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Morphometric differentiation of *Glossophaga soricina soricina* (Chiroptera: Phyllostomidae) in three Brazilian biomes

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ABSTRACT. *Glossophaga soricina soricina* (Pallas, 1766) plays an important role in the ecosystems where it is found, taking part in the pollination of hundreds of plant species. Here, we statistically compared 12 external characters of 169 specimens collected in three Brazilian biomes: 82 in the Pantanal (Mato Grosso), 45 in the Atlantic Forest (Ilhéus, Bahia) and 42 in the Caatinga (Chapada Diamantina, Bahia). Each character was analyzed by descriptive and inferential statistics. The t-test didn't detect any differences between males and females of each population. The ANOVA with *a posteriori* Tukey test showed significant results for all traits (Forearm length, Calcar length, Body length, Tail length, Hindfoot length, Ear length, Tragus length, Thumb length, Noseleaf length, Noseleaf width and Horseshoe width) except for Tibia length. The discriminant analysis showed distinct clusters representing the populations of each biome. The tests demonstrated that the three populations are significantly different from one another and that the specimens of the population from the Caatinga, on average, are larger than those from the Pantanal, which are larger than those from the Atlantic Forest, confirming the possible existence of intra-specific geographic variation.

KEY WORDS. Atlantic Forest; Caatinga; Glossophaginae; Morphometry; Pantanal.

Glossophaga soricina (Pallas, 1766) (Glossophaginae) is endemic to the New World and is distributed throughout the Neotropical region, from Mexico to South America, reaching southern Brazil, Paraguay, and northern Argentina. Currently, there are five subspecies: *G. s. antillarum* (Rehn, 1902), *G. s. handleyi* (Webster & Jones, 1980), *G. s. mutica* (Merrian, 1898), *G. s. soricina* (Pallas, 1766) and *G. s. vallens* (Miller, 1913) (Simmons 2005). Given that *G. s. soricina* is widely distributed throughout Brazil, including areas and biomes with unique and distinct characteristics, we tested the hypothesis that populations vary according to habitat differences.

There are few morphometric studies including specimens of *G. soricina* from Brazil. Some examples are those of Webster (1983), who measured specimens from South America; Willig (1983), who examined 17 external characters of *G. soricina* from the Caatinga and Cerrado, and failed to detect any significant geographical differences between the two populations; and López-González (2005) who examined specimens from Paraguay.

Brazil has a wide variety of biomes that include specific characteristics, three of which are subjects of this study. The Pantanal is a large floodplain with two well-defined seasons, rainy summer and dry winter, both with high relative humidity. The Atlantic Forest has high average temperatures year round, regular and well distributed rainfall and high relative humidity. Finally, the Caatinga, the only exclusive biome of Brazil, present only in the Northeast, is characterised by low relative humidity and little rainfall volume (Leal *et al.* 2003, Campalini & Schäffer 2010, Alho *et al.* 2011).

Patterns of geographic variation have been detected in several species of bats. Nargosen & Tamsitt (1981) observed clinal variation in the size of representatives of *Anoura cultrata* Handley, 1960. In their data, individuals from Central America (represented by Costa Rica and Panama) were larger than those from South America (represented by Peru), a discrepancy that was interpreted as a result of inter-specific competition. There are fewer species of Phyllostomidae bats in Costa Rica and Panama, which led the authors to speculate that a wide variety of foods is available for *A. cultrata*, giving those populations a selective advantage.

Owen *et al.* (1984) and McLellan (1984), when studying species of *Carollia*, detected a significant decrease in the size of specimens from North to South, and attributed their findings to ecological factors involving competition. Storz *et al.* (2001) analyzed the pattern of geographic variation in the external morphology of specimens of *Cynopterus sphinx* Vahl, 1797 (Pteropodidae).

