

Size and shape variability in the skull of *Myotis nigricans* (Schinz, 1821) (Chiroptera: Vespertilionidae) from two geographic areas in Brazil

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Abstract

We present a quantitative analysis of sexual dimorphism and geographic variation in the skull of *Myotis nigricans* (Schinz, 1821) assessed by geometric morphometrics. Differences in size and shape of skulls were investigated using 30 landmarks plotted on two-dimensional images of lateral and ventral views. Results of geometric morphometrics revealed sexual dimorphism in the centroid size of the skull in both views. Females were larger than males. Nevertheless, there was no sexual dimorphism in skull shape of *M. nigricans*. Geographic variation was detected in size and shape of the skull. South Brazilian specimens were significantly larger than Ceará specimens only in the lateral view. Differences in skull shape were statistically significant in both views: specimens from South Brazil were brevirostri and presented a more expanded skull in the posterior region while Ceará specimens were longirostri and do not present any expansion in the brain case. Ecological factors for these phenomena are discussed in the text.

Keywords: Chiroptera, *Myotis nigricans*, skull morphology, geometric morphometrics.

Variação de tamanho e forma no crânio de *Myotis nigricans* (Schinz, 1821) (Chiroptera: Vespertilionidae) de duas áreas geográficas no Brasil

Resumo

Uma análise quantitativa do dimorfismo sexual e da variação geográfica do crânio de *Myotis nigricans* (Schinz, 1821) é apresentada através da morfometria geométrica. Diferenças no tamanho e na forma do crânio foram avaliadas através de imagens bidimensionais das vistas lateral e ventral do crânio, onde foram estabelecidos 30 marcos anatômicos. Os resultados indicaram a existência de dimorfismo sexual no tamanho do centróide do crânio para ambas as vistas estudadas. As fêmeas foram maiores que os machos. Contudo, não foi detectado dimorfismo sexual na forma do crânio de *M. nigricans*. A variação geográfica esteve presente no tamanho e na forma do crânio. Espécimes do Sul do Brasil tiveram tamanho do centróide maior que os espécimes do Ceará apenas na vista lateral. Diferenças na forma do crânio foram estatisticamente significativas em ambas as vistas: os espécimes do Sul do Brasil apresentaram-se brevirostri e com grande expansão na região posterior do crânio, enquanto os espécimes do Ceará apresentaram crânios longirostri e sem expansão posterior da caixa craniana. São discutidos fatores ecológicos para estes fenômenos.

Palavras-chave: Chiroptera, *Myotis nigricans*, morfologia craniana, morfometria geométrica.

1. Introduction

The genus *Myotis* (Kaup, 1821) has more than 100 species globally widespread (Simmons, 2005). The species are morphologically similar and hardly ever reflect specialization, which may cause some problems in their correct identification (LaVal, 1973; Bogan, 1978; Reduker, 1983; Ruedi et al., 1990; Baud and Menu, 1993; Barquez et al., 1999; Gannon et al., 2001; Ruedi and Mayer, 2001; López-Gonzales et al., 2001; Stadelmann et al., 2004) and leads to a taxonomic complexity of the group. *Myotis* has had a meaningful evolutionary success, being found in all continents (except in Antarctic), from semi-deserted habitats to sub-Antarctic regions (Ruedi and Mayer, 2001). Most of them are insectivore, even though some fish eater species were registered (Rice, 1998). The species from this genus are able to live together in colonies, sharing space and food, without apparent competitive exclusion (Ruedi and Mayer, 2001; Stadelmann et al., 2004), because morphological similarity does not reflect necessarily ecological similarity (Sauders and Barclay, 1992). According to Benda and Horáček (1995) the morphological variation pattern for this genus provides complex information about taxonomy, adaptation and the history of the distribution of the species as well.

The genus *Myotis* has 14 species in the Neotropical region (LaVal, 1973), from which six are registered for Brazil: *M. albescens* (E. Geoffroy, 1806), *M. levis* (I. Geoffroy, 1824), *M. nigricans* (Schinz, 1821), *M. riparius* Handley, 1960, *M. ruber* (E. Geoffroy, 1806) and *M. simus* Thomas, 1901 (Simmons, 2005). *Myotis nigricans* is found from South Mexico (Nayarit and Tamaulipas) to Peru, Bolivia, North Argentina, Paraguay and South Brazil. It is also occurring in Trinidad and Tobago and Lesser Antilles (St. Martin, Montserrat and Grenada) (Simmons, 2005). González (2000) stated that *M. nigricans* is common in Rio Grande do Sul, including Porto Alegre urban areas. This species has sociable habits and lives in spaces like tree barks, foliages and ceiling of buildings and houses (González, 2000).

