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THE GENUS *Myotis* (CHIROPTERA, VESPERTILIONIDAE) IN BRAZIL: PHYLOGENY, DISTRIBUTION, AND CYTOGENETICS

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Abstract. *Myotis* is the largest genus of the Vespertilionidae, showing a cosmopolitan geographical distribution and is considered an example of adaptive radiation. Nine species occurs in Brazil and this study synthesized aspects of the geographic distribution, karyotype, and phylogeny. A search in bibliographic databases was carried out using keywords. The phylogeny study was based on the sequencing of a specimen of *Myotis ruber* collected in a fragment of the Altantic Forest of Minas Gerais; this specimen was deposited at the Newton Baião de Azevedo Museum of Zoology. The genus showed to be widely distributed in the Brazilian territory, with *Myotis nigricans* being the most widespread. In addition, high karyotypic conservatism was observed in all species of the genus. The phylogenetic analyses using the *mt-Cytb* gene corroborated the monophyletic aspect of the genus and the *Myotis ruber* species.

Keywords: Geographic potential, karyotype, phylogenetic systematics, Vespertilionidae.

Resumo. O gênero *Myotis* (Chiroptera, Vespertilionidae) no Brasil: filogenia, distribuição e citogenética. *Myotis* é o maior gênero de Vespertilionidae, apresentando distribuição cosmopolita e sendo um excelente exemplo de radiação adaptativa. Nove espécies ocorrem no Brasil e este estudo sintetizou aspectos da distribuição geográfica, cariótipo e filogenia. Foi realizada uma pesquisa em bancos de dados bibliográficos, utilizando palavras-chave. O estudo de filogenia baseou-se no sequenciamento de um espécime de *Myotis ruber*, coletado em um fragmento de Mata Atlântica do Estado de Minas Gerais; e depositado no Museu de Zoologia Newton Baião de Azevedo. O gênero mostrou ser amplamente distribuído no território brasileiro, sendo *Myotis nigricans* a espécie mais representativa. Além disso, observou-se alto conservadorismo cariotípico em todas as espécies do gênero. As análises filogenéticas utilizando o gene mitocondrial citocromo-b corroboraram o aspecto monofilético do gênero e da espécie *Myotis ruber*.

Palavras-Chave: Potencial geográfico, cariótipo, sistemática filogenética, Vespertilionidae.

INTRODUCTION

Myotis Kaup, 1829 is the most representative genus among the Vespertilionidae and has a wide distribution (LAVAL, 1973; KOOPMAN, 1993; RUEDI & MAYER, 2001; SIMMONS, 2005; TAVARES *et al.*, 2008). Its species exhibit great similarities in forms and seldom display specialized features (LAVAL, 1973; RUEDI *et al.*, 1990; Gannon *et al*., 2001; López-González *et al*., 2001; Ruedi & Mayer, 2001; Stadelmann *et al*., 2004), hindering the process of identification and the systematic organization of the group.

Tate (1941) divided the genus into seven subgenera by grouping species within each subgenus according to their morphological similarity: *Selysius*, *Isotus*, *Paramyotis*, *Myotis*, *Chrysopteron*, *Leuconoe*, and *Rickettia*. Findley (1972) analyzed 48 external and cranial characters grouping the species only in the three subgenera *Selysius*, *Myotis*, and *Leuconoe*, also according to the specimens' morphological affinities related to different flight and feeding modes. A fourth subgenus, *Cistugo*, was described by Koopman (1993), and as containing species restricted to South Africa. *Cistugo* is now recognized as a distinct genus from *Myotis* (Lack *et al*., 2010). The other subgenera are currently considered an ecomorphological group, that is, all *Myotis* of the New World are grouped into one single subgenus yet without a formally known name (Wilson, 2008). Despite being a broad and diverse genus, *Myotis* is karyotypically one of the most conserved (Baker & Jordan, 1970); in general, phylogenetic studies classify the genus as being monophyletic (Stadelmann *et al*., 2007).

