ORIGINAL ARTICLE

SEXUAL DIMORPHISM IN A COOPERATIVE BREEDING THRAUPID (WHITE-BANDED TANAGER, *NEOTHRAUPIS FASCIATA*): THE RELEVANCE OF THE BLACK MASK

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ABSTRACT · Sexual dimorphism is common among birds, and is often caused by sexual selection. The detailed characterization of sexual dimorphism in a species is essential to propose and test hypotheses about the mechanisms responsible for its origin and maintenance. In this context, we examined the sexual size and plumage dimorphism in the White-banded Tanager (*Neothraupis fasciata*) based on wild individuals sexed using DNA-based techniques in seven different localities in the Brazilian savanna (Cerrado). Based on eight morphometric variables, we found that males were on average larger than females, and found statistically significant differences for some morphometric variables in three localities studied. Sexual dimorphism in plumage coloration was restricted to the size of the black mask, which extended to the lores in males and was limited to the auriculars in females. Thus, our results showed that the White-banded Tanager exhibits dimorphism in size and plumage, indicating that sexual dimorphism in this species seems more intricate than it has been described in literature. We also emphasize that the extension of the black mask is the best trait for sexing this species, and it can also be applied to immatures. Finally, we point out some aspects that should be investigated to better understand the evolution and maintenance of the sexual dimorphism in the White-banded Tanager.

RESUMO · **Dimorfismo sexual em um traupídeo com reprodução cooperativa (tiê-do-cerrado, *Neothraupis fasciata*): a relevância da máscara preta**
O dimorfismo sexual é comum entre as aves e é frequentemente causado pela seleção sexual. A caracterização do dimorfismo sexual de uma espécie é essencial para propor e testar hipóteses relacionadas aos mecanismos responsáveis pela sua origem e manutenção. Neste contexto, examinamos o dimorfismo sexual de tamanho e plumagem no tiê-do-cerrado (*Neothraupis fasciata*) em sete localidades diferentes distribuídas nas savanas brasileiras (Cerrado) com base em indivíduos sexados por técnica molecular. Considerando as oito variáveis morfométricas avaliadas, observamos que os machos foram em média maiores do que as fêmeas, e encontramos diferenças estatisticamente significativas para algumas variáveis morfométricas em três localidades amostadas. O dimorfismo sexual de plumagem nessa espécie está relacionado com o tamanho da máscara preta, que se estende até o loro nos machos e está limitada às auriculares nas fêmeas. Os resultados mostram que o tiê-do-cerrado apresenta tanto dimorfismo de tamanho quanto de plumagem, indicando que o dimorfismo sexual nessa espécie parece mais complexo do que tem sido descrito na literatura. Ressaltamos ainda que a extensão da máscara preta é a melhor característica para identificar o sexo dos indivíduos desta espécie, inclusive porque esta característica pode ser aplicada em imaturos. Finalmente, nós apontamos alguns aspectos que podem ser investigados para melhor entender a evolução e manutenção do dimorfismo sexual no tiê-do-cerrado.

KEY WORDS: Cerrado · Molecular sexing · Neotropical · Plumage dichromatism · Sexual size dimorphism · Thraupidae

INTRODUCTION

Sexual dimorphism in plumage and size is common among birds (Amadon 1959, Shine 1989, Owens & Hartley 1998, Temeles 2000, Dunn et al. 2001, Blondel et al. 2002). Several hypotheses have been proposed to explain the origin and maintenance of the dimorphism, and since Darwin (1871) differences between the sexes are mainly ascribed to sexual selection in its two forms: male-male competition (intrasexual selection) and female choice (intersexual selection). In this context, the social mating system, level of sperm competition, and pattern

of parental care can be considered important factors determining sexual differences in plumage color (Darwin 1871, Wallace 1889, Butcher & Rohwer 1989, Andersson & Iwasa 1996; Dunn et al. 2001, 2015). Although sexual selection is the most common explanation for sexual dichromatism in birds, ecological factors can also play a role (Badyaev & Hill 2003).

Males and females may attain different body sizes, resulting in sexual size dimorphism (Amadon 1959, Selander 1966). Males are usually larger than females in most bird species (Darwin 1871, Amadon 1959), although in some cases females are larger than males (reversed sexual size dimorphism), such as some hummingbirds and raptors (Amadon 1959, Jehl-Jr. 1970, Andersson & Norberg 1981, Temeles 2000, Stiles et al. 2005). Different hypotheses have been used to explain sexual size dimorphism, but the main hypotheses in passerine birds are related to sexual selection (Darwin 1871, Hedrick & Temeles 1989, Andersson & Iwasa 1996, Blondel et al. 2002) and ecological mechanisms, such as competition for resources, which may result in sex differences in habitat use and sexual niche segregation (Hedrick & Temeles 1989, Shine 1989, Blondel et al. 2002).

Males are more colorful and larger than females in most bird species, and the main explanation may be attributed to higher variance in reproductive success in males (e.g., Darwin 1871, Amadon 1959, Trivers 1972). While these are the most widespread patterns, there are some differences. In cooperative breeding species, intrasexual competition (for breeding sites, food, social status, and others) among females can be similar or greater than in males (Hauber & Lacey 2005, Rubenstein & Lovette 2009). In response to this, females may acquire traits that improve reproductive success directly through intrasexual competition, resulting in a more elaborate plumage in females and a reduced degree of sexual dimorphism (Hauber & Lacey 2005, Clutton-Brock 2009, Dale et al. 2015). For instance, the influence of reproductive skew and selection on females is well-known for African Starlings (Sturnidae), in which the degree of sexual dimorphism is greater in non-cooperative than in cooperative breeding species (Rubenstein & Lovette 2009). Thus, cooperative breeding species constitute important models to study the different forces shaping sexual dimorphism in birds.

In the order Passeriformes, sexual dimorphism has been reported for many different bird families (del Hoyo et al. 2016). In family Thraupidae (tanagers and allies) about half of the species exhibit a marked plumage dimorphism with males being more conspicuously colored than females (Sick 1984, Isler & Isler 1987, Ridgely & Tudor 1989, Burns 1998, Burns et al. 2014, del Hoyo et al. 2016). In contrast, sexual size dimorphism has not often been investigated for Thraupidae (Isler & Isler 1987), with few reports for two species of the genus *Tangara* (*T. preciosa*, *T. peruviana*) (Firme et al. 2014).

In this study, we examined sexual dimorphism in the White-banded Tanager (*Neothraupis fasciata*,

Thraupidae), a monotypic and cooperatively breeding species endemic to the tropical savannas of South America (Cracraft 1985, Pichorim et al. 2014). Based on the plumage color pattern, the White-banded Tanager is characterized as sexually monomorphic and drab by some authors (Allen 1981, Isler & Isler 1987, Burns 1998), although others have described this species as sexually dimorphic (Sick 1984, Alves 1988, Ridgely & Tudor 1989, Sigrist 2013, Duca & Marini 2014a, Hilty & de Juana 2016). In general, adult males of this species have gray crown, nape, and dorsal areas, a broad black mask extending from the forehead through the lores and eyes to the cheeks, and mostly black wing-coverts with white band on lesser coverts. Throat and lower underparts are white, while chest and upper breast are tinged light pearly gray. Adult females are described as similar to adult males, but duller or slightly duller (Sick 1984, Alves 1988, Ridgely & Tudor 1989, Sigrist 2013, Duca & Marini 2014a, Hilty & de Juana 2016). Immatures are much duller and browner than adults and neither present a black mask nor black wing-coverts contrasting with the white wing-bar (Sick 1984, Alves 1988, Ridgely & Tudor 1989, Sigrist 2013, Hilty & de Juana 2016).