Studies about sexual dimorphism and geographic variation from *M. nigricans* are scarce. LaVal (1973), who is a pioneer in the research about morphological variation of this species, suggested that *M. nigricans* should present differences among populations from different geographic areas in South America. Nevertheless, he did not mention any data about sexual dimorphism. Myers and Wetzel (1983), throughout the analyses of body and skull linear measurements, found some evidence of geographic variation among species from Argentina, Bolivia and Paraguay; however no significant differences were found between male and female specimens. Up to now, the studies describing *M. nigricans* morphology in South America have been exploring just the traditional morphometrics (linear measurements) and literally no information about the shape changes was presented (Ihering, 1895; Cabrera, 1957; LaVal, 1973; Myers and Wetzel,

1983; Lopez-Gonzales et al., 2001). Besides, very few information is known about sexual dimorphism and geographic variation from this species in Brazil and most of them are related to body size. In this sense, the current study presents the first information about sexual dimorphism and geographic variation on the size and shape of *M. nigricans* skulls, from two Brazilian geographic areas.

2. Material and Methods

A total of 120 adult specimens of *Myotis nigricans* from two geographic areas in Brazil were examined: South Brazil and Ceará state (Figure 1, see Appendix for details).

2.1. Studied areas

South Brazil area comprises the states of Paraná, Santa Catarina and Rio Grande do Sul. The examined specimens from this area were collected between 23 and 31° S of latitude, mainly in localities covered by the Brazilian Atlantic Forest (see the appendix for a detailed list of the sampling localities). This biome is composed of two major vegetation types: the Atlantic Rain Forest (coastal forest) and the Atlantic Semi-deciduous forest (tropical seasonal forest). The Atlantic Rain Forest covers the mountain chain that runs along the coastline from northeastern to southern Brazil, which includes our sampling areas in Santa Catarina. The Atlantic Semi-deciduous Forest extends across the plateau in the center and southeastern interior of the country (Leitão-Filho and Morellato, 1997; Oliveira-Filho and Fontes, 2000) and covers most of sampling areas in Rio Grande do Sul and Paraná states. This area experiences warm and wet climate, with dry seasons eventually. The temperatures range from -6 °C during winter months to more than 30 °C in the summer season (Morellato et al., 2000; Morellato and Haddad, 2000; Oliveira-Filho and Fontes, 2000).

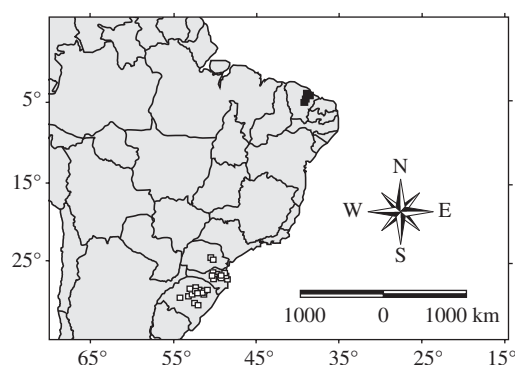


Figure 1. Map presenting the two studied geographic areas with the original localities of *Myotis nigricans* exemplars. Black square: Ceará State; white square: South Brazil (Paraná, Santa Catarina and Rio Grande do Sul states).

In the Ceará state, the specimens of *M. nigricans* examined were collected between 06 and 04° S of latitude (see the appendix for a detailed list of the sampling localities). The dominant biome is Caatinga, which is a vast xeric biome that covers most of north-eastern Brazil (Mares et al., 1985). This biome is covered by several xeric, deciduous plants associations from dry, open woodland to thorn scrub with cacti and terrestrial bromelias. In general, the climate conditions are semi-arid, which a variable annual precipitation between 250 and 1270 mm which falls unpredictably between September and March. There is no permanent water course, only some springs, water-holes and man-made reservoirs (Olmos, 1993). The dryness station remains five to eight months annually and the temperature varies from 27 to 33 °C (Mares et al., 1985).