There are only nine species recorded in Brazil (Nogueira *et al*., 2014): *Myotis albescens* (É. Geoffroy, 1806); *Myotis dinelli* Thomas, 1902; *Myotis izecksohni* Moratelli *et al*., 2011a; *Myotis levis* (É. Geoffroy, 1824); *Myotis lavali* Moratelli *et al*., 2011a; *Myotis nigricans* (Schinz, 1821); *Myotis riparius* Handley, 1960; *Myotis ruber* (É. Geoffroy, 1806); *Myotis simus* (Thomas, 1901).

Regarding the genus taxonomic complex of species that occur in Brazil, *Myotis dinellii* was described as a subspecies of *Myotis levis* by LaVal (1973) based on the allopatric distribution of organisms and their morphological similarities. Subsequently, because sympatric traits were recorded in the spatial distribution of *M. levis levis* and *M. l. dinellii* (see Passos *et al*., 2010) in Southern Brazil, they could no longer be described as species and subspecies. Miranda *et al* (2013) performed morphological and morphometric studies on these organisms and detected differences in the sexual dimorphism of both species, with *M. levis* showing separation between males and females, and differences in color and size. *Myotis levis* showed more similarity with *M. ruber* while *M. dinellii* presented similarity to *M. albescens* and *M. nigricans*.

Myotis lavali and *M. izecksohni* were described from the *M. nigricans* complex based on morphological and morphometric traits for species differentiation among themselves, and for the differentiation of *M. nigricans* species, during a study about qualitative evaluations of this species (Moratelli *et al*., 2011b).

Although there are a volume considerable work describing the ecology of individuals of the genus, systematic and phylogenetic aspects remain poorly understood. Therefore, through bibliographic review and molecular study of *Myotis ruber*, this work brought together, briefly, data on the geographic distribution of the genus *Myotis* in Brazil and to find out the cytogenetic and phylogenetic status, pointing out the main problems involved in these studies.

Materials and Methods

The phylogenetic analyses were based on cytochrome-b (cyt-b) sequences of *Myotis* obtained from GenBank and proper sequencing (Table 1). The mitochondrial cytochrome b (cyt-b) gene was chosen for the study because it is widely used in systematic studies of mammals, alone or in combination and because it has a database of good taxonomic coverage deposited in GenBank.

A specimen of *Myotis ruber* was collected during a bat survey in an Atlantic Forest remnant of Minas Gerais, located in Reserva Particular do Patrimônio Natural Santuário Ecológico Mata dos Jacus (RPPN SEMJ) (42°02'51" W; 20°29'01" S; DATUM SIRGAS 2000), in municipality the Alto Jequitibá. The ranges of temperature vary from 1,150 to 1,470 mm, the climate type is Cwb, wet tropical, with dry winter and temperate summer, according to the classification of Köppen (ALVARES *et al*., 2013). The specimen was prepared and deposited at the Newton Baião de Azevedo Museum of Zoology (voucher MZNB 378; see Faria *et al*. 2017). Hepatic tissue DNA was extracted

Table 1. List of species analyzed with name, GenBank or feld number (number), locality and reference.

Taxa	ID GenBank	Reference
Myotis ruber	-	This study
Myotis ruber	AF376867	Ruedi & Mayer, 2001
Myotis simus	AM262336	Stadelmann *et al*., 2007
Myotis simus	JX130506	Larsen *et al*., 2012
Myotis riparius	JX130571	Larsen *et al*., 2012
Myotis riparius	JX130570	Larsen *et al*., 2012
Myotis riparius	JX130569	Larsen *et al*., 2012
Myotis riparius	JX130568	Larsen *et al*., 2012
Myotis nigricans	AF376864	Ruedi & Mayer, 2001
Myotis nigricans	JX130535	Larsen *et al*., 2012
Myotis nigricans	JX130530	Larsen *et al*., 2012
Myotis nigricans	JX130529	Larsen *et al*., 2012
Myotis albescens	JX130504	Larsen *et al*., 2012
Myotis albescens	JX130503	Larsen *et al*., 2012
Myotis albescens	JX130522	Larsen *et al*., 2012
Myotis levis	JX130475	Larsen *et al*., 2012
Myotis levis	AF376853	Ruedi & Mayer, 2001
Myotis gracilis	AB243030	Kawai *et al*., 2006

