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ARTÍCULO ORIGINAL

Fluctuating asymmetry of three bat species in extensive livestock systems from Córdoba department, Colombia

Asimetría fluctuante de tres especies de murciélagos en sistemas de ganadería extensiva del departamento de Córdoba, Colombia

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KEYWORDS:

Wing morphology; *Artibeus planirostris; Artibeus lituratus; Carollia perspicillata;* tropical dry-forest; sexual dimorphism.

ABSTRACT

The aim of this study was to analyze the degree of fluctuating asymmetry in wing traits and digits of three common bat species (*Artibeus lituratus*, *Artibeus planirostis* and *Carollia perspicillata*) in extensive livestock systems from Córdoba department, Colombia. To do this, specimens from the Mammalian Collection at the Museo Javeriano de Historia Natural of Pontificia Universidad Javeriana (Bogotá, Colombia) were analyzed. All specimens belonged to fragments of tropical dry-forest immersed in extensive livestock systems of Córdoba department. To parse out fluctuating asymmetry, 11 wing traits were used. To assess the existence of asymmetry, nonparametric U test was applied. Finally, to evaluate the existence of significant differences among digits, nonparametric one-way analysis of variance were carried out. In total, 114 specimens were analyzed (*A. planirostris*=40, *A. lituratus*=33, *C. perspicillata*=41). The results showed no statistical differences ($p > 0.05$) in most of wing traits. Little variation in wing traits was due to its relevance on flight performance and the high tolerance to perturbation of these bat species. "Differential-mortality" hypothesis and "Big Mother" hypothesis are discussed. Results from this work suggest that livestock systems do not severely affect these species. Further studies should consider the type of management to know which one provides better conditions for bats.

PALABRAS CLAVE:

Morfología alar; *Artibeus planirostris; Artibeus lituratus; Carollia perspicillata;* bosque seco-tropical; dimorfismo sexual.

RESUMEN

El objetivo de este estudio fue analizar el grado de asimetría fluctuante en los rasgos alares y en los dígitos de tres especies comunes de murciélagos (*Artibeus lituratus*, *Artibeus planirostis* y *Carollia perspicillata*) en sistemas ganaderos extensivos del departamento de Córdoba, Colombia. Para esto, se analizaron ejemplares de la Colección de Mamíferos del Museo Javeriano de Historia Natural de la Pontificia Universidad Javeriana (Bogotá, Colombia). La asimetría fluctuante se evaluó usando 11 rasgos alares. Para evaluar la existencia de asimetría, se usaron contrastes no paramétricos. Finalmente, se realizaron análisis no paramétricos de varianza con el fin de evaluar si existían diferencias entre los dígitos. En total, 114 ejemplares fueron analizados (*A. planirostris*= 40, *A. lituratus*=33, *C. perspicillata*= 41). Los resultados no mostraron diferencias estadísticas ($p>0.05$) para la mayoría de rasgos alares. La poca variación alar se debió a su importancia en el vuelo y a la alta tolerancia de estas especies a la perturbación. Las hipótesis de "Mortalidad diferencial" y "Gran madre" son discutidas. Los resultados de este trabajo sugieren que los sistemas ganaderos no afectan severamente a estas especies. Futuros estudios deberían considerar el tipo de manejo para conocer cuál proporciona mejores condiciones para los murciélagos.

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INTRODUCTION

Nowadays, several anthropogenic pressures are jeopardizing animal populations (CEBALLOS *et al.,* 2017). Environmental changes caused by landscape transformation may disrupt homeostasis affecting individual fitness of vertebrate populations (CODA *et al.,* 2016; LAZIC *et al.,* 2013). Particularly, livestock systems have been increasing in the last few decades and is likely to increase in the future, threatening biodiversity in developing countries (THORNTON *et al.,* 2009). However, studies assessing the stress of animal species in livestock systems are scarce, especially in some Neotropical countries such as Colombia.