The lack of consensus about the existence of dichromatism and the lack of comprehensive descriptions of the plumage of adult females in the White-banded Tanager make sex identification in the field challenging. Additionally, some features make this species an excellent model to explore different aspects that may influence sexual dimorphism in birds: the White-banded Tanager is a resident and facultative cooperative breeder that defends a territory year-round and displays hostile interactions between birds of different groups (Alves 1990, Alves & Cavalcanti 1990, Duca & Marini 2014b). Thus, this species permits to explore the influence of different aspects in the evolution of sexual dimorphism in birds, such as breeding and territorial systems.

Here we quantify sexual dimorphism in size and plumage coloration in the White-banded Tanager using eight morphometric variables and two aspects of plumage coloration for individuals sexed genetically using DNA-based techniques. Based on our results, we point out some lines of study that can improve the understanding of the processes involved in the evolution and maintenance of sexual dimorphism in this species.

METHODS

Data collection. White-banded Tanagers were attracted by playback calls and caught using mist nets in seven different localities in Brazil: Environmental Protection Area (APA) Gama and Cabeça de Veado, Águas Emendadas Ecological Station, Serra da Canastra National Park, Emas National Park, Chapada dos Guimarães National Park, Grande Sertão Veredas National Park, and Ponte Alta do Tocantins (Figure 1, Table 1). The Chico Mendes Institute for Conserva-
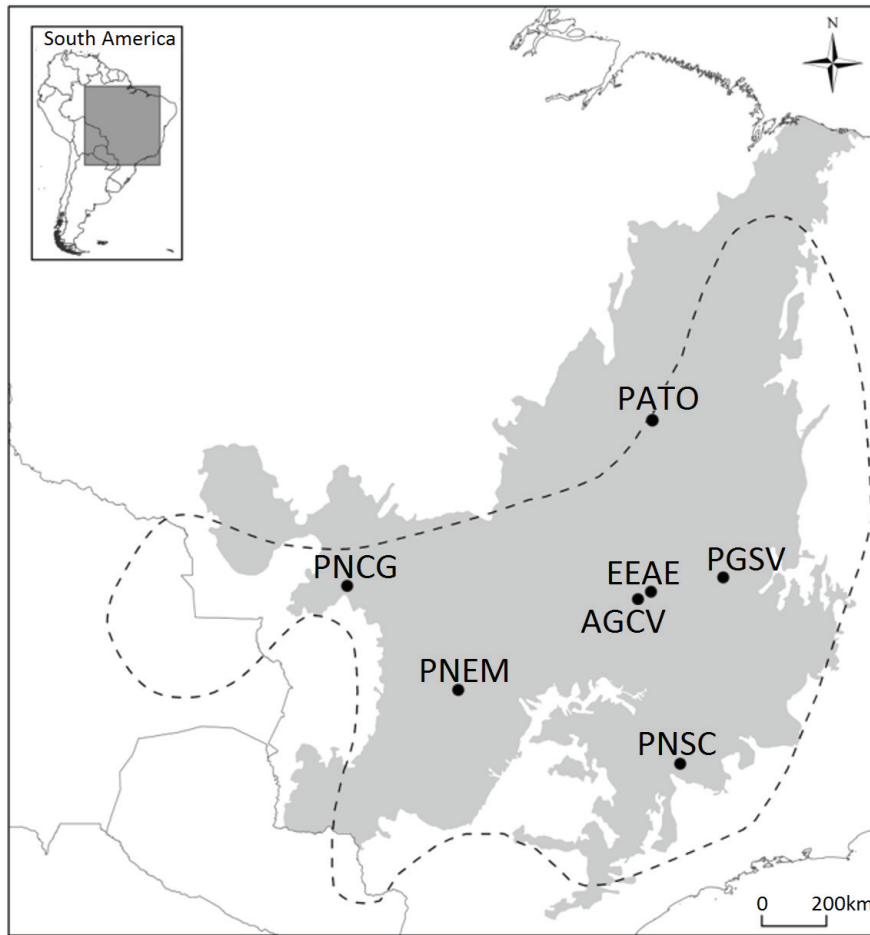
Figure 1. Geographical distribution (dashed line) of the White-banded Tanager (*Neothraupis fasciata*) in the Cerrado region of Brazil (gray shading) and sampling localities. Species distribution follows BirdLife International & NatureServe (2014), and Cerrado distribution is according to the Ministério do Meio Ambiente (2006). AGCV: APA Gama and Cabeça de Veado; EEAE: Águas Emendadas Ecological Station; PNSC: Serra da Canastra National Park; PNEM: Emas National Park; PNCG: Chapada dos Guimarães National Park; PGSV: Grande Sertão Veredas National Park; and PATO: Ponte Alta do Tocantins.

tion of Biodiversity provided the permits for the field-work (SISBIO n° 27682-1), and the methods were reviewed and authorized by the Comitê de Ética da Universidade de Brasília (UnBDoc n° 75111/2013). All captured birds were banded with numbered metal rings supplied by the National Center for Bird Conservation (CEMAVE) and afterwards released at the capture sites.

Field work was carried out from March 2012 to May 2014. Given that the reproductive period of this tanager starts at the end of dry season and lasts during part of the rainy season (Duca & Marini 2011), we considered birds caught between September and January as sampled during breeding season and those caught between February and August as sampled during non-breeding season. We examined skull ossification patterns as criteria for aging birds (CEMAVE 1994, Roos 2010). To our knowledge, no previous study described skull ossification timing for tanagers. Considering that skull ossification in Passeriformes is a process that takes four to twelve months depending on the species (Ralph et al. 1993), we assumed that

the complete skull ossification in White-banded Tanagers will be reached in this time frame. Thus, White-banded Tanagers with incomplete skull ossification represent hatching-year individuals, hereafter immatures, while those with complete ossification were classified as adults.

A set of eight morphometric variables were taken from all individuals as described by Baldwin et al. (1931): total length with feathers (TLF, from the tip of the bill to the end of the tail), bill length (BL, straight line from the distal end of the nostril to the tip of the maxilla), bill width at base (BW, measured at the base of the exposed culmen), bill height at base (BH, from the base of exposed culmen to the lower edge of the ramus of the mandible below), tarsus length (TA, from the joint between tibia and metatarsus to the internal base of hallux), tail length (TL, from the distal base of uropygial gland to tip of central rectrices), wing length (WL, from the carpal joint to the tip of the longest primary), and body mass (BM). Measurements were done with a Mitutoyo® digital caliper (± 0.05 mm), except for total length with feathers, which

Table 1. Sampling localities (Cerrado, Brazil) and sample size of measured White-banded Tanagers (*Neothraupis fasciata*) used in the size and plumage sexual dimorphism analyses. Sample localities with asterisk mark were not considered for analyses of sexual size dimorphism. F: females; M: males.

Sample locality	Coordinates	Size		Plumage	
		F	M	F	M
APA Gama and Cabeça de Veado	15°56'S, 47°51'W	8	9	8	8
Águas Emendadas Ecological Station*	15°33'S, 47°36'W	0	0	4	1
Serra da Canastra National Park*	20°20'S, 46°38'W	4	3	4	2
Emas National Park	17°55'S, 52°58'W	5	6	2	1
Chapada dos Guimarães National Park*	15°24'S, 55°49'W	2	1	5	5
Grande Sertão Veredas National Park	15°18'S, 45°37'W	9	23	9	22
Ponte Alta do Tocantins	10°45'S, 47°30'W	9	8	4	5

was done with a ruler (± 0.5 mm), and body mass which was taken with a Pesola® dynamometer (precision 0.5 g). All measurements were made by the same observer (CALR).