2.2. Material examined

For this study, the specimens from the following institutions were examined: Zoology Department from the Instituto de Biociências from the Universidade Federal do Rio Grande do Sul (ZMAM), Museu de Ciências Naturais from the Fundação Zoobotânica do Rio Grande do Sul (MCN), Museu de Ciências Naturais from the Centro Universitário UNIVATES (ZMUMCN) and Universidade de Blumenau (FURB-SLA).

The age group was determined observing the presence of bone or cartilage epiphysis on the metacarpal-phalangeal joint from the left fourth digit (Burnett and Kunz, 1982; Kunz and Anthony, 1982; Anthony, 1988).

The sexual dimorphism was analyzed just using adult specimens from South Brazil, due to the absence of male specimens from Ceará area. Geographic variation was also analyzed just for adult female specimens for the same reason mentioned above.

2.3. Geometric morphometrics

The geometric morphometrics (see Bookstein, 1991; Rohlf and Marcus, 1993; Monteiro and Reis, 1999) were used to analyze sexual dimorphism and geographic variation in skulls of *Myotis nigricans*. This technique has showed to be objective and efficient when compared to traditional methods (Zelditch et al., 1995; Rohlf et al., 1996; Rohlf, 1998).

A total of 120 (lateral view) and 119 (ventral view) images of *M. nigricans* skull were analysed (see Table 1). The images were taken using a Nikon Coolpix 4500 digital camera joined to a Nikon SMZ 800 stereo microscope and saved in JPEG format. All the images had an 800 x 600 pixels resolution. The skulls were positioned at 90° from the stereo microscope lenses. A scale was used in this process. Thirty homologous and topologically equivalent landmarks were plotted on the skull in order to describe the size and shape variation. They were digitized using TpsDig1.40 (Rohlf, 2004a). All the landmarks were positioned on the left side of the skull image (Figure 2).

2.4. Landmarks definition

Lateral view (Figure 2a): (1) supra occipital basis; (2) skull anterior extremity, leveled to the first incisor

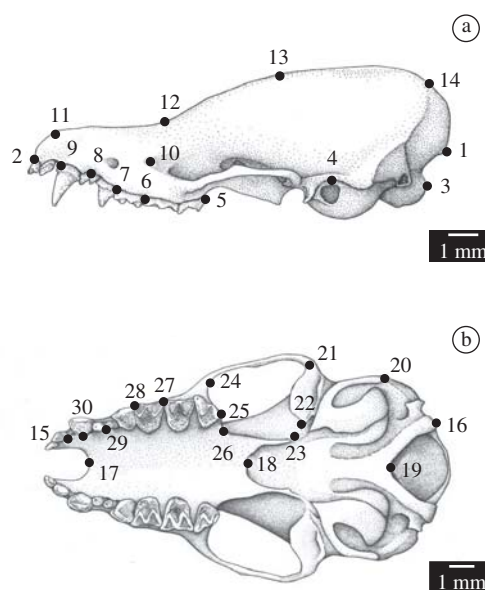


Figure 2. Skull landmarks analyzed in the study (see details in material and methods). a) lateral view. b) ventral view.

Table 1. Number of *Myotis nigricans* specimens included in the geometric morphometrics analysis (lateral and ventral views), according to the geographic area and gender.

Geographic area	Gender	Lateral view	Ventral view
South Brazil	Females	59	59
	Males	48	47
Ceará state	Females	13	13
	Males	-	-
Total		120	119

alveolus; (3) post extremity of the occipital condyle; (4) dorsal external auditory meatus; (5) third molar post extremity; (6) alveolar limit between first and second molar; (7) alveolar limit between third pre-molar and first molar; (8) alveolar limit between first and second pre-molar; (9) canine alveolus anterior extremity; (10) ocular orbit; (11) nasal fossa dorsal extremity; (12) rostrum/skull congruent point; (13) skull anterior lateral extremity; (14) intersection between interparietal and supraoccipital bone.