One of the approaches to evaluate the degree of stress of animal species is the Fluctuating asymmetry (FA), which is defined as the random deviations from perfect symmetry (e.g. radial, dihedral, rotational, bilateral) in populations of organisms (GRAHAM *et al.,* 2010). FA can serve as a useful indicator of developmental instability, reflecting environmental and genetic stress of organisms (LEARY and ALLENDORF, 1989). For instance, species under environmental stress such as decreased food availability (WAUTERS, 1996), agrochemicals (CODA *et al.,* 2016), and pathogens (ST- AMOUR *et al.,* 2010), have higher levels of asymmetry. In addition, the decline of heterozygosity has an inverse relation to the levels of FA; in this sense, as heterozygosity decreases, FA increases (MESSIER and MITTON, 1996). Accordingly, FA seems to provide an adequate measure of stress since it has been useful in the evaluation of anthropogenic pressures on different animal groups such as insects (FLOATE and FOX, 2000), birds (LENS et al.,1999), amphibians (ST-AMOUR et al., 2010), reptiles (LAZIC et al., 2013) and mammals (CODA et al., 2016; WAUTERS, 1996).

Within mammals, bats are the unique taxonomic group with the ability for powered flight (CAMARGO and OLIVEIRA, 2012). To achieve this, bats have an integrated and multivariate fight apparatus: the wings (NORBERG and RAYNER, 1987). This structure is a special modification of forelimbs, characterized by a membrane of skin (dactylopatagia), stretched between elongated digits (digits II-V) (Wang *et al.,* 2010). Although some studies have analyzed morphological differences in bat wings according to ecological characteristics (MARINELLO and BERNARD, 2014; NORBERG and RAYNER, 1987), differences in both intraspecific level and wing regions need more attention (STEVENS *et al*., 2013; CAMARGO and OLIVEIRA, 2012; FIGUEIREDO *et al*., 2015). For example, variation between sexes in terms of wing traits (i.e. sexual dimorphism) and variation among digits are poorly known.

The Caribbean region of Colombia is one of the most biodiverse in the country (RANGEL-CH, 2015). A priority ecosystem for conservation in this region is the tropical dry-forest, due to the historical loss of 92% of its original coverage (GÓMEZ and MORENO, 2016). Moreover, according to IUCN´s Red List of Ecosystems criteria, it has been classified as critically endangered ecosystem (CR) (ETTER *et al.,* 2015), and one of the mainly factors of its historic transformation is the expansion of livestock systems (PIZANO and GARCIA, 2014).

Particularly, in Cordoba department different studies have been done with bats in fragments of tropical dry-forest immersed in matrices of extensive livestock systems, analyzing ectoparasites load (CALONGE, 2012), reproductive phenology (VELA-VARGAS, 2013), and the structure of bat assemblages (BALLESTEROS-CORREA, 2015). In these studies, *Artibeus lituratus, Artibeus planirostris*, and *Carollia perspicillata* are the species with the highest abundances in the assemblages. Furthermore, these species are involved in key roles such as seed dispersers of pioneering plants (e.g. *Ficus, Cecropia, Solanum*), enhancing the reforestation of disturbed areas in tropical dry-forest (RÍOS-BLANCO, 2010). Nevertheless, it is unknown the degree of environmental stress that these bat species are facing in extensive livestock systems, which are quite common in the Caribbean region. The use of FA can be an important tool for this purpose, especially in key structures like bat wings. Therefore, the present study aimed to assess the degree of FA in wing traits (i.e. metacarpals and phalanges) and the digits (i.e. III digit, IV digit, and V digit) of *A. lituratus*, *A. planirostis* and *C. perspicillata*, using specimens collected in fragments of tropical dry-forest immersed in extensive livestock systems of Cordoba department. Sexual dimorphism is also evaluated in terms of wing traits for each one of the bat species.

MATERIALS AND METHODS.

Study site. Bat specimens from the Mammalian Collection at the Museo Javeriano de Historia Natural Lorenzo Uribe Uribe S. J. (MPUJ-MAMM) of Pontificia Universidad Javeriana (Bogotá, Colombia) were analyzed. All specimens belonged to fragments of tropical dry-forest immersed in extensive livestock systems of Cordoba department. Livestock systems had conventional and silvopastoral management. The localities of the specimens were: Betanci-Guacamayas (08° 11´05,3" N 075° 31´49,2" W), Las Palmeras (08° 30´37,1" N 076° 06´12,9" W), Chimborazo (08° 44´32,4" 076° 19´23,4"W), San Lorenzo (08° 53´20,0" N 076° 18´42,6" W), and El Refugio (08° 32´44,3" N 075° 20´39,9"W). These specimens are the result of undergrad (RÍOS-BLANCO, 2010; OLAYA-RODRÍGUEZ, 2009), master (CALONGE, 2012; VELA-VARGAS, 2013) and doctoral thesis (BALLESTEROS-CORREA, 2015).