Plumage characterization of immature and adults was based on general body coloration and black mask extension. Individuals were assigned to one of three categories of general body plumage coloration: dull brownish: yellowish gray underparts, brown back, lacks evident mask, and does not exhibit black wing-coverts contrasting with the white wing-bar (Figure 2A); matte grayish: differs from previous category mainly by the presence of black wing-coverts contrasting with the white wing-bar, more distinctive mask, back and breast more grayish, but with scattered brown feathers (Figure 2B); and bright grayish: gray back and breast, white belly and throat, black wing-coverts contrasting with the white wing-bar, and evident black mask (Figure 2C). We also evaluated the extension of the black mask considering auriculars (ear-coverts) and lores (side of the head between the front of the eyes and the base of the bill). Three categories were used: no black mask (Figure 2A), black mask extending to the auriculars (Figure 2B), and black mask extending to the lores (Figure 2C). Although we are aware of the subjectivity involved to score plumage coloration, these categories were chosen based on a pilot study conducted in the field because they allow the unequivocal categorization of each individual. All individuals were categorized by the same observer (CAL).

Molecular sexing. For each individual, a blood sample of 50–100 µL was obtained from the brachial wing vein. The blood was drawn from the puncture site with a sterilized microcapillary tube, and the blood samples were stored in absolute ethanol at -20 °C. Total DNA was isolated using proteinase K digestion followed by phenol-chloroform extraction according to standard procedure (Bruford et al. 1992). We used the CHD-based sex identification, a sexing technique

based on Polymerase Chain Reaction (PCR) using the P2/P8 primers as described by Griffiths et al. (1998). The sex was identified based on the analysis of the number of fragments (one for males and two for females) observed in 3% agarose gels (Griffiths et al. 1998). Positive and negative controls were used at each PCR procedure.

Statistical analysis. Sexual size dimorphism was evaluated in the four sample localities where ten or more individuals were sampled: APA Gama and Cabeça de Veado, Emas National Park, Grande Sertão Veredas National Park, and Ponte Alta do Tocantins (see Table 1). To evaluate the influence of the sex, sample locality, age structure, and whether sexual differences vary with locality (interaction term between sex and sample locality) on each morphometric variable we used a generalized linear model (GLM) with a Gaussian distribution (link = identity, test = F). We checked for under or over-dispersion in the data comparing the ratio of residual deviance to degrees of freedom with the given dispersion parameter for each model. All GLM analysis were carried out in the R environment (R Core Team 2014). Considering that sample locality showed significant influence on some morphometric variables (see Results), we tested for differences between the sexes of adult individuals in each sample locality using Student's t-tests. We checked for normality and homogeneity of variance using Shapiro-Wilk and Anderson-Darling tests, respectively. These revealed that logarithmic transformations were necessary in six comparisons (Table S1).

To evaluate the level of sexual size dimorphism in each locality, we first conducted a Principal Component Analysis (PCA) to reduce the dimensionality of the morphometric data of adults using the program PAST 3.11 (Hammer et al. 2001). Given that the variables included had two different units (mm and g), we chose to use a correlation matrix (which normalizes the variables dividing them by their standard deviations, Hammer et al. (2015)) to run the PCA. For two
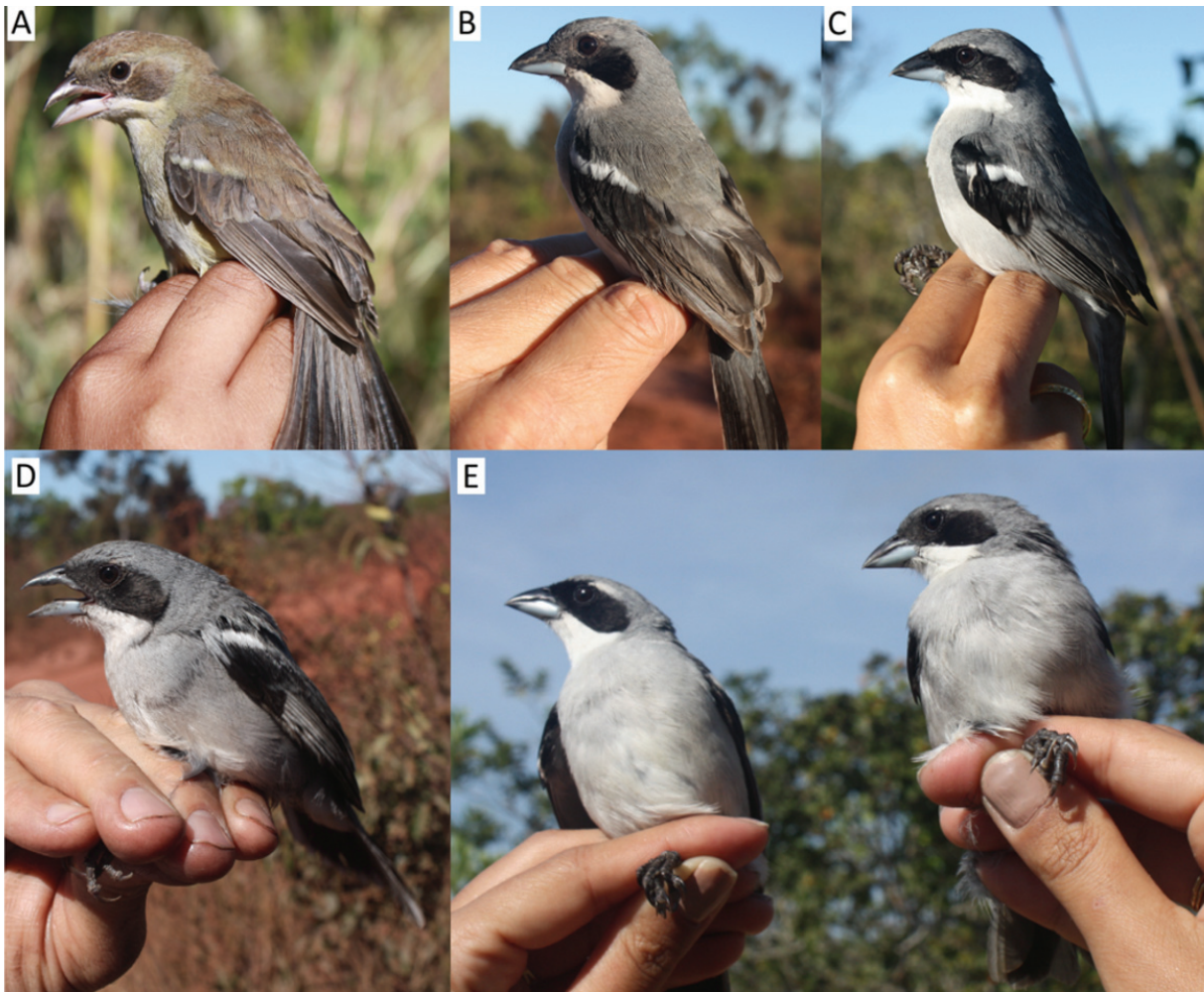
Figure 2. Plumage dimorphism in the White-banded Tanager (*Neothraupis fasciata*), captured in the Cerrado region of Brazil. A) Immature with drab brownish plumage and no mask; B) adult females with matte grayish plumage and black mask at the auriculars; C) adult male individual with bright grayish plumage and black mask extending to the lores; D) adult female with bright grayish plumage, black mask at the auriculars and dark gray coloration at the lores; and E) adult bright gray male (left) and bright gray female (right) exposed to the same light conditions.

females that escaped before we could complete the measurements we used the locality-specific mean value of total length with feathers for the PCA (one from APA Gama and Cabeça de Veado and one from Ponte Alta do Tocantins). At Emas National Park, two morphological variables (bill width and bill height) were not measured. To test for differences between the sexes in each locality, we used non-parametric multivariate one-way Analysis of Similarities (ANO-SIM) based on Gower distances (Clarke 1993), using the program PAST 3.11. The R-statistic value describes the extent of similarity between each pair of groups in the ANOSIM analysis, with values close to 1 indicating that the two groups do not overlap and close to 0 indicating that there is no difference between the groups. Significance was assessed based on 10,000 permutations.