using the phenol/chloroform protocol according to Sambrook *et al*. (1989). The DNA quality was verified by 0.8% agarose gel electrophoresis and ultraviolet (UV) transilluminator visualization and quantified in a Nanodrop spectrophotometer (ND-1000™). The gene was amplified through polymerase chain reactions (PCR; Axygen model Maxygene II) standardized with specific primers.

The incomplete mitochondrial cytochrome b gene (733bp; cyt-b) was amplified with the primers L14724 (Irwin *et al*., 1991) and CIT-REV (Casado *et al*., 2010). Amplicons were purifed using the GFX PCR DNA and Gel Band Purifcation Kit (GE Healthcare) and labelled with the PCR primers and the internal primers SOT in1 and SOT in2 (Cassens *et al*., 2000), MVZ16 (Smith & Patton, 1993), mt-Cytb AOT (Menezes *et al*., 2010). Sequencing was carried with an ABI PrismTM 3130XL (Tokyo, Japan) platform, and electropherograms were manually checked with CHROMAS version 1.45 and CHROMAS PRO version 1.41 (Technelysium Pty Ltd). Sequences were manually aligned in MEGA (Tamura *et al*. 2007).

The phylogenetic analyses were based on a prior molecular evolution model, chosen using the Akaike Information Criterion test (AIC) with modifications following Posada & Crandall (2001), and run in the ModelGenerator 0.85 software (Keane *et al*., 2006). The Maximum Likelihood (ML) analyses with the cyt-b gene were performed using the HKY evolution model (Hasegawa *et al*., 1985) with invariant sites ratio and gamma substitution distribution rate (HKY+I+G). The ML topology was obtained with the PhyML 3.0 software (Guindon & Gascuel, 2003) using the following parameters: optimized sequence balance, proportion of estimated variable sites, estimated α, initial BioNj tree, topology of sizes of optimized branches, and the aLRT-SH support values obtained on the basis of 1,000 replicates (Anisimova & Gascuel, 2006; Guindon *et al*., 2010).

Bibliographic searches were conducted in databases such as Web of Science (http://www.webofknowledge.com/) and SciElo (http://www.scielo.br/) to obtain data on taxonomy, geographic distribution, and cytogenetics. The searches were made from July of 2016 to May of 2018 using the following keywords: 'geographic distribution', 'karyotype', 'phylogenetic', '*Myotis*'. Records were obtained in both databases. The distribution reviews consider both animals deposited in collections, as well as capture and release data.

Results and Discussion

Phylogeny

The molecular analysis showed that the dataset provided 733 base pairs of the mitochondrial cytochrome b gene (cyt-b) with a TAT stop codon. The maximum likelihood topology confirmed the morphological identification of the specimen collected by us, clustering it together with the other *M. ruber*, with 2.05% of genetic distance between them, while the maximum value of the genetic distance (K2p) interspecific result 16.59% (Table 2), was higher than that found

Table 2. Minimum (Min) and maximum (Max) genetic distance estimates (K2p), in percentage, between *Myotis* for cyt-b.