Ventral view (Figure 2b): (15) skull anterior extremity, in the level of second incisor; (16) occipital condyle post extremity; (17) premaxillary incisura; (18) palatal incisura; (19) foramen magnum incisura; (20) skull lateral extremity, in the level of auditory region; (21) articular fossa left lateral extremity; (22) articular fossa right lateral extremity; (23) lacerated foramen right lateral extremity; (24) zygomatic incisura; (25) third molar post extremity; (26) mandibular arcade incisura; (27) alveolar limit between first and second molar; (28) alveolar limit

between third pre-molar and first molar; (29) alveolar limit between first and second molar; (30) canine alveolus left extremity.

To avoid inflation of degrees of freedom related to the bilaterally symmetrical of the ventral view, landmarks were digitized in half of the skull and all the analysis was performed using this configuration.

The coordinates produced by TpsDig 1.40 program (Rohlf, 2004a) from the images (originally recorded as pixels) were converted in millimeters by the multiplication of the established conversion factor pixel/mm of each image using the included scale in the image, and then saved in nts format.

Centroid size was calculated using the software TPS Regr 1.28 (Rohlf, 2003), and used as a size variable independent of shape (Bookstein, 1991). Mean centroid size was calculated for both sexes and for both studied regions and compared through Student's *t*-test.

In order to compare shape, the coordinates for each specimen in this study were scaled, aligned and transformed by General Procrustes Alignment (GPA) using the software TpsRel 1.39 (Rohlf, 2004b) with the options $\alpha = 0$, projection orthogonal and include uniform component. The GPA method computes a consensus configuration (least-squares Procrustes average configuration) based on the landmark coordinates of all specimens (see Bookstein, 1991, for methodological details). Shape differences between the consensus landmark configuration of each individual specimen were obtained and used to compute a matrix of partial warp scores. Relative warp scores were computed over the covariance matrix of the partial warp scores, therefore being analogous to PCA in the sense that they describe the axes of greater variation in shape for all of the analyzed specimens. The α parameter was set to zero to give the same weight to partial warps in smaller and greater scales (Rohlf, 1993). The partial warps matrix, including the uniform component, was used in a Canonical Variates Analysis (CVA), to describe differences between the populations and in order to confirm patterns previously suggested by the relative warp scores. In this case, if CVA confirms any a priori group, the scores from canonical variant 1 (CV1) and centroid size (CS) of *M. nigricans* skull were plotted for both views, in order to visualize and evaluate how size and shape contributed for the arrangement of these groups. In addition, thin-plate spline diagrams were generated in order to represent the deformation of skull shape in each population using the software Morphueus et al. (Slice, 1999).

All the statistical analyses of geometric morphometrics were performed using SPSS 11.5 (SPSS for Windows, Lead Technologies Inc, Chicago).

3. Results

3.1. Sexual dimorphism

Size: The mean of the centroid size was statistically different between male and female specimens from South Brazil (see Table 2) for both studied views. Female skulls were larger than male skulls ($P < 0.05$).

Shape: For the lateral view, 24 relative warps were generated. The first one explained 25.59% of the total variation, the second 15%, and the third 11.06%. Together they represent more than 50% of the skull total shape variation. Scatter plots among the three first relative warps did not show any a priori group formation for the genders.

For the ventral view, 28 relative warps were generated. The first one explained 24.37% of the total variation, the second 16.18%, and the third 11.5%. Together they represented more than 50% of total skull shape variation. Scatter plots among the three first relative warps from this view also did not show any a priori group formation for the genders, indicating that there is no differences in the skull shape between males and females of *M. nigricans*.

3.2. Geographic variation

Size: The mean differences in the centroid size were statistically significant in the lateral view (see Table 3), in which specimens from South Brazil were larger than those from Ceará ($P < 0.05$). Nevertheless, in the ventral view, the mean centroid size was not statistically significant between the studied areas (see Table 3), which indicates a lack of geographic variation in size for this view ($P > 0.05$).

Shape: 24 relative warps were generated for the lateral view. The first one explained 21.95% of the total variation, the second 17.71% and the third 11.93%. Together they represented more than 50% of the total variation of the skull. The two first relative warps (RW1 and RW2) in the lateral view (Figure 3a) suggested a formation of a priori groups and a partial separation between South Brazil and Ceará specimens along the axis of the second relative warp due to skull shape differences. The CVA of partial warps confirmed the existence of geographic variation in the lateral view, fundamentally related to the change in the skull shape and not related to size (centroid size) (Figure 4). These differences were statistically significant (Wilk's Lambda = 0.470; $g_1 = 4$; $F = 22.62$; $P < 0.0001$).