We also performed a Discriminant Analysis to evaluate the ability of morphometric data to correct classify adult individuals into each sex using a set of morphometric variables statistically different on at least one of the four localities based on *t*-tests. To predict group membership, we used the minimal Mahalanobis distance from each point to each group's multivariate mean. The leave-one-out cross validation using jackknifing procedure was used to check the efficiency of discriminant analysis in correctly assigning individuals to a group (male or female). The Discriminant Analysis was carried out using the program PAST 3.11.

RESULTS

Based on molecular sexing, the morphometric analysis included 8 immatures (5 males, 3 females) and 69 adults (41 males, 28 females) (Tables 1, S1). Plumage color was scored for 80 individuals: 9 immatures (5 males, 4 females) and 71 adults (39 males, 32 females) (Tables 1, 2).

Sexual size dimorphism. Across all sites, we found significant differences between sexes on three mor-

Table 2. Plumage characterization of the White-banded Tanager in relation to the capture period, age and sex. F: females; M: males.

Plumage category	Breeding				Non-breeding			
	Immature		Adult		Immature		Adult	
	M	F	M	F	M	F	M	F
Dull brownish								
No black mask	0	0	0	2	1	4	0	5
Black mask to the auriculars	0	0	0	0	0	0	0	0
Black mask to the lores	0	0	0	0	3	0	3	0
Matte gray								
No black mask	0	0	0	0	0	0	0	0
Black mask to the auriculars	0	0	0	3	0	0	0	17
Black mask to the lores	0	0	0	0	0	0	1	0
Bright gray								
No black mask	0	0	0	0	0	0	0	0
Black mask to the auriculars	0	0	0	0	0	0	0	5
Black mask to the lores	0	0	3	0	1	0	32	0
Total	0	0	3	5	5	4	36	27

phometric variables: total length with feathers, tail length, and wing length (Table 3). We observed a significant effect of age on total length with feathers and bill length, in which adults were larger than immatures. The test of sample locality was statistically significant for all variables, except for total length with feathers and bill height. The interaction between sex and locality was significant for wing length and body mass (Table 3), indicating that sexual differences in these morphometric variables vary with sample locality. Males were on average larger than females for most morphometric variables in the four localities (Figure 3, Table S1). Four variables, total length with feathers (t-test = -3.612, P = 0.003), tail length (t-test = -2.446, P = 0.027), wing length (t-test = -4.796, P < 0.001), and body mass (t-test = -3.340, P = 0.004), showed significant differences between the sexes in Ponte Alta do Tocantins (Figure 3, Table S1). Wing length was statistically significant in Grande Sertão Veredas National Park (t-test = -3.785, P < 0.001) and Emas National Park (t-test = -3.528, P < 0.007), and tarsus length was statistically significant in the latter (t-test = -2.335, P < 0.047) (Figure 3, Table S1).

The first two principal components summarized most of the variance in all sample localities. Specifically, components PC1 and PC2 explained almost 50% of the variance in Grande Sertão Veredas National Park, 60% of the variance in both APA Gama and Cabeça de Veado and Ponte Alta do Tocantins, and 70% of the variance in Emas National Park (Figure 4). We observed high to medium levels of morphometric overlap between the sexes in APA Gama and Cabeça de Veado and Grande Sertão Veredas National Park, and low levels of morphometric overlap in Ponte Alta

do Tocantins and Emas National Park (Figure 4). The ANOSIM results showed significant size differences between sexes only in Ponte Alta do Tocantins (R = 0.382, P = 0.002; Table S1). Discriminant Analysis was performed for each locality using total length with feathers, tarsus length, tail length, wing length, and body mass. The cross-validation showed that 82.4% of the individuals were correctly assigned to their sex in Ponte Alta do Tocantins, 80% in Emas National Park, 77.8% in Grande Sertão Veredas National Park, and 66.7% in APA Gama and Cabeça de Veado (Figure S1, Table S2).

Sexual plumage dimorphism. During the breeding seasons, we sampled a total of 8 adult individuals. All males (N = 3) caught during this period showed bright gray plumage with a black mask to the lores, while females showed two plumage patterns: dull brownish plumage with no black mask (N = 2) and matte gray plumage with a black mask extending to the auriculars (N = 3) (Table 2).

During the non-breeding seasons, we caught immatures (N = 9) and adults (N = 63) of both sexes. One immature male showed dull brownish plumage with no black mask, 3 showed dull brownish plumage with a black mask to the lores, and 1 showed bright gray plumage with a black mask to the lores (Table 2). All immature females (N = 4) showed dull brownish plumage with no black mask. Among adult males caught in non-breeding period, most of the individuals (N = 32) showed bright gray plumage with a black mask to the lores, while 3 showed dull brownish plumage with a black mask to the lores, and only 1 showed matte gray plumage with a black mask to the lores (Table 2). Among the adult females caught dur-

Table 3. Influence of the sex, age structure, sample locality, and the interaction between sex and sample locality (Sex : Locality) on each morphometric variable of the White-banded Tanager. Degrees of freedom (df), deviance, *F*-value, and Pr (> *F*) results of generalized linear model (GLM) are given for each morphometric variable. Asterisks indicate statistical significance ($p \leq 0.05$).