Specimen selection. Exclusion criteria were used in order to avoid noise factors such as developmental stage, misleading taxonomic identification, and specimens badly preserved. In doing so, bat vouchers selected were only adults, according to data of the tags and field notebooks of each specimen. The identification of each specimen was corroborated with the keys of GARDNER (2007). All specimens in bad state of preservation (i.e. broken wings) were excluded from data analysis.

Wing traits. To analyze FA, 11 wing traits were measured from both right and left sides (Figure 1). The measurements were taken with a digital caliper (Mitutoyo Calibrador Vernier Mod Cd6 -csx 150 Mm) with an accuracy of 0.01 mm and were done by the same researcher (Dennis Castillo-Figueroa).

Data analysis. After testing the assumptions of normality (Shapiro-Wilk test) and homoscedasticity (Levene test), wing traits data were nonparametric. Hence, to assess the existence of fluctuating asymmetry between the right and left sides of bat wings, nonparametric U test of Wilcoxon-Mann-

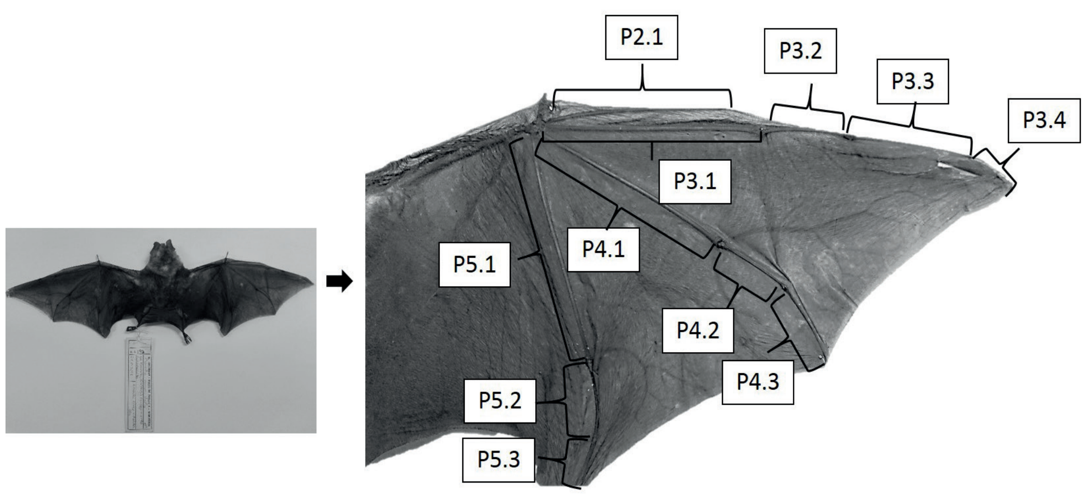

Figure 1. Wing traits used for fluctuating asymmetry analysis. **P2.1** Second digit, metacarpal. **P3.1** Third digit, metacarpal. **P3.2** Third digit, first phalanx. **P3.3** Third digit, second phalanx. **P3.4** Third digit, third phalanx **P4.1** Fourth digit, metacarpal **P4.2** Fourth digit, first phalanx **P4.3** Fourth digit, second phalanx **P5.1** Fifth digit, metacarpal **P5.2** Fifth digit, first phalanx **P5.3** Fifth digit, second phalanx.

Whitney was applied. The null hypothesis for this test establishes no significant differences between the means of wing sides for each trait (FIGUEIREDO *et al.,* 2015). Statistical means and standard deviations are presented for right and left sides of each wing trait.

To evaluate the existence of significant differences among digits of bat wings, the traits were grouped into the following categories: III digit (P3.1+P3.2+P3.3+P3.4), IV digit (P4.1+P4.2+P4.3) and V digit (P5.1+P5.2+P5.3). These categories present metacarpals and phalanges that support the main structure of dactylopatagium, which is important for flight performance (NORBERG and RAYNER, 1987). Therefore, a general analysis of bat wing asymmetry was conducted, through the three principal digits that support this wing membrane. The asymmetry value was calculated according to PALMER (1994):