The region between the rostrum and the skull congruent point generated the strongest deformation in the lateral view of the skull. The specimens from South Brazil

Table 2. Student's *t*-test results from sexual dimorphism analysis for centroid size in both views of *Myotis nigricans* skull from South Brazil (n = sample number; t = t value; d.f. = degrees of freedom; P = significance).

View	n	♀ Mean (SD)	n	♂ Mean (SD)	t	d.f.	P
Lateral	59	1.86 (0.05)	48	1.84 (0.04)	2.12	105	0.036*
Ventral	59	3.15 (0.09)	47	3.09 (0.08)	3.31	104	0.001*

* $P < 0.05$.

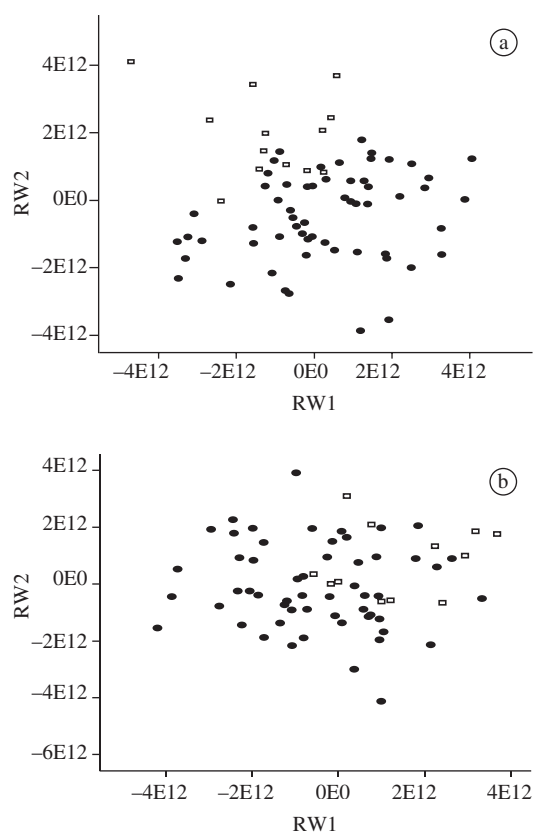


Figure 3. Scatter plot of the first two relative warps scores (RW1 and RW2) of *Myotis nigricans* female skull on lateral view a) and ventral view b). Black circle: South Brazil; white square: Ceará State.

Table 3. Student's *t*-test results from geographic variation analysis for centroid size in both views of *Myotis nigricans* female skull of the two studied geographic areas (n = sample number; t = t value; d.f. = degrees of freedom; P = significance).

View	n	South Brazil mean (SD)	n	Ceará mean (SD)	t	d.f.	P
Lateral	59	1.89 (0.03)	13	1.86 (0.05)	2.16	70	0.034*
Ventral	59	3.15 (0.09)	13	3.19 (0.06)	1.67	70	0.099 ns

*P < 0.05 e ns = not significant.

are brevirostri, and those from Ceará are longirostri. As a consequence, the brevirostri have a shorter rostrum but a broad and less inclined skull. The longirostri ones have a longer rostrum but a less extensive and abrupt inclined skull.

Twenty eight relative warps were generated for the ventral view. The first one explained 24.61% of the total variation, the second 16.86% and the third 10.47%. Together they represented more than 50% of the total variation of the skull. The two first relative warps (RW1

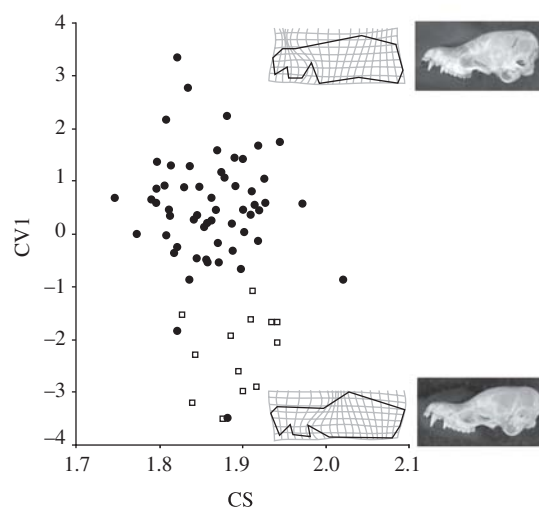


Figure 4. Scatter plot of the scores from canonical variant 1 (CV1) and centroid size (CS) of *Myotis nigricans* female skull from lateral view. Diagrams represent extreme skull shapes resulting from regression of shape coordinates over canonical scores (effect intensified 3X). Photos on the right represent one specimen of each studied geographic area (specimen from South Brazil: ZMUMCN 0265; specimen from Ceará State: MCN 1163). Black circle: South Brazil; white square: Ceará State.