Clade	Min	Max
Myotis	0,00%	16,59%
M. ruber	0,00%	2,05%
M. simus	0,00%	1,01%
M. albescens	1,01%	1,45%
M. riparius	0,00%	0,14%
M. nigricans	0,14%	0,58%
M. levis	0,00%	2,05%

by RUEDI & MAYER (2001) (15%). The divergence intraspecific did not exceed 5%, corroborating studies already done for species of neotropical bats (PORTER & BAKER, 2004; HOFFMANN & BAKKER, 2001). Also revealed the monophyletic aspect of the genus *Myotis*, divided into two clades (Figure 1); one with support value (aLRT = 46) and formed by *Myotis levis*, *M. nigricans*, *M. albescens* e *M. riparius*; and the other with support value (aLRT = 100), divided into two strains, with

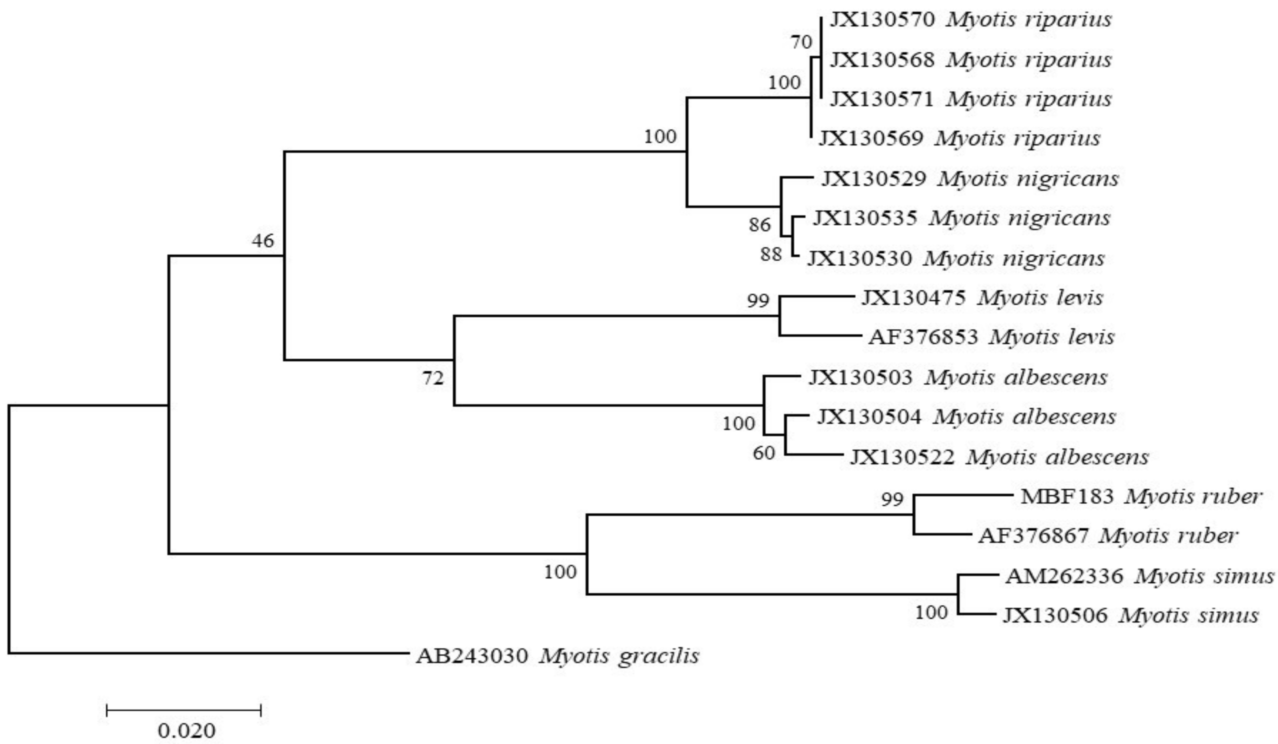

Figure 1. Maximum likelihood topology for cyt-b showing the phylogenetic relationships of *Myotis*.

Myotis simus and *M. ruber*.