| Morphometric variable	Factor	df	Deviance	*F* - value	Pr (> *F*)
Total length with feathers	Sex	01	399.86	8.82	0.004*
	Age	01	232.67	5.13	0.027*
	Locality	03	346.31	2.54	0.063
	Sex : Locality	03	94.34	0.69	0.559
	Residual	66	2993.50		
Bill length	Sex	01	0.50	1.51	0.225
	Age	01	2.08	6.28	0.015*
	Locality	02	8.29	12.53	< 0.001*
	Sex : Locality	02	0.35	0.52	0.595
	Residual	59	19.52		
Bill width	Sex	01	0.25	2.59	0.113
	Age	01	0.00	0.00	0.951
	Locality	02	1.68	8.82	< 0.001*
	Sex : Locality	02	0.01	0.07	0.935
	Residual	59	5.60		
Bill height	Sex	01	0.03	0.34	0.559
	Age	01	0.23	2.41	0.126
	Locality	02	0.48	2.54	0.088
	Sex : Locality	02	0.20	1.04	0.360
	Residual	59	5.60		
Tarsus length	Sex	01	0.07	0.04	0.839
	Age	01	1.16	0.70	0.407
	Locality	03	19.86	3.97	0.011*
	Sex : Locality	03	4.39	0.88	0.457
	Residual	68	113.48		
Tail length	Sex	01	81.48	9.20	0.003*
	Age	01	27.97	3.16	0.080
	Locality	03	259.31	9.76	< 0.001*
	Sex : Locality	03	50.66	1.91	0.136
	Residual	67	593.46		
Wing length	Sex	01	227.79	57.03	< 0.001*
	Age	01	11.51	2.88	0.094
	Locality	03	279.26	23.30	< 0.001*
	Sex : Locality	03	58.89	4.91	0.004*
	Residual	68	271.61		
Body mass	Sex	01	0.324	0.11	0.739
	Age	01	0.335	0.12	0.734
	Locality	03	59.026	6.81	< 0.001*
	Sex : Locality	03	32.775	3.78	0.014*
	Residual	68	196.35		
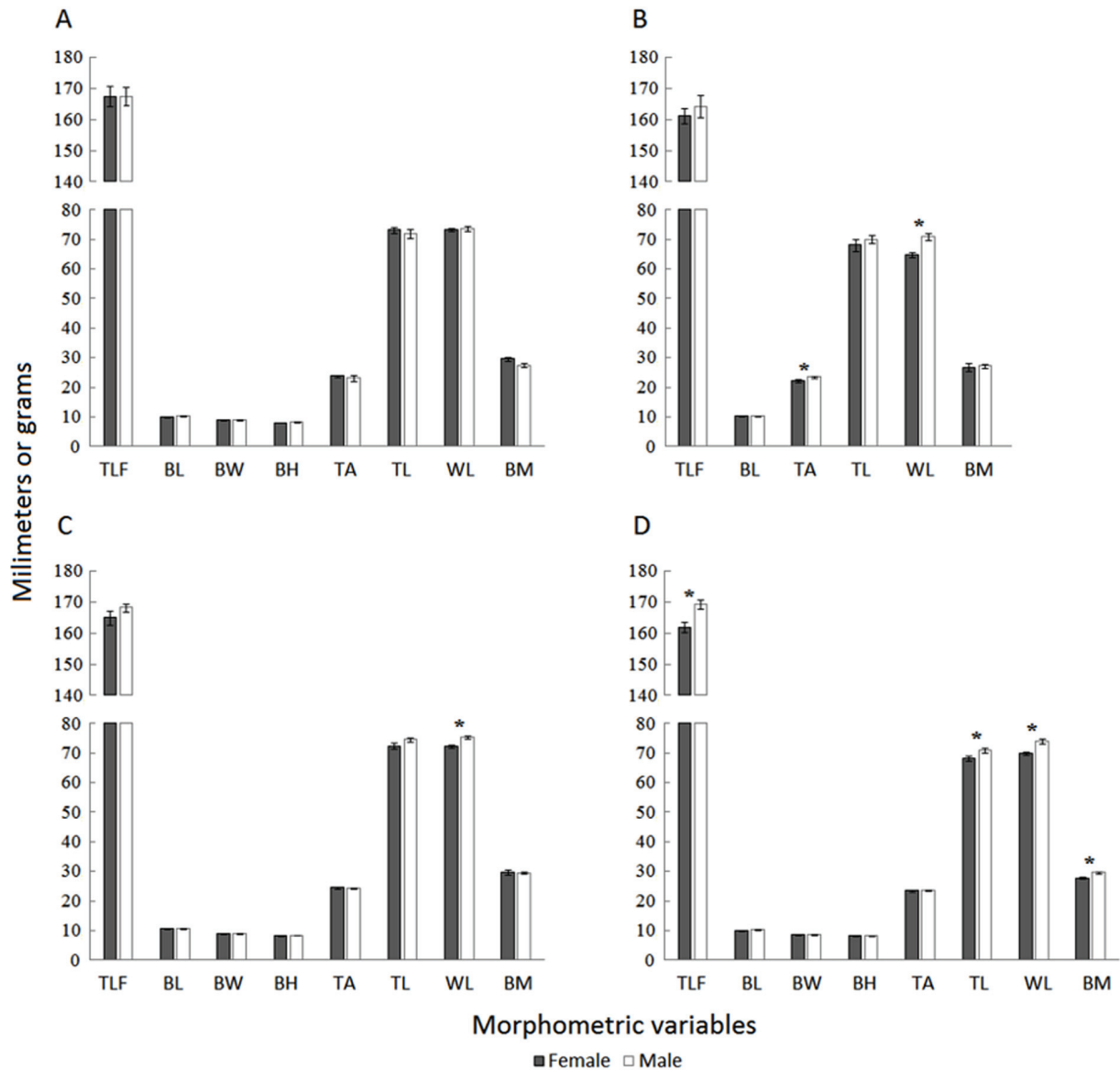
Figure 3. Mean and standard error of eight morphometric traits for female and male White-banded Tanagers (*Neothraupis fasciata*) captured in four different localities in the Brazilian Cerrado: A) APA Gama and Cabeça de Veado; B) Emas National Park; C) Grande Sertão Veredas National Park; and D) Ponte Alta do Tocantins. Statistically significant differences between males and females are indicated by an asterisk. Morphometric variables are: TLF: total length with feathers; BL: bill length; BW: bill width; BH: bill height; TA: tarsus length, TL: tail length, WL: wing length; and BM: body mass. All units are in mm, except for BM in g.

ing this period, we registered 5 with dull brownish plumage and no black mask, 17 with matte gray plumage with a black mask in the auriculars, and 5 with bright gray plumage and a black mask in the auriculars (Table 2).

We did not notice obvious differences in plumage patterns across sampling localities. Among adult females, we observed the matte gray plumage in all sample localities, while the bright gray pattern was observed in Grande Sertão Veredas (N = 2), APA Gama and Cabeça de Veado (N = 2), and Ponte Alta do Tocantins (N = 1). For adult males, the bright gray plumage was widespread across the sample localities.