Webster (1983) reported geographic variation among subspecies of *G. soricina*, but not within subspecies. Moreover, it is known that the intra-specific variation within *Glossophaga* is vast (López-González 2005), and detailed analyses involving these variations, where they occur, and why they occur, are scarce. In addition, there are few studies of the external morphology of the species and so far none that compare populations of *G. s. soricina* from several Brazilian biomes.

This paper compares populations from three biomes – the Caatinga, the Pantanal and the Atlantic Forest, in order to detect quantitative variation between and within these populations. Furthermore, hypotheses about which factors are responsible for this differentiation are formulated. We also compared our results with those published in previous studies.

MATERIAL AND METHODS

All specimens analyzed were identified through the key present in "Mammals of South America" of Griffiths & Gardner (2008) and "Key for the Determination of Brazilian bats" of Vizotto & Taddei (1973). The deposit numbers of the specimens analyzed are available in the appendix.

Specimens from the Pantanal biome are representative of the Reserva Privada do Patrimônio Natural do Serviço Social do Comércio no Pantanal, located in Barão de Melgaço, Mato Grosso. Samples were collected between 1999 and 2002 by the Laboratório de Mastozoologia and Museu Nacional, Universidade Federal do Rio de Janeiro (UFRJ). In total, 82 specimens of *G. s. soricina* were captured, and were deposited at the Museu Nacional, UFRJ.

The specimens from the Atlantic forest were collected between 1935 and 1954, in Buerarema and Ilhéus municipalities, both in the state of Bahia. The investigations were carried out by the Estudos e Pesquisa sobre a Febre Amarela (Ministério da Educação e Saúde), with the support of the International Health Division of the Rockefeller Foundation. A total of 45 specimens of *G. s. soricina* were collected and deposited in the Museu Nacional of the Universidade Federal do Rio de Janeiro (Vaz 2005).

Specimens from the Caatinga originated from the Chapada Diamantina. The study area is located in the Central region of Bahia, Northeastern Brazil. The region has one of the highest altitudes of the biome and covers a diversity of landscapes with fragmented areas of rocky fields, Cerrado, Caatinga and Forest enclaves (Jesus *et al.* 1985, Sbragia & Cardoso 2008). A total of 42 specimens of *G. s. soricina* were collected in expeditions and were deposited in the Laboratório de Mastozoologia (Universidade Federal do Rio de Janeiro), between 2002 and 2006.

The external measurements were defined by Vizotto & Taddei (1973), plus measurements of tragus and nasal leaf (Bogdanowicz *et al.* 1997, Handley Jr 1988). The measurements were made with a ruler of high precision and the values converted to millimeters (mm). The 12 characters measured were: Body length (BL), Tail length (TL), Forearm length (FA), Calcar length (CL), Hind foot length (HF), Ear length (E), Tragus length (TR), Tibia length (TI), Thumb length (TH), Noseleaf length (NL), Noseleaf width (NW) and Horseshoe width (HW). For further discussion, the Body length and Tail length were analyzed together, and categorized as Total length (ToL).

In an attempt to analyze the differences in the populations, we calculated the mean, standard deviation, maximum and minimum values of these measurements. The normality test was performed in order to verify whether the populations showed normal distribution. The Student t test was performed in order to detect whether there is sexual dimorphism in the populations. The one way ANOVA was performed with an *a posteriori* examination of Tukey, with the intent to detect whether there are significant differences in characters between populations in focus and in which populations these differences are present. The discriminant analysis combined with Mahalanobis distance was performed to detect which variables (characters) are most important to distinguish between the groups (populations), along with the canonical analysis to construct a chart that identifies and allows the visualization of clusters of populations. Analyses were performed in Statistica 8 (Statistica Software Inc.) and GraphPad Prism 5.0 (GraphPad Software Inc.). The tests were all applied to a decision level alpha of 0.05.

RESULTS

The mean and standard deviation for each biome are summarised in Table I. The Student t test showed no significant difference ($p > 0.05$) in the external morphology of specimens of each population. The one way ANOVA followed by *a posteriori* Tukey test identified significant differences between at least two biomes for all characters analyzed, except Tibia length (Table II).