$$FA = |R - L|$$

In the formula, FA is the fluctuating asymmetry value, R is the measurement of the right side of the wing, as well as L represents the left side of the wing. Nonparametric one-way analysis of variance was done (Kruskal-Wallis), taking the digit as the factor and the III, IV and V digits as the levels of the factor. In all the tests, $p<0.05$ defined the level of statistically significant difference. All the analysis presented here, were done separately for males and females in the statistical software Rwizard 2.1. Because of some works have shown sexual dimorphism in Neotropical bats based on wing traits (CAMARGO and OLIVEIRA, 2012; STEVENS *et al*., 2013), multivariate analysis of variance (MANOVA) was conducted in InfoStat 1.0. Due to wing traits were nonparametric, transformations of all variables to normal scores (z values) were done in order to use MANOVA test.

RESULTS

In total, 114 bat specimens were analyzed: 33 of *A. lituratus* (15 males, 18 females), 40 of *A. planirostris* (15 males, 25 females), and 41 of *C. perspicillata* (21 males, 20 females). The nonparametric Wilcoxon-Mann-Whitney test only showed the existence of significant differences between wing sides of the first phalanx of the third digit in males (P3.2) and the third phalanx of the third digit (P3.4) in females of *C. perspicillata* ($p<0.05$, Table 1). For both *Artibeus* species there were no statistical differences in any wing trait (Table 2 and 3).

Table 1. Wing traits of *Carollia perspicillata* specimens in extensive livestock systems (Córdoba, Colombia).

Wing traits	Males				Females			
	Right	Left	U	p	Right	Left	U	p
P2.1	31.58± 1.65	31.42 ± 1.65	217	0.949	30.41 ± 2.09	29.94 ± 1.74	229	0.445
P3.1	36.67± 1.02	36.51 ± 1.18	241	0.614	36.22 ± 1.76	36.35 ± 1.72	178	0.560
P3.2*	15.65± 0.66	15.14 ± 0.69	306	**0.032**	15.41 ± 0.95	15.21 ± 0.67	242	0.261
P3.3	20.51± 0.78	20.18 ± 0.86	266	0.252	20.37 ± 1.03	19.90 ± 1.13	250	0.176
P3.4*	12.61± 1.12	12.46 ± 0.75	253	0.420	12.76 ± 0.66	11.47 ± 2.07	317	**0.001**
P4.1	35.05± 1.27	35.09 ± 1.39	206	0.727	35.03 ± 1.82	35.08 ± 1.94	188	0.766
P4.2	12.30± 0.45	12.12 ± 0.55	263	0.285	12.42 ± 0.59	12.15 ± 0.68	234	0.357
P4.3	13.35± 0.95	13.15 ± 0.78	238	0.668	13.42 ± 0.90	13.22 ± 1.19	208	0.828
P5.1	37.42 ±1.31	37.68 ± 1.31	198	0.583	37.07 ± 2.21	37.49 ± 2.15	168	0.398
P5.2	11.17± 0.62	10.91 ± 0.60	274	0.182	11.16 ± 0.64	10.86 ± 0.58	253	0.155
P5.3	12.30± 1.17	12.20 ± 0.91	235	0.715	12.11 ± 1.02	12.05 ±1.09	210	0.786

Statistical mean ± standard deviation for Right and Left wings presented in millimeters (mm). U= Non-parametric U test. p= Significance level of Wilcoxon-Mann-Whitney test. * Statistical differences.

Table 2. Wing traits of *Artibeus lituratus* specimens in extensive livestock systems (Córdoba, Colombia).

Wing traits	Males				Females			
	Right	**Left**	**U**	**p**	**Right**	**Left**	**U**	**p**
P2.1	57.11 ± 3.29	56.83 ± 3.26	117	0.870	61.15 ± 2.04	59.81 ± 2.11	216	0.087
P3.1	61.72 ± 3.58	61.55 ± 3.59	112	1	64.10 ± 1.42	63.41 ± 1.38	208	0.151
P3.2	21.61 ± 1.71	21.28 ±1.34	136	0.329	22.76 ± 0.98	22.17 ± 1.27	201	0.226
P3.3	34.65 ± 2.75	34.62 ± 2.71	116	0.900	36.13 ± 1.21	35.87 ± 1.35	182	0.526
P3.4	22.70 ± 2.43	22.70 ± 2.00	121	0.724	24.21 ± 1.89	23.97 ± 1.73	183	0.516
P4.1	60.74 ± 3.50	61.05 ± 3.56	102	0.682	62.54 ± 1.52	62.84 ± 1.33	147	0.657
P4.2	18.29 ± 1.32	17.88 ± 1.42	139	0.280	19.12 ± 0.90	18.68 ± 0.70	203	0.194
P4.3	23.79 ± 2.30	23.52 ± 2.72	112	1	25.49 ± 1.28	25.01 ± 1.24	204	0.189
P5.1	63.65 ± 3.85	63.54 ± 3.96	115	0.917	64.93 ± 1.68	64.93 ± 1.78	174	0.716
P5.2	13.98 ± 1.09	13.73 ± 1.07	132	0.436	14.77 ± 0.88	14.50 ± 0.80	200	0.229
P5.3	20.07 ± 1.62	19.34 ± 1.52	142	0.229	20.22 ± 2.27	20.19 ± 1.29	167	0.874