DISCUSSION

The White-banded Tanager is described by some authors as monomorphic (Allen 1981, Isler & Isler 1987, Burns 1998), and even the studies that described this species as dimorphic reported only slight plumage dimorphism, generally denoted as females duller or browner than males (Sick 1984, Alves 1988, Ridgely & Tudor 1989, Sigrist 2013, Duca & Marini 2014a, Hilty & de Juana 2016). Our results indicated that sexual dimorphism exists in the White-banded Tanager in both size and plumage. In relation to the latter, we found that both males and females showed matte gray and bright gray plumages, and
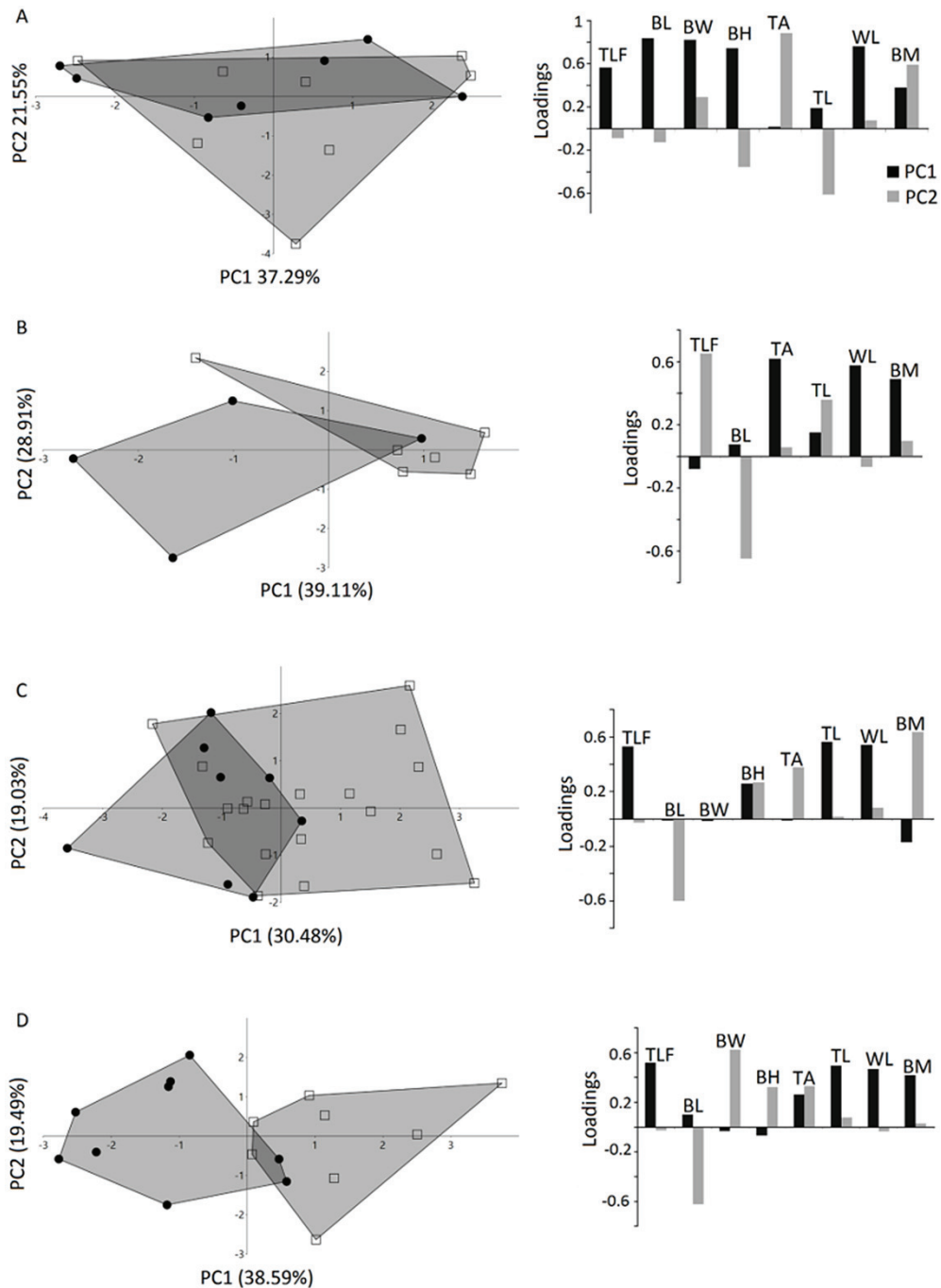
Figure 4. Principal components (PC) 1 and 2 of morphometric variables of male and female White-banded Tanagers (*Neothraupis fasciata*) captured in four different localities in the Brazilian Cerrado: A) APA Gama and Cabeça de Veado; B) Emas National Park; C) Grande Sertão Veredas National Park; and D) Ponte Alta do Tocantins. Males are represented by squares and females by filled circles. The proportion of the variance (%) explained by each component is given in parenthesis. Loading scores for PC1 and PC2 are given separately for each sampling locality. TLF: total length with feathers; BL: bill length, BW: bill width; BH: bill height, TA: tarsus length, TL: tail length, WL: wing length; and BM: body mass.

that the main difference between the sexes is the extension of the black mask.

Size dimorphism. Although sexual size dimorphism is not common among tanagers (Isler & Isler 1987), we found sexual size dimorphism in the White-banded Tanager. In general, males were larger than females,

and we found statistically significant differences for some morphometric variables in three localities. Allen (1981) had already pointed out the possibility of size dimorphism in this species considering birds sampled in Mato Grosso, western Brazil. The size differences found between sexes were consistent with previous studies in many bird species that

describe the male as larger than the female (Darwin 1871, Székely et al. 2007, Lislevand et al. 2009). In particular, a similar pattern was observed in two species of the genus *Tangara*, in which males have longer wings (*T. peruviana*, *T. preciosa*) and tail (*T. preciosa*) (Firme et al. 2014). The observed pattern of larger males may be related with advantages of dominance in contests of strength and endurance (Darwin 1871, Andersson & Iwasa 1996), and longer wings can improve flight ability (Rayner 1988, Møller & Nielsen 1997, Moreno-Rueda 2003), conferring advantages during territorial defense and competition.

The White-banded Tanager showed spatial variation in the degree of size dimorphism. Population differences in body size and size dimorphism may arise from a balance between different selection pressures, like sex-specific reproductive roles and responses to environmental factors (Blondel et al. 2002, Blanckenhorn et al. 2006). For instance, spatial variation in sexual size dimorphism was also observed in the House Finch (*Carpodacus mexicanus*) due to differences in local selection pressures for sexual dimorphism (Badyaev & Hill 2000, Badyaev et al. 2000). In this context, the geographic variation in the degree of sexual size dimorphism found for the White-banded Tanager reinforces the importance of considering the geographical scale when sexing birds based on size dimorphism. However, we stress that more samples are needed to fully determine spatial variation in sexual size dimorphism in this species.

Plumage dimorphism. According to the literature, immatures of the White-banded Tanager are much duller (or browner) than adults and lack both mask and black-and-white wing pattern (Sick 1984, Alves 1988, Ridgely & Tudor 1989, Sigrist 2013, Hilty & de Juana 2016). More specifically, according to Alves (1988) this plumage pattern occurs in individuals less than six months old; after that, individuals gradually acquire the dark mask and black-and-white wing pattern. This plumage succession seems to occur in juveniles between one month and one year of age (Duca & Marini 2014a, Hilty & de Juana 2016). We found that the majority of immatures showed dull brownish plumage, in agreement with these previous descriptions. However, some individuals classified as adults (complete ossification) also showed dull brownish plumage. Among these individuals, only males showed black mask. They were caught in May (non-breeding period), and probably were individuals born during the previous breeding season (*ca.* six months old). In contrast, all adult females with dull brownish plumage had no black mask and were caught during breeding and non-breeding periods, likely representing individuals between six months and one year of age. Thus, these findings suggest that the black mask appears earlier in males than females, which disagrees with Alves (1988) who suggests that both sexes begin to acquire the mask at around six months of age.

We also found 1 adult male and 20 adult females with matte gray plumage. This plumage pattern has been found in individuals described as sub-adults in the literature (Alves 1988, Duca & Marini 2014a, Hilty & de Juana 2016) and seems to occur in individuals between 1 and 2 years of age (Duca & Marini 2014a, Hilty & de Juana 2016). All these individuals also showed black mask, but in males it extended to the lores while in females it was restricted to the auriculars. Considering that we caught similar numbers of adult males and females, this result may be interpreted as males retaining the sub-adult plumage during a shorter period than females and acquiring the full adult plumage (bright gray pattern) faster than females.

On the other hand, most adult males (90%) and 5 adult females (16%) showed bright gray pattern. Furthermore, in bright gray individuals both sexes presented the black mask (see Figure 2E), but restricted to the auriculars in females and extending to the lores in males. All of these females showed a dark gray coloration at the lores. These findings contrast with some previous descriptions where the plumage of adult females of the White-banded Tanager is duller or browner than in adult males (e.g., Sick 1984, Alves 1988, Duca & Marini 2014a), with brown (Alves 1988) or dark gray mask (Duca & Marini 2014a). Thus, both general body plumage coloration (matte grayish or bright grayish patterns) and mask color do not seem the best way to classify individuals into each sex, as females may also exhibit bright gray plumage and black mask. Instead, the extension of the black mask seems to be a more reliable trait to determine sex in this species, since a black mask extending to the lores was observed in all males with full adult plumage and in some immature males with dull brownish plumage, but was never observed in females. Hence, we recommend using the extension of the black mask instead the color intensity as a main diagnostic character for sexing White-banded Tanagers.