and RW2) in the ventral view (Figure 3b) suggested a formation of a priori groups and a partial separation between South Brazil and Ceará specimens along the axis of the first relative warp due to skull shape differences. The CVA from partial warps also confirmed the existence of two groups for this view, suggesting that the geographic variation in *M. nigricans* is also related to shape changes, but not to size (Figure 5). These differences were statistically significant (Wilk's Lambda = 0.495; $g_1 = 4$; $F = 20.297$, $P < 0.0001$).

The extremities of the occipital condyle and the articular fossa regions generated the most evident shape deformation in ventral view. Specimens from South Brazil presented a more expanded skull in the posterior region (in which there is the articular fossa, the tympanic bulla and the occipital condyle) when compared to the same skull region from Ceará specimens.

The lateral view was the most informative for the analysis of geographic variation in *M. nigricans* skulls, because both, centroid size and shape of skull, presented significant differences between specimens from the two studied geographic areas (see Figure 4).

4. Discussion

The results for sexual dimorphism suggested that differences between *M. nigricans* male and female skulls were fundamentally related to size and not to shape, with female skulls being larger than male. Myers (1978) stated that bat females of Vespertilionidae are usually larger

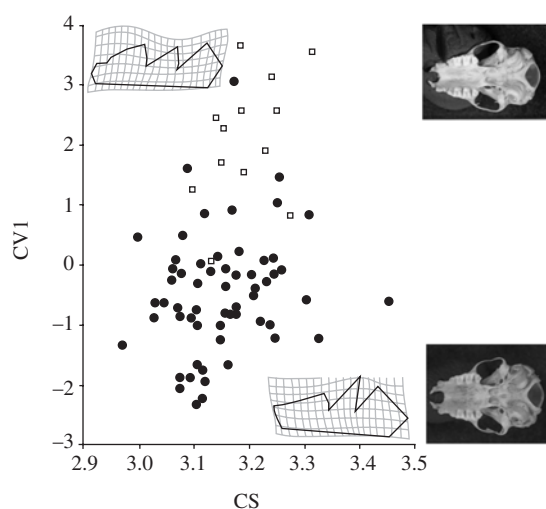


Figure 5. Scatter plot of the scores from canonical variant 1 (CVI) and the centroid size (CS) for half part of *Myotis nigricans* female skull from ventral view. Diagrams represent extreme skull shapes resulting from regression of shape coordinates over canonical scores (effect intensified 3X). Photos on the right represent one specimen of each studied geographic area (Specimen from South Brazil: ZMAM 0780; specimen from Ceará State: MCN 1148). Black circle: South Brazil; white square: Ceará State.

than males in order to perform appropriately parental care. Females, when pregnant, fly carrying their fetuses. After that they need to take care of the offspring in the colonies. Because of this, a larger size of the body may be favorable to provide a successful reproductive process (Myers, 1978).

Carvalho (1973) commented on the absence of information related to *M. nigricans*' life history. Nevertheless, when analyzing other species from the same genus, Barclay (1991) and Rodrigues et al. (2003) found that, when entering the reproductive period, the females go to reproductive colonies, and there they remain looking after their offspring. For this reason, parental care is, in fact, energetically demanding for females (Kurta et al., 1989) and in this sense a larger size could be favorable.

Our results corroborated the results obtained by Saunders and Barclay (1992). They observed a larger forearm length in females of *M. lucifugus* and *M. volans*. In addition, Bogdanowicz and Owen (1996) also found sexual dimorphism in *Otonycteris hemprichi* skull (Vespertilionidae) using geometric morphometrics. Females presented larger centroid size than males. Overall, the observed differences between male and female skulls of *M. nigricans* presented in the current study confirm the literature data about the existence of sexual dimorphism in vespertilionids related to size.