Studies using cyt-b have recovered *M. albescens* as belonging to the clades formed by *M. nigricans* and *M. levis*, while *M. simus*, *M. riparius* and *M. ruber* form a distinct clade (Stadelmann *et al*., 2007; Rueddi *et al*., 2013; Moratelli *et al*., 2013; Moratelli *et al*., 2016; Moratelli *et al*., 2017). However, the topology generated in the present work, was structured to group *M. albescens* with *M. levis*, *M. nigricans* and *M. riparius*; with *M. ruber* and *M. simus* forming the second clade. This result, although little statistically supported, was similar to that found by Larsen *et al* (2012), which indicates the presence of lineages not yet known for the genus, exemplified by the values of genetic divergence.

The taxonomic problems found in the group are evident, which implies difficulties in carrying out consistent phylogenetic and phylogeographic studies. Besides that, in Brazil, there is a need for a more extensive geographical sampling of *Myotis*, because there is insufficient genetic data to allow better construction of biogeography, conservation and evolutionary history at national level.

Distribution

The species have alimentary habits, in the majority, insectivorous (Peracchi *et al*., 2010) and many can divider space and food resources without competitive exclusion (Stadelmann *et al*., 2004), for example, *Myotis albescens*, *M. nigricans* and *M. riparius*, since morphological similarity does not necessarily reflect ecological similarity (Saunders & Barclay, 1992). *Myotis albescens* populates the States of Acre, Amapá, Amazonas, Bahia, Mato Grosso do Sul, Minas Gerais, Pará, Paraná, Rio de Janeiro, Rio Grande do Sul, Roraima, and São Paulo (Moratelli & Wilson, 2011; Moratelli *et al*., 2011a; Peracchi *et al*., 2011; Miranda *et al*., 2013) occupying the Amazon, Atlantic Rainforest, Cerrado, and Pantanal domains (Paglia *et al*., 2012), which agrees with the fact that the foraging species, mainly in forested regions (Figure 2A).

Myotis dinelli is recorded in the States of Santa Catarina and Rio Grande do Sul (Passos *et al*., 2010, Miranda *et al*., 2013) and is distributed in Atlantic Rainforest and Pampas (Paglia *et al*., 2012) (Figure 2B).

Myotis izecksohni has records in the States of Paraná and Rio de Janeiro, both in the Atlantic Rainforest domains (Moratelli *et al*., 2011a). Dias *et al.* (2015) reported new records of the species' distribution in the State of Minas Gerais through specimens collected in a region classified as an ecotone between the Atlantic Rainforest and Cerrado, emphasizing the need for more studies to characterize species habits (Figure 2C).

Myotis lavali was first recorded in endemism in the Caatinga (Moratelli *et al*., 2011a) occurring in the States of Pernambuco, Bahia, and Ceará. Moratelli & Wilson (2013) found records of the species in the Atlantic Rainforest domains of the Brazilian Northeast, also reporting occurrence in a part of the Cerrado belong-