The characters Hind foot length, Ear length, Tragus length, Body length, Tail length, Noseleaf length and Thumb length were the most important for the differentiation of the three populations by discriminant analysis (Table III). The Mahalanobis distance (Table IV) showed that there are significant differences between the populations analyzed, the largest between the population of the Atlantic Forest and Caatinga, which is corroborated when the average is taken into consideration (Table I). The canonical variables and the scatter plot showed three groups of points distinguished from each other (Fig. 1), with each group presenting one of the populations studied – Pantanal, Caatinga and Atlantic Forest.

DISCUSSION

The last and major revision of the genus *Glossophaga* was published by Webster (1983). According to the author, there is clinal variation in cranial and external dimensions of *G. soricina*, in which populations from South America are generally smaller than the populations from Central America and the western regions of the Andes, and *G. s. soricina* is the smallest subspecies. Mean external measurements given by Webster (1983) and López-González (2005) publications, are shown in Table V.

Comparing the values found in previous studies with the present study (Table V), we detected large differences, mainly

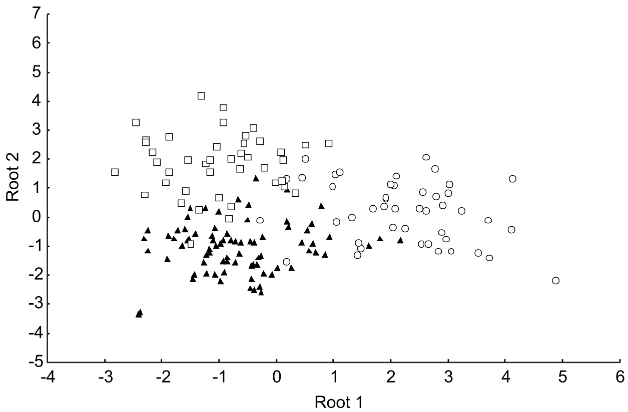

Figure 1. Scatter plot of canonical scores for discriminant function 1 x 2. We can distinguish three groupings that characterize each of the populations analyzed. (▲) Pantanal, (□) Caatinga, (○) Atlantic Forest

Table I. Mean and standard deviation approximate in mm of the characters analysed in this article. The first column corresponds to the characters and the location is specified in the first row for the biomes Pantanal (Mato Grosso), Caatinga (Chapada Diamantina, Bahia) and Atlantic Forest (Bahia).

	Pantanal	Caatinga	Mata Atlântica
FA	35.8 ± 1.1	35.9 ± 1.7	35.2 ± 1.6
CL	4.9 ± 0.7	5.4 ± 1.7	4.8 ± 0.8
HF	8.9 ± 0.7	8.4 ± 0.8	8.3 ± 0.8
E	12.8 ± 0.7	12.6 ± 1.0	12.2 ± 0.7
TR	4.7 ± 0.5	5.2 ± 0.4	3.9 ± 0.5
ToL	54.9 ± 3.8	55.8 ± 5.8	51.7 ± 4.2
NL	4.9 ± 0.5	5.8 ± 0.7	5.5 ± 0.7
NW	3.6 ± 0.4	3.8 ± 0.4	3.7 ± 0.4
HW	4.4 ± 0.3	4.6 ± 0.3	4.5 ± 0.3
TI	14.8 ± 0.7	14.7 ± 0.7	14.5 ± 0.9
TH	4.6 ± 0.4	4.1 ± 0.5	5.0 ± 0.7

Table II. Results of the one way ANOVA test with *a posteriori* Tukey test. Only one character – Tibia length – was not important for the differentiation of biomes. The decision level α was 0.05, ns = not significant and * = significant.