In relation to plumage dimorphism, Rubenstein & Lovette (2009) showed that the degree of dimorphism is lower in cooperative than in non-cooperative breeding species, in response to increased intrasexual competition among females. Moreover, changes in social status, particularly in the breeding status, may be signaled by changes in ornaments (e.g., Karubian 2008, Solís et al. 2008). Since the White-banded Tanager is a cooperative breeding species (Alves 1988, Manica & Marini 2012), we hypothesize that the increased levels of intrasexual selection in females might be related to female plumage ornamentation in this species. In this context, some field observations during our study may be seen as indicative of possible links between plumage variation and social status of adult females. We used playback calls to attract birds mist-nets, as both males and females defend their territory (Duca & Marini 2014b) and respond agonistically to playback calls. At each mist netting site, we usually captured from two to six individuals, which seems to correspond to a single territorial group,

since group size in this species ranges from two to eight individuals per territory (Alves 1990, Duca & Marini 2014b). We found that group sex ratio (males : females) ranged from 0.25 to 0.83 per group (N = 11 groups). In each group, we never caught more than one bright-gray female, and in some cases (N = 3) we caught both bright and matte gray females in the same group. In contrast, we often (N = 9) captured more than one bright gray male per group. Based on these observations, we suspect that bright gray females were the breeders in each group while matte gray females were non-reproductive and act as helpers in White-banded Tanager groups.

Future perspectives. Taking into account that the characterization of sexual dimorphism of a species is essential to propose and test hypotheses about the mechanisms responsible for its origin and maintenance, our findings added to the previous descriptions provide a starting point for future work to understand the evolution of sexual dimorphism in this species. Geographical variation in selection pressures in this species have yet to be investigated to better understand which principles or mechanisms may be driving the spatial variation in sexual size dimorphism. Considering that large size in males may evolve due to the advantages of large size in intrasexual competition for breeding territories and mates, and sex differences in the provision of parental care (Selander 1972, Clutton-Brock et al. 1977, Shine 1989, Blondel et al. 2002), we can hypothesize that the larger body size in males of the White-banded Tanager confers advantages during territorial defense and /or male-male competition for mating. Furthermore, considering the geographical variation of body size, larger body size in White-banded Tanager males could be particularly important in localities with a high density of territories.

CONCLUSION

Our findings showed that the White-banded Tanager shows some degree of dimorphism in size and slight dimorphism in plumage coloration. Males were on average larger than females, and we found statistically significant differences for some morphometric variables in three localities studied. We suggest that the extension of the black mask (to the lores in males and restricted to the auricular in females) is the best trait to sex this species. Finally, the intricate variation in plumage colorations observed in the White-banded Tanager provides an interesting study system to understand the processes involved in the evolution of sexual dimorphism in cooperative-breeding bird species.

ACKNOWLEDGMENTS

REFERENCES

Allen, JA (1891) On a collection of birds from Chapada, Matto Grosso, Brazil, made by Mr. Herbert H. Smith. Part I - Oscines. *Bulletin of the American Museum of Natural History* 3: 337–381.

Alves, MAS & RB Cavalcanti (1990). Ninhos, ovos e crescimento de filhotes de *Neothraupis fasciata*. *Ararajuba* 1: 91–94.

Alves, MAS (1988) Organização social e biologia reprodutiva de *Neothraupis fasciata* (Passeriformes: Thraupidae). Tese de Doutorado, Univ. de Brasília, Brasília, Brasil.

Alves, MAS (1990) Social system and helping behavior in the White-banded Tanager (*Neothraupis fasciata*). *The Condor* 92: 470–474.

Amadon, D (1959) The significance of sexual differences in size among birds. *Proceedings of the American Philosophical Society* 103: 531–536.

Andersson, M & RÅ Norberg (1981) Evolution of reversed sexual size dimorphism and role partitioning among predatory birds, with a size scaling of flight performance. *The Biological Journal of the Linnean Society* 15: 105–130.

Andersson, M & Y Iwasa (1996) Sexual selection. *Trends in Ecology & Evolution* 11: 53–58.

Badyaev, AV & GE Hill (2000) The evolution of sexual dimorphism in the House Finch. I. Population divergence in morphological covariance structure. *Evolution* 54: 784–1794.

Badyaev, AV & GE Hill (2003) Avian sexual dichromatism in relation to phylogeny and ecology. *Annual Review of Ecology and Systematics* 34: 27–49.

Badyaev, AV, GE Hill, AM Stoehr, PM Nolan & DJ McGraw (2000) The evolution of sexual size dimorphism in the House Finch II. Population divergence in relation to local selection. *Evolution* 54: 2134–2144.

Baldwin, SP, HC Oberholser & LG Worley (1931) Measurements of birds. *Scientific publications of the Cleveland Museum of Natural History* 2: 1–165.

BirdLife International & NatureServe (2014) *Neothraupis fasciata*. Bird species distribution maps of the world. 2012. *In* IUCN Red list of threatened species. Version 2015-2. Available at http://www.iucnredlist.org [Accessed 20 August 2015].

Blanckenhorn, WU, RC Stillwell, KA Young, CW Fox & KG Ashton (2006) When Rensch meets Bergmann: does sexual size dimorphism change systematically with latitude? *Evolution* 60: 2004–2011.

Blondel, J, P Perret, MC Anstett & C Thébaud (2002) Evolution of sexual size dimorphism in birds: test of hypotheses using Blue Tits in contrasted Mediterranean habitats. *Journal of Evolutionary Biology* 15: 440–450.

Bruford, MW, O Hanotte, JFY Brookfield & T Burke (1992) Single-locus and multilocus DNA fingerprinting. Pp 225–269 in Hoelzel, A. R. (ed). *Molecular genetic analysis of populations*. IRL Press, Oxford, UK.

Burns, KJ (1998) A phylogenetic perspective on the evolution of sexual dichromatism in tanagers (Thraupidae): the role of females versus male plumage. *Evolution* 52: 1219–1224.

Burns, KJ, JS Allison, OT Pascal, NA Mason, FK Barker, J Klicka, SM Lanyon & IJ Lovette (2014) Phylogenetics and diversification of tanagers (Passeriformes: Thraupidae), the largest radiation of Neotropical songbirds. *Molecular Phylogenetics and Evolution* 75: 41–77.

Butcher, GS & S Rohwer (1989) The evolution of conspicuous and distinctive coloration for communication in birds. *Current Ornithology* 6: 51–108.

CEMAVE (1994) *Manual de anilhamento de aves silvestres*. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, Brasília, Brazil.

Clarke, KR (1993) Non-parametric multivariate analysis of changes in community structure. *Australian Journal of Ecology* 18: 117–143.

Clutton-Brock, TH (2009) Sexual selection in females. *Animal Behaviour* 77: 3–11.

Clutton-Brock, TH, PH Harvey & B Rudder (1977) Sexual dimorphism, socionomic sex ratio and body weight in primates. *Nature* 269: 797–800.

Cracraft, J (1985) Historical biogeography and patterns of differentiation within the South American avifauna: areas of endemism. *Ornithological Monographs* 36: 49–84.

Dale, J, CJ Dey, K Delhey, B Kempenaers & M Valcu (2015) The effects of life history and sexual selection on male and female plumage colouration. *Nature* 527: 367–370.