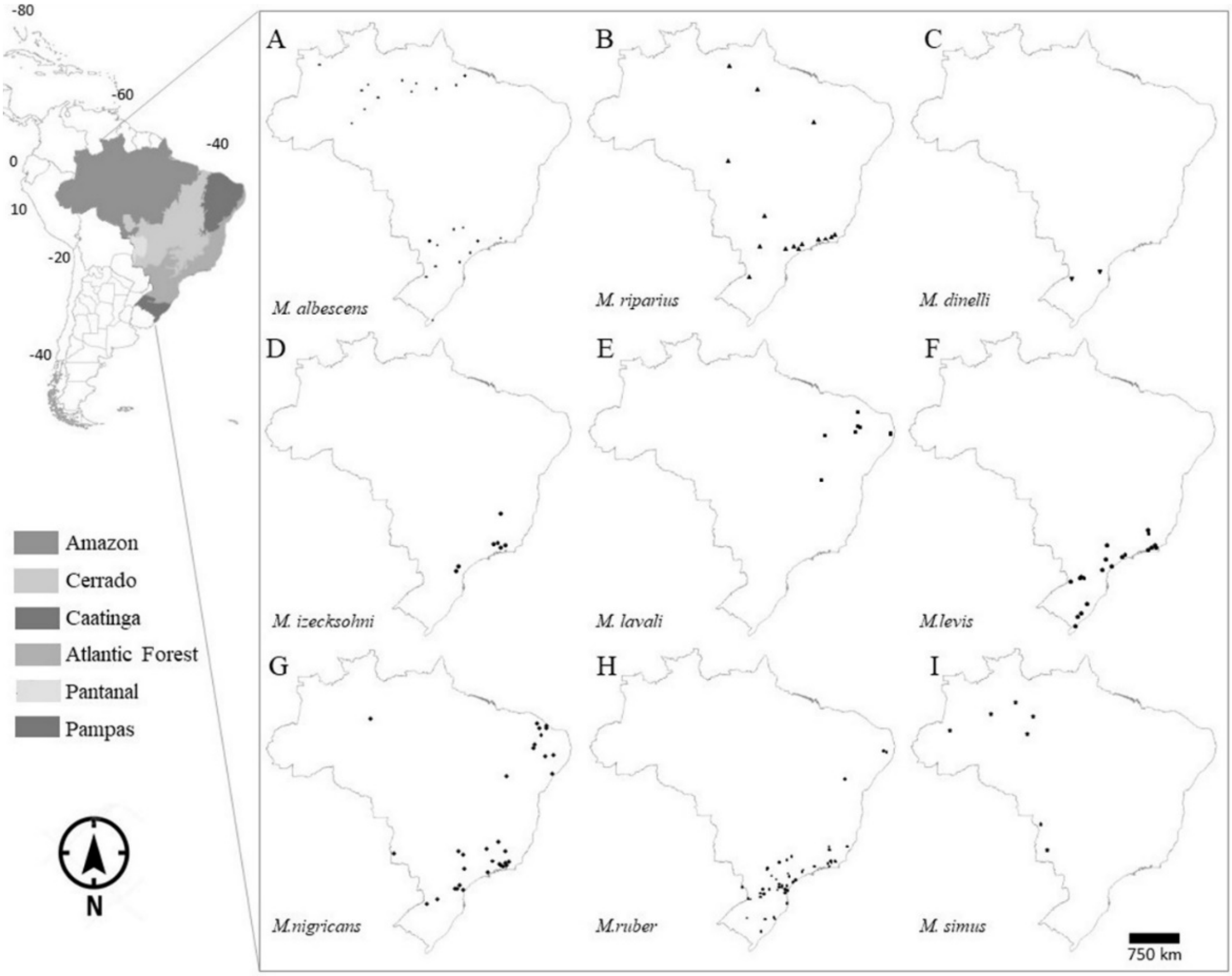

Figure 2. Occurrence of the genus *Myotis* in Brazil based on bibliographic data.

ing to the state of Tocantins, which composes a diagonal corridor. Maas e*t al* (2013) recorded the occurrence of the species in the State of Piauí reinforcing the idea that it may present wider distributions within the Cerrado and Caatinga domains (Figure 2D).

Myotis levis occurs only in Southeastern/Southern Brazil in the States of Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul (Miranda *et al*., 2010; Moratelli *et al*., 2011a; Peracchi *et al*., 2011; Miranda *et al*., 2013). It occurs only in the domains of the Atlantic Rainforest and Pampas (Moratelli & Peracchi, 2007; Paglia *et al.*, 2012; Miranda *et al*., 2013) (Figure 2E).

Myotis nigricans is present through most of the national territory, except for the States of Acre, Rondônia, Tocantins, Maranhão, Piauí, Rio Grande do Norte, and Alagoas (Astúa & Guerra, 2008; Fábian, 2008; Moratelli *et* *al*., 2011a; Peracchi *et al*., 2011; Brito & Bocchiglieri, 2012). This is the most varied species in the genus and can occur in a variety of habitats from mountainous to semi-arid regions (Wilson, 2008) with the potential to spread to a great amount of Brazilian biomes such as the Amazon, Atlantic Rainforest, Cerrado, Caatinga, Pantanal, and Pampas (Brito & Bocchiglieri, 2012; Paglia *et al*., 2012) (Figure 2F).