Characters	FA	CL	HF	E	TR	BL
Pantanal x Atlantic Forest	ns	ns	*	*	*	*
Pantanal x Caatinga	ns	*	*	ns	*	ns
Atlantic Forest x Caatinga	*	*	ns	ns	*	*
Characters	**TL**	**NL**	**NW**	**HW**	**TI**	**TH**
Pantanal x Atlantic Forest	ns	*	ns	ns	ns	*
Pantanal x Caatinga	*	*	*	*	ns	*
Atlantic Forest x Caatinga	*	*	ns	ns	ns	*

Table III. Results of discriminant analysis for the biomes analysed. Number of variables in the model: 12; three groups (Pantanal, Atlantic Forest and Caatinga). Wilk's lambda: 0.14427, approximate F(24.310) = 21.090, p < 0.00001.

	Wilk's Lambda	Partial Lambda	F-remove (2,155)	p-level
FA	0.147286	0.979527	1.61983	0.201266
CL	0.146980	0.981566	1.45544	0.236468
HF	0.169594	0.850684	13.60314	0.000004
E	0.153633	0.939064	5.02900	0.007654
TR	0.235004	0.613910	48.74005	0.000000
BL	0.176138	0.819080	17.11835	0.000000
TL	0.164648	0.876238	10.94630	0.000036
NL	0.199394	0.723549	29.61090	0.000000
NW	0.144979	0.995119	0.38017	0.684379
HW	0.148041	0.974532	2.02533	0.135428
TI	0.147927	0.975283	1.96409	0.143760
TH	0.182670	0.789791	20.62728	0.000000

Table IV. Mahalanobis distances. We can detect with this test that the populations of the Atlantic Forest and Caatinga are more distant from each other (13.22595) that the Pantanal and Atlantic Forest populations (10.02568) and these are more distant than the populations of Pantanal and Caatinga (8.70698).

	Pantanal	Atlantic Forest	Caatinga
Pantanal	0.00000	10.02568	8.70698
Atlantic Forest	10.02568	0.00000	13.22595
Caatinga	8.70698	13.22595	0.00000

Table V. Average of characters analysed in this study and in previous studies. The first column corresponds to the characters analysed and the location is specified in the first line, where the author is represented by asterisks: present study (*1); WEBSTER (1983) (*2) and LÓPEZ-GONZÁLEZ (2005) (*3). Note the large difference in the amount of body length found by WEBSTER and LÓPEZ-GONZÁLEZ compared to the values found in this study.

	Pantanal*1	Caatinga*1	Atlantic Forest *1	South America*2	Paraguay*3
FA	35.8	35.9	35.2	35.8	34.9
HF	8.9	8.4	8.3	10.1	11.0
E	12.8	12.6	12.2	13.7	14.1
ToL	54.9	55.8	51.7	62.6	65.1

in the Total length of the specimens studied by WEBSTER (1983) and LÓPEZ-GONZÁLEZ (2005), which had greater values (62.6 and 65.1) than the specimens measured in the current study (54.9, 55.8 and 51.7). There are two possible reasons for this: 1) geographical variation; 2) differences in the measurement techniques.

Comparing only the data obtained in this study, we observed that, on average, the population of the Caatinga (Chapada Diamantina, Bahia) had larger body values (Forearm length, Calcar length, Tragus length, Total length, Noseleaf length, Noseleaf width and Horseshoe width), followed by the population of the Pantanal (Mato Grosso), with intermediate values for the two populations and greater values for the characters of Hind foot length and Ear length, and finally, the population of the Atlantic Forest (Ilhéus) with lower measurements than the others, except for the Thumb length, which is the largest in the biomes analyzed (Table V).

The Student t test for sexual dimorphism showed very high values of p ($p > 0.05$), indicating that the difference between external measurements of males and females is not significant for most characters; however, on average, females are larger than males. According to Nargosen & Tamsitt (1981), females larger than males are common in species of Phyllostomidae bats.

The one way ANOVA with *a posteriori* Tukey test revealed which characters are the most important for differentiating the populations (Table II). Significant differences were found in at least two populations, except for Tibia length, for which p was very high ($p > 0.05$). For all characters that can significantly separate among populations, a larger sample size is needed. As for intra-specific variation, the differences between individuals of the populations are less sensitive to tests and therefore require a large sample size.