Statistical mean ± standard deviation for Right and Left wings presented in millimeters (mm). U= Non-parametric U test. p= Significance level of Wilcoxon- Mann-Whitney test.

Table 3. Wing traits of *Artibeus planirostris* specimens in extensive livestock systems (Córdoba, Colombia).

Wing traits	Males				Females			
	Right	**Left**	**U**	**p**	**Right**	**Left**	**U**	**p**
P2.1	50.63 ± 2.45	51.14 ± 2.97	101	0.663	51.63 ± 3.10	50.80 ± 3.33	300	0.815
P3.1	54.11 ± 2.08	53.89 ± 2.24	117	0.851	55.03 ± 3.21	54.65 ± 3.17	0.37	0.375
P3.2	17.84 ± 0.98	17.40 ± 0.80	138	0.290	18.32 ± 1.25	17.93 ± 1.34	333	0.698
P3.3	28.75 ± 1.24	28.83 ± 2.31	128	0.520	29.48 ± 2.24	28.99 ± 2.59	328	0.771
P3.4	19.99 ± 1.48	20.24 ± 1.37	101	0.652	20.55 ± 2.02	20.70 ± 1.96	302	0.853
P4.1	52.84 ± 2.25	53.36 ± 2.14	98	0.566	53.77 ± 3.19	54.08 ± 3.06	288	0.641
P4.2	15.59 ± 0.81	15.20 ± 0.70	142	0.221	15.86 ± 0.81	15.50 ± 0.94	397	0.101
P4.3	22.06 ± 0.80	21.87 ± 0.80	131	0.442	21.97 ± 1.90	21.84 ± 1.87	331	0.719
P5.1	55.29 ± 2.23	55.31 ± 2.19	116	0.884	56.20 ± 3.27	56.41 ± 3.12	300	0.815
P5.2	11.85 ± 0.50	11.49 ± 0.65	155	0.077	12.00 ± 0.75	11.65 ± 0.73	397	0.103
P5.3	17.28 ± 0.80	16.95 ± 0.91	132	0.430	17.54 ± 1.77	17.18 ± 1.61	342	0.573

Statistical mean ± standard deviation for Right and Left wings presented in millimeters (mm). U= Non-parametric U test. p= Significance level of Wilcoxon- Mann-Whitney test.

For the digit analysis, only the females of *A. planirostris* presented significant differences (H= 3.370, p= 0.035, Figure 2). In the other two species, there were no significant difference between the digits in terms of fluctuating asymmetry values (Figure 2).

On the other hand, *A. lituratus* was the unique of the three bat species analyzed, which showed sexual dimorphism according to wing traits (Wilks' Lambda =0.72, F=2.83, p=0.01). For all the wing measurements, females presented higher values in the statistical means than males (Table 2).