Myotis riparius is distributed in the States of Acre, Amapá, Amazonas, Pará, Minas Gerais, Bahia, Paraná, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, and São Paulo (Dias & Peracchi, 2007; Moratelli *et al*., 2011a; Peracchi *et al*., 2011; Maas *et al*., 2013; Miranda *et al*., 2013). It is the second most representative species in the genus in terms of distribution levels, devido à sua grande plasticidade adaptativa (Novaes *et al*., 2017). It extends to the Amazon, Atlantic Rainforest, Cerrado, Caatinga, and Pantanal domains (Dias & Peracchi, 2007; Paglia *et al*., 2012) (Figure 2G).

Myotis ruber is found in the States of Pernambuco, Bahia, Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul (Weber *et al*., 2010; Miranda *et al*., 2013; Moratelli *et al*., 2011a; Peracchi *et al*., 2011). It occupies only the Caatinga and Atlantic Rainforest domains (Moratelli & Peracchi, 2007; Paglia *et al*., 2012, being more common in well conserved forest environments (Figure 2H).

Myotis simus displays an endemism for South America as well as *M. izecksohni* and *M. lavali* (LaVal, 1973; Simmons, 2005; Wilson, 2008; Moratelli *et al*., 2011a; Moratelli, 2012). It is the least representative species of the genus in terms of occurrence with distribution recorded in the States of Amazonas, Mato Grosso do Sul, and Pará (Moratelli *et al*., 2011a), and in Amazon domains (Paglia *et al*., 2012). It is worth mentioning that individuals occurring in the State of Mato Grosso do Sul may correspond to another species know (Moratelli *et al*., 2015). In addition, there are records of the species present in the Atlantic Rainforest and in Pantanal (Moratelli, 2012; Paglia *et al*., 2012), being found near aquatic environments (Figure 2).

Cytogenetic

Although considered a specious and successful genre in adaptive issues, *Myotis* presents, karyotypically, high conservatism. Baker & Jordan (1970) determined 2n = 44 e FN = 50, based on cytogenetic studies of *M*. *auriculus*, *M*. *nigricans*, *M*. *simus* and *M*. *riparius*. They described the autosomal complement of the species as three large pairs and a small pair of metacentric chromosomes; and 17 acrocentric pairs ranging in size from medium to small. The sex chromosomes were characterized as an average submetacentric chromosome (X) and a small chromosome acrocentric (Y). Such karyotype was described for another eighteen species of the genus. However, some studies FN = 52 (Strelkov & Volobluev, 1969; Bickham & Hafner, 1978).

Banding techniques proved to be important for studies of systematic and evolutionary aspects (Bickham & Baker, 1976; Bickman & Hafner, 1978). Bickham & Hafner (1978) used G and C band standards for *Myotis myotis* and *M*. *oxynathus*, where both were identical and showed remarkable similarity with the *Miniopterus*, suggesting that both genders reflect the karyotype composition of the ancestor from which they evolved. Bickham & Baker (1976) examined patterns of New World species bands and found small differences in the size of the heterochromatic pair and the smaller autosomes. Bickham (1979) determined 218 bands for *M*. *nigricans*, which presented 25 autosomes with exclusive pattern. Another 11 species presented the same pattern, and three differed minimally. Bickham *et al* (1986) found chromosomal variations only in chromosome size Y and in the presence or absence of short heterochromatic arms in the small autosomes. Some authors indicate that the numbering of each arm is efficient, Robertsonian mergers and translocations are forms that, commonly, cause chromosome change within the family Vespertilionidae (Capanna & Civitelli, 1970; Bickham & Baker, 1976).

