight that the specimens measured by Tomotani & Silveira (2016) were embalmed; therefore, direct comparisons to living individuals are not appropriate.

The morphometric analysis of the Yellow-legged Tinamou demonstrated the occurrence of predominantly male-biased sexual dimorphism in the assessed population. Bill length, tail length and middle toe length can be used to distinguish females from males in the field year-roundly, while total body mass might be used only during the breeding season. However, we highlight that our results are limited by our small sample size. Thus, we suggest the use of those measurements when the use of molecular sexing is not viable. However, we also highlight that one must take into account regional variations in morphometric measures when studying *C. n. noctivagus* populations from other regions of Brazil. Finally, further investigation and comparisons must be conducted with other *C. n. noctivagus* populations throughout its geographic distribution, in order to investigate if isolated populations of this subspecies tend to present some degree of morphometric differentiation.

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