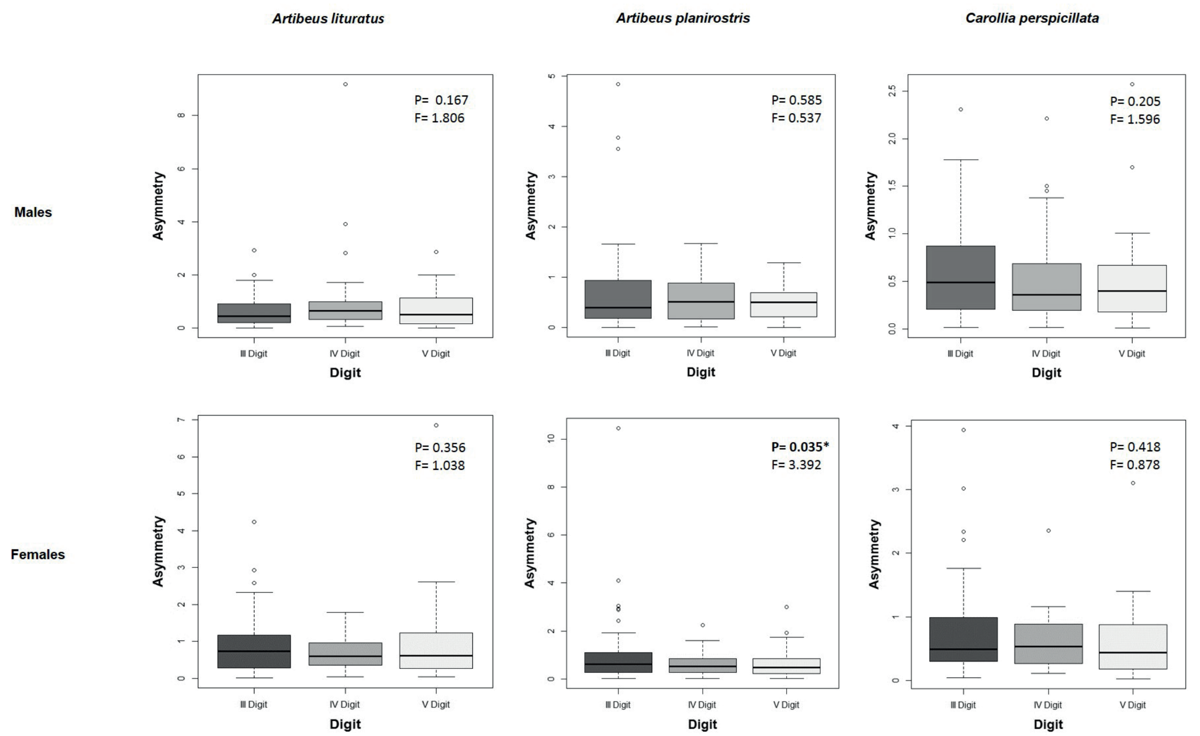

Figure 2. Box-plots for asymmetry values of the III, IV and V digits in females and males of *Artibeus planirostris*, *Artibeus lituratus* and *Carollia perspicillata* specimens in extensive livestock systems (Córdoba, Colombia). p= Significance level of Kruskal-Wallis. H= Kruskal-Wallis test.

By contrast, neither *A. planirostris* (Wilks' Lambda =0.88, F=0.95, p=0.494) nor *C. perspicillata* (Wilks' Lambda =0.89, F=0.89, p=0.548) presented sexual dimorphism in wing traits.

DISCUSSION

Fluctuating asymmetry of bat species and their tolerance to habitat perturbations. In general terms, the results showed no statistical difference (p>0,05) in most of the wing traits, suggesting that environmental conditions of tropical dry-forest fragments immersed in extensive livestock systems, were not enough to cause several changes in the wing morphology of these bat species. One of the possible reasons of the low degree of asymmetry is that natural selection may favor symmetrical individuals rather than asymmetrical. The "Differential-mortality" hypothesis establishes that individuals with less FA have more possibilities for survival unlike individuals with high FA (FLOATE and FOX, 2000). Symmetry may favor the locomotion leading a better ability for flight performance and food capture (VOIGT *et al.*, 2005). Hence, the selective pressure on bats eliminates asymmetrical individuals, selecting symmetrical ones for next generations (VOIGT *et al.*, 2005), which are probably the specimens analyzed in this work.

Additionally, variation in wing traits is more conservative due to its functionality on flight performance (NORBERG and RAYNER, 1987), thermoregulation (MAKANYA and MORTOLA, 2007), and food manipulation (VANDOROS and DUMONT, 2004). Comparing with other body regions such as head and hind-limbs, forelimbs have less asymmetry in bats (FIGUEIREDO *et al.,* 2015).