Size variation in specimens from different latitudes has already been detected in the genus *Myotis*. Bogdanowicz (1990) found that, in Europe, specimens of *M. daubentoni* from higher latitudes are larger than

those from lower latitudes. Benda and Horáček (1995) obtained the same result for other species of *Myotis* (*M. myotis*, *M. blythi* and *M. nattereri*) in Palearctic region. Some longitudinal geographic variation patterns were also found in several species of the genus *Myotis* (Topál, 1971; Myers and Wetzel, 1983; Ruedi et al., 1990; Albayrak and Asan, 2002).

LaVal (1973), based on body size, suggested the existence of a different geographic variation in *M. nigricans* from Brazil. According to him, specimens from Amazonian Basin were larger than those from Minas Gerais, Rio de Janeiro and São Paulo, regions considered by the author as South Brazil. The data from the current study did not confirm the author's observation. However, it is important to mention that LaVal (1973) only presented the suggestion of geographic variation based on descriptive and qualitative results, which were not statistically tested.

According to Thorpe (1987), there are two suggestions for the existence of geographic variation: (1) current ecology and (2) historical process. The current ecology conditions can be biotic (mimicry, interspecific competition, etc) or physical (substrate adaptation, adaptation for climatic conditions such as Bergmann's rule, etc). The two geographic areas studied are highly differentiated in terms of vegetation, temperatures and also latitude. The Ceará region is located in the arid Caatinga biome in the northeast of Brazil with sampling areas positioned between 06 and 04 °S (see material and methods for details) and high temperatures varying from 27 to 33 °C (Mares et al., 1985). While South Brazil sampling areas were in the Brazilian Atlantic forest region, which presents both: a warm and wet climate without a dry season and also a seasonal climate with a relatively severe dry season. The temperatures in this area range from -6 °C during winter months to more than 30 °C in the summer season (Morellato and Haddad, 2000).

In this sense we can speculate Bergmann's rule (Bergmann, 1847), which states that "...races from cooler climates tend to be larger than species of warm-blooded vertebrates than races of the same species in living in warmer climates..." (quoted in Mayr, 1963: 319). The usual explanation for this rule is that large animals expend less energy for thermoregulation because of their small surface-to-volume ratio; it is therefore argued that it is more economical for large than for small individuals to live in cold climates (McNab, 1971). Nevertheless, an evocation of this theory to explain our findings could only be reassured if we had examined all the species distribution and found a clinal variation along the different latitudes occupied by *M. nigricans*. In spite of the limitations derived from our restricted sampling areas, we believe that any historical process, could result in population isolation (segregation in the species distribution, for instance) which may lead to a geographic variation pattern associated to specialization.

Based on size and shape geographical variation of the analyzed structures, the occurrence of subspecies

or distinct species could be suggested. Nevertheless, the small number of male samples coming from Ceará area did not allow an analysis of the geographic variation for this gender. Moreover, there is a lack of knowledge about *M. nigricans* from other regions from Brazil, which would be fundamental for a taxonomic definition. In this sense, we strongly recommend the continuity of this study throughout the analysis of skull and external morphology by traditional morphometrics as well as molecular markers, and mainly to examine specimens along all *M. nigricans* species distribution.

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Appendix - Specimens examined