The results of the discriminant analysis, which are summarized in Tables III and IV show that the Wilk's lambda was relatively small (0.14427), and the approximate value of F was high (24.310) = 21.090, indicating a high discrimination. The p level showed that characters Ear length, Tragus length, Noseleaf length, Thumb length, Body length, Hind foot length and Tail length contribute to the greater discrimination between the groups (small values of the partial p level). By analyzing Fig. 1, we observed that along the y axis (Root 1) there is overlap between the populations of the Caatinga and Pantanal, while the population of the Atlantic Forest contrasts with those. However, by analyzing the dispersion of scores along the x axis (Root 2), we noticed that there are differences among the three populations: the scores of the Atlantic Forest are among the Caatinga and Pantanal scores. Although a larger sample size is required for the groups, a tendency towards separation thereof can already be observed (Fig. 1). The Mahalanobis distances show that populations of the Atlantic Forest and Caatinga are farther apart than the Pantanal and Atlantic Forest and those of the Caatinga and Pantanal (Table IV). The means of the canonical variables and the scatter plot for the selected discriminant function separated the groups (Fig. 1).

Similarly to much of the Atlantic Forest biome, the forest in the city of Ilhéus (Bahia) is reduced, fragmented and modified. The remnant forest is represented by small fragments, which are distant from one another, facilitating the isolation of species and a decrease in the available resources, affecting the presence and abundance of the native flora and fauna (Faria & Baumgarten 2007). The reduction of space and resources generates a greater overlapping of niches (flower resources), which can be translated by competition. In many cases, there is a tendency for populations to specialize in the use and exploitation of different resources, or occupy different spaces in the same environment. This specialization generates a reduction in the ecological niche of the species, but otherwise allows the continuity to survive in the ecosystem (Dajoz 2005). In addition, intraspecific competition may influence the body size of individuals of a population. In areas with an abundance of species (high species richness), the selection is in favor of smaller species, which can occupy more specialized feeding niches (Grant 1965, Heaney 1978).

The hypothesis that there are significant differences between the three populations was corroborated both by one way ANOVA and by Discriminant Analysis, opening a new and curious way for the study of biomes and populations of nectar feeding species. Thus, the differences may be due to several factors, among them: the greater availability of niches to *Glossophaga* in one biome compared with another; the presence of competing species, such as other *Glossophaginae* bats; non-biotic factors such as humidity, temperature and precipitation, which through evolution have acted differently in the three locations, selecting larger individuals in certain biomes. Alternatively, it may simply be due to genetic drift. These issues reflect the need for more studies, in greater detail, of the taxonomy and classification of *Glossophaga* in order to obtain a better understanding of the significant distinctions found below the specified level.

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Appendix. The provenance of the specimens analyzed are summarized below.

The 82 specimens from Pantanal, Mato Grosso, Barão de Melgaço (MN 64008-64018, MN 64021-64028, MN 64034-64043, MN 64045-64059, MN 64099, MN 64117-64125, MN 64159, MN 64182, MN 64443, MN 64450, MN 64468, MN 64491-64492, MN 64542-64547, MN 64554-62555, MN 64575-63577, MN 64586-64587, MN 64594, MN 64597-64603) are available at Museu Nacional, Universidade Federal do Rio de Janeiro.

The 45 specimens from Atlantic Forest, Bahia, Ilhéus (MN 49885-49899, MN 49903, MN 49905-49929, MN 49931-49934) are available at Museu Nacional, Universidade Federal do Rio de Janeiro.

The 42 specimens from Caatinga, Chapada Diamantina (IAS 01, IAS 03-13, IAS 64, IAS 66-68, IAS 71-72, IAS 97, IAS 105, IAS 108-111, IAS 113-117, IAS 132-135, IAS 184, JAO 1163, JAO 1426-1431, JAO 1477) are available at the Laboratório de Mastozoologia, Universidade Federal do Rio de Janeiro.

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