Darwin, C (1871) *The descent of the man, and selection in relation to sex*. John Murray, London, UK.

del Hoyo, J, A Elliott, J Sargatal, DA Christie & E de Juana (2016) *Handbook of the birds of the world alive*. Lynx Edicions, Barcelona, Spain. Available at http://www.hbw.com [Accessed 11 January 2016].

Duca, CG & MA Marini (2011) Variation in breeding of the Shrike-like Tanager in central Brazil. *The Wilson Journal of Ornithology* 123: 259–265.

Duca, CG & MA Marini (2014a) High survival and low fecundity of a Neotropical savanna tanager. *Emu* 114: 121–128.

Duca, CG & MA Marini (2014b) Territorial system and adult dispersal in a cooperative-breeding tanager. *The Auk* 131: 32–40.

Dunn, PO, JK Armenta & LA Whittingham (2015) Natural and sexual selection act on different axes of variation in avian plumage color. *Science Advances* 1: e1400155.

Dunn, PO, LA Whittingham, & TE Pitcher (2001) Mating systems, sperm competition, and the evolution of sexual dimorphism in birds. *Evolution* 55: 161–175.

Firme, DH, CP Assis, L Seixas, IG Alpino & MA Raposo (2014) Taxonomia alfa de *Tangara peruviana* (Desmarest, 1805) e *Tangara preciosa* (Cabanis, 1851) (Aves, Passeriformes, Emberizidae). *Arquivos do Museu Nacional* 65: 39–46.

Griffiths, R, MC Double, K Orr & RJG Dawson (1998) A DNA test to sex most birds. *Molecular Ecology* 7: 1071–1075.

Hammer, Ø, DAT Harper & PD Ryan (2001) PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4: 4–9.

Hammer, Ø, DAT Harper & PD Ryan (2015) *PAST: Reference Manual. Version 3.14.* Natural History Museum, Univ. of Oslo, Oslo, Norway. Available at http://folk.uio.no/ohammer/past/past3manual.pdf [Accessed 10 March 2016].

Hauber, ME & EA Lacey (2005) Bateman's principle in cooperatively breeding vertebrates: the effects of non-breeding alloparents on variability in female and male reproductive success. *Integrative and Comparative Biology* 45: 903–914.

Hedrick, AV & EJ Temeles (1989) The evolution of sexual dimorphism in animals: hypotheses and tests. *Trends in Ecology & Evolution* 4: 136–138.

Hilty, S & E de Juana (2016) White-banded Tanager (*Neothraupis fasciata*). *In* del Hoyo, J, A Elliott, J Sargatal, DA Christie & E de Juana (eds). *Handbook of the birds of the world alive*. Lynx Edicions, Barcelona, Spain. Available at http://www.hbw.com/node/61574 [Accessed 11 January 2016].

Isler, ML & PR Isler (1987) *The tanagers*. Smithsonian Institution Press, Washington, DC, USA.

Jehl-Jr, RJ (1970) Sexual selection for size differences in two species of sandpipers. *Evolution* 24: 311–319.

Karubian, J (2008) Changes in breeding status are associated with rapid bill darkening in male Red-backed Fairy-wrens *Malurus melanocephalus*. *Journal of Avian Biology* 39: 81–8.6

Lislevand, T, J Figuerola & T Székely (2009) Evolution of sexual size dimorphism in grouse and allies (Aves: Phasianidae) in relation to mating competition, fecundity demands and resource division. *Journal of Evolutionary Biology* 22: 1895–1905.

Manica, LT & MÂ Marini (2012) Helpers at the nests of White-banded Tanager (*Neothraupis fasciata*) benefit male breeders but do not increase reproductive success. *Journal of Ornithology* 153: 149–159.

Ministério do Meio Ambiente (2006). *Mapa de cobertura vegetal do bioma Cerrado*. Embrapa Cerrados, Univ. Federal de Uberlândia & Universidade Federal de Goiás (eds). Available at http://mapas.mma.gov.br/mapas/aplic/probio/datadownload.htm?/cerrado [Accessed 15 August 2015].

Møller, AP & JT Nielsen (1997) Differential predation cost of a secondary sexual character: Sparrowhawk predation on Barn Swallows. *Animal Behaviour* 54: 1545–1551.

Moreno-Rueda, G (2003) The capacity to escape from predators in *Passer domesticus*: an experimental study. *Journal für Ornithologie* 144: 438–444.

Owens, IPF & IR Hartley (1998) Sexual dimorphism in birds: why are there so many different forms of dimorphism? *Proceedings of the Royal Society B: Biological Sciences* 265: 397–407.

Pichorim, M, M Silva, BRA França, TM Oliveira-Júnior & MC Rodrigues (2014) A Cerrado bird community in the northernmost portion of northeastern Brazil - recommendations for conservation. *Revista Brasileira de Ornitologia* 22: 347–362.

R Core Team (2014) *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org.

Ralph, CJ, PP Geupel, TE Martin & DF DeSante (1993) *Handbook of field methods for monitoring landbirds*. Pacific Sothwest Research Station, Albany, California, USA.

Rayner, J MV (1988) Form and function in avian flight. *Current Ornithology* 5: 1–66.

Ridgely, RS & G Tudor (1989) *The birds of South America. The oscine passerines*. Univ. of Texas Press, Austin, Texas, USA.

Roos, AL (2010) Capturando aves. Pp 79–106 in Matter, SV, FC Straube, I Accordi, V Piacentin & FJ Cândido-Jr (eds). *Ornitologia e conservação: ciência aplicada, técnicas de pesquisa e levantamento*. Technical Books, Rio de Janeiro, Brazil.

Rubenstein, DR & IJ Lovette (2009) Reproductive skew and selection on female ornamentation in social species. *Nature* 462: 786–789.

Selander, RK (1966) Sexual dimorphism and differential niche utilization in birds. *The Condor* 68: 113–151.

Selander, RK (1972) Sexual selection and dimorphism in birds. Pp 180–230 *in* Campbell, BG (ed). *Sexual selection and the descent of man 1871-1971.* Aldine Publishing Company, Chicago, Illinois, USA.

Shine, R (1989) Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *The Quarterly Review of Biology* 64: 419–461.

Sick, H (1984) *Ornitologia brasileira, uma introdução*. Editora Univ. de Brasília, Brasília, Brazil.

Sigrist, T (2013) *Guia de campo Avis Brasilis - avifauna brasileira*. 3rd ed. Avis Brasilis, São Paulo, Brazil.

Solís, E, JM Avilés, C La Cruz, J Valencia & G Sorci (2008) Winter male plumage coloration correlates with breeding status in a cooperative breeding species. *Behavioral Ecology* 19: 391–397.

Stiles, FG, DL Altshuler & R Dudley (2005) Wing morphology and flight behavior of some North American hummingbirds. *The Auk* 122: 872–886.

Székely, T, T Lislevand & J Figuerola (2007) Sexual size dimorphism in birds. Pp 27–37 *in* Fairbairn, DJ, WU Blanckehorn & T Szekely (eds). *Sex, size and gender roles*. Oxford Univ. Press, Oxford, UK.

Temeles, EJ (2000) Evidence for ecological causation of sexual dimorphism in a hummingbird. *Science* 289: 441–443.

Trivers, RL (1972) Parental investment and sexual selection. Pp 136–179 *in* Campbell, BG (ed). *Sexual selection and the descent of man 1871-1971.* Aldine Publishing Company, Chicago, Illinois, USA.

Wallace, AR (1889) *Darwinism - an exposition of the theory of natural selection with some of its applications*. Macmillan, London, UK. Available at http://biodiversitylibrary.org.