BRASIL, Ceará: Canindé (4° 21' S and 39° 18' W), MCN 1144 (♀), MCN 1163 (♀), MCN 1359 (♀); Jaguaruana (4° 50' S and 37° 46' W), MCN 1146-1148 (♀); Palhano (4° 44' S and 37° 57' W), MNC 1113 (♀), MCN 1151-1153 (♀), MCN 1155 (♀), MCN 1157 (♀); Pereiro (6° 02' S 38° and 27' W), MCN 1149 (♀); Paraná: Castro (24° 79' S and 50° 01' W), ZMAM 0776 (♂), ZMAM 0780 (♂); Londrina (23° 31' S and 51° 16' W), ZMAM 0775 (♀), ZMAM 0777-0778 (♂); Santa Catarina: Água Doce (26° 99' S and 51° 16' W), FURB-SLA 1726 (♀), FURB-SLA 1730 (♀); Blumenau (26° 93' S and 49° 05' W), FURB-SLA 1634 (♂), FURB-SLA 1644 (♂); Florianópolis (27° 59' S and 48° 54' W), FURB-SLA 0057 (♀), FURB-SLA 0111 (♀), FURB-SLA 0238-0240 (♂), FURB-SLA 0241 (♀), FURB-SLA 0242 (♂), FURB-SLA 0254-0255 (♀), FURB-SLA 0256 (♂), FURB-SLA 0257-0258 (♀), FURB-SLA 0282 (♂), FURB-SLA 0283-0284 (♀), FURB-SLA 0286 (♀), FURB-SLA 0288 (♂), FURB-SLA 0289-0290 (♀), FURB-SLA 0305 (♂), FURB-SLA 0306-0308 (♀), FURB-SLA 0313 (♂), FURB-SLA 0314 (♀), FURB-SLA 0318 (♂), FURB-SLA 0320 (♂), FURB-SLA 321-0323 (♀), FURB-SLA 0352-0353 (♂), FURB-SLA 0360-0361 (♂), FURB-SLA 0552-0553 (♂), FURB-SLA 0560 (♀), FURB-SLA 0707 (♀), FURB-SLA 1022 (♂); Gaspar (26° 55' S and 48° 57' W), FURB-SLA 0075 (♀); Indaial (26° 89' S and 49° 23' W), FURB-SLA 1740 (♂), FURB-SLA 1781 (♀), FURB-SLA 1783 (♀), FURB-SLA 1802 (♂), FURB-SLA 1811 (♂), FURB-SLA 1823 (♀); Jaraguá do Sul (26° 29' S and 49° 04' W), FURB-SLA 1875 (♂); Joinville (26° 30' S and 48° 84' W), FURB-SLA 0063 (♀); Nova Veneza (28° 63' S and 49° 49' W), FURB-SLA 0897 (♂), FURB-SLA 0902 (♀), FURB-SLA 0908 (♀); Pomerode (26° 74' S and 49° 17' W), FURB-SLA 1220-1222 (♀); Santa Rosa do Sul (29° 08' S and 49° 42' W), FURB-SLA 0914 (♀), FURB-SLA 0918 (♂), FURB-SLA 0920 (♀); Rio Grande do Sul: Anta Gorda (28° 58' S and 52° 00' W), MCN 0556-0557 (♀); Arroio do Meio (29° 24' S and 51° 56' W), ZMUMCN 0391 (♂); Caxias do Sul (29° 10' S and 51° 10' W), ZMAM 0078 (♀), ZMAM 0079 (♂), ZMAM 0080 (♀); Dom Pedro de Alcântara (29° 22' S and 49° 50' W), ZMAM 0195 (♀), ZMAM 0196 (♂), ZMAM 0197 (♀), ZMAM 0200-0201 (♂), ZMAM 0257 (♂), ZMAM 0502 (♂); Forquethinha (29° 22' S and 52° 05' W), ZMUMCN 0648 (♀); General Câmara (29° 54' S and 51° 45' W), ZMAM 0165 (♀); Guaíba (30° 06' S and 51° 19' W), MCN 2351 (♂); Lajeado (29° 28' S and 51° 57' W), ZMUMCN 0225-0226 (♂), ZMUMCN 0652 (♀); Maquiné (29° 40' S and 50° 12' W), ZMAM ZMAM 0755-0756 (♀), ZMAM 0757 (♂); Marquês de Souza (29° 19' S and 52° 05' W), ZMUMCN 617 (♂); Pelotas (31° 46' S and 52° 20' W), ZMAM 0732 (♂), ZMAM 0735 (♀), ZMAM 0740 (♂); Putinga (29° 00' S and 52° 09' W), ZMUMCN 0265 (♀), ZMUMCN 0509 (♂); Santa Maria (29° 41' S and 53° 49' W), MCN 0299 (♀), MCN 0301 (♂); São José do Herval (29° 02' S and 52° 17' W), ZMUMCN 0383 (♀); São Lourenço do Sul (31° 21' S and 51° 58' W), MCN 0399 (♀); Teutônia (29° 26' S and 51° 48' W), ZMUMCN 0402 (♀); Viamão (30° 04' S and 51° 01' W), ZMAM 0782 (♀), ZMAM 0783 (♂), ZMAM 0785 (♂).