Moreover, the three bat species are common frugivorous with a wide geographical distribution throughout the country (MUÑOZ, 2001). Typically, *C. perspicillata* and *A. lituratus* are often used as ecological indicators of fragmented landscapes (GALINDO, 2004). All the three species are reported in different environments such as monocultures (BREVIGLIERI and UIEDA, 2014; ORTEGÓN-MARTÍNEZ and PÉREZ-TORRES, 2007), urban systems (FIGUEIREDO *et al.,* 2015; ALBERICO *et al.,* 2005) and extensive livestock systems (BALLESTEROS-CORREA, 2015). Based on the findings of this research, but also on the high abundances of these bat species reported for extensive livestock systems (BALLESTEROS-CORREA, 2015), it is possible to infer that, at first glance, these species are not hampered by livestock systems because of their high tolerance to habitat perturbation. Further studies should evaluate the FA of bat species from other guilds (e.g. insectivores, carnivores, nectarivores, omnivores), and with other ecological characteristics (e.g. high habitat specificity, rare species), to test differential responses of species to livestock systems.

Some differences in wing traits and sexual dimorphism. Differences were detected in two wing traits of the third digit for *C. perspicillata* (P3.2, P3.4, Table 1). The third digit is a measure of hand-wing length and enable a fast and economic flight (DIETZ *et al.,* 2006). Recently, it has been found a wing defect (i.e. nonsymmetrical digit) in specimens of *C. perspicillata* associated to livestock systems (CASTILLO-FIGUEROA and PÉREZ-TORRES, 2018). This wing anomaly refers to the difference of the length in P3.4 between left and right side (CASTILLO-FIGUEROA and PÉREZ-TORRES, 2018), which is the wing trait that showed statistical difference in the present study (Table 1).

For *A. planirostris,* other studies conducted in urban environments from Brazil neither found a high asymmetry (FIGUEIREDO *et al.,* 2015). The present study shows the low degree of stress for this species in extensive livestock systems, only with statistical difference between the digits of females (Figure 2), but without differences in the individual wing traits for both males and females (Table 3). *A. planirostris* exhibits a great ecological plasticity using a variety of roost structures in sites with high availability of food (BREVIGLIERI and UIEDA, 2014). In this sense, fragments of tropical dry-forest may provide enough food source for developmental stability in this species.

On the other hand, females of *A. lituratus* showed for all the statistical means of wing traits, higher values compared with males (Table 2). The "Big Mother" hypothesis proposes that larger females have greater reproductive success, because they can birth larger offspring and bring a greater amount of resources to them (RALLS, 1976). Previously, STEVENS *et al.* (2013) demonstrated the existence of this phenomenon using wing traits of *A. lituratus* from 15 sites in eastern Paraguay and five sites in north-eastern Argentina. This study highlighted that larger wing traits are useful for better flight performance in females to forage resources, carrying babies, and compensate a higher body weight (STEVENS *et al.,* 2013). The results presented here support the "Big Mother" hypothesis for *A. lituratus* in terms of wing traits.

Final considerations. The asymmetry analysis with multiple traits provides better understanding of the influence of stress (ST-AMOUR *et al.,* 2010; LEUNG *et al.,* 2000; LEARY and ALLENDORF, 1989). In this study, detailed analyzes of 11 wing traits were conducted (i.e. metacarpals and phalanges), but also general analyzes using three main structures of the wings were made (i.e. III digit, IV digit, V digit).

One of the limitations of the present study is the low sample size, which can affect the detection of normality deviations (BENÍTEZ and PARRA, 2011; GRAHAM *et al.,* 2010). However, this issue depends mainly on the specimens available in biological collections (CASTILLO-FIGUEROA, *in press*; PYKE and EHRLICH, 2010), being outside the researcher's control. It is important to increase the number of specimens in museum collections for address, robustly, different ecological questions using more samples. This study shows the relevance of biological collections in the contribution of key data, which can be useful to parse out the effect of landscape transformation on the ecological responses of species (CASTILLO-FIGUEROA, *in press*).

In conclusion, wing traits of these bat species did not show a considerably asymmetry in tropical dry-forest fragments immersed in extensive livestock systems of Cordoba department. The results of this work suggest a low degree of stress for the three bat species, and highlight that livestock systems do not severely affect them due to their high tolerance to habitat perturbation. Nevertheless, this study did not distinguish the type of management of extensive livestock system. It would be important to compare silvopastoral management with conventional management to know, with more detail, if someone of them provides better conditions for bat species. Finally, it would be important to consider bat species of other guilds and with a high habitat specificity, because in the present study only frugivorous and common species were considered.

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