Some authors attribute this conservatism to the fact that the genre is older, and, as well, intraspecific and interspecific chromosome variation is uncommon among Vespertilionidae (Baker & Patton, 1967; Baker, 1970; Bickham & Hafner, 1978). Sotero-Caio *et al* (2017) found variation in the number and location of heterochromatic segments and nucleoli organizing regions for the genus, but the diploid number remains unchanged. Evidence suggests that repetitive DNA may play an important role in promoting events of chromosomal rearrangements. Therefore, the authors point out that the exploration of the repetitive region should be considered for a better understanding of the role of non-coding DNA in the chromosome structure.

Cytogenetic studies of the genus *Myotis* in Brazil are uncommon, a factor that is perhaps related to the conservatism presented by the genre, that ends up not arousing interest in these works. However, Ao *et al* (2006), in study of bands, showed chromosomal differences among species of Old World *Myotis*. But, due to the scarcity of resources, it is not possible to apply cytogenetic methods to understand the karyotype evolution of the species, which does not allow this tool to be applicable to the taxonomic diagnosis.

Conclusion

The distribution of the genus varies according to the species, which, for the most part, are distributed over a large part of the Brazilian territory with some coexisting in the same location; *M. nigricans* is the most widespread. A high karyotypic conservatism is observed in all species of the genus, but, studies point to the importance of the use of banding techniques for karyotype delineation. The results obtained through the molecular data using the cyt-b gene corroborate the monophyletic aspect of the genus *Myotis* and the *Myotis ruber* species. However, the number of individuals of the genus *Myotis* sequenced in Brazil is incipient, and with little data of geographical scope, suggesting the need for additional genetic investigations to be able to test any phylogenetic hypotheses.

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References

Alvares, C.A., Stape J.L., Sentelhas, P.C., Gonçalves, J.L.M., Sparovek, G. 2013. Köppen climate classification map for brazil. **Meteorologische Zeitschrift 22**(5): 711-728.

Anisimova, M. & Gascuel, O. 2006. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. **Systematic Biology 55**: 539-552.

Ao, L.; Gu, X.; Feng, Q.; Wang, J.; O'Brien, P.C.M.; Fu, B.; Mao, X.; Su, W.; Wang, Y.; Volleth, M.; Yang, F. & Nie, W. 2006. Karyotype relationships of six bat species (Chiroptera, Vespertilionidae) from China revealed by chromosome painting and G-banding comparison. **Cytogenetic and Genome Research 115**: 145-153.

Astúa, D. & Guerra, D.Q. 2008. Caatinga bats in the Mammal Collection of the Universidade Federal de Pernambuco. **Chiroptera Neotropical 14**(1): 326-338.

Baker, R.J. & Patton, J.L. 1967. Karyotypes and Karyotypic variation of North American vespertilionid bats. **Journal of Mammalogy 48:** 270-286.

Baker, R.J. & Jordan, R.G. 1970. Chromosomal studies of some neotropical bats of the families Emballonuridae, Noctilionidae, Natalidae and Vespertilionidae. **Caryologia: International Journal of Cytology, Cytotosystematics and Cytogenetics 23**(4): 595-604.

Bickham, J.W. & Baker, R.J. 1976. Chromosome homology and evolution of emydid turtles. **Chromosoma (Berl.) 54**: 201-219.

Bickham, J. W. & Hafner, J. C. 1978. A chromosomal banding study of three species of vespertilionid bats from Yuguslavia. **Genetica 48**: 1-3.

Bickham, J. W. 1979. Chromosomal variation and evolutionary relationships of vespertilionid bats. **Journal of Mammalogy 60:** 350-363.

Bickham, J.W.; McBee, C.J. & Schlitter, C.A. 1986. Chromosomal variation among seven species of Myotis (Chiroptera: Vespertilionidae). **Journal of Mammalogy 67**: 746-750.

Brito, D.V. & Bocchiglieri, A. 2012. Comunidade de morcegos (Mammalia: Chiroptera) no Refúgio de Vida Silvestre Mata do Junco, Sergipe, Nordeste do Brasil. **Biota Neotrop 12**: 254-262.

Capanna, E. & Civitelli, M. V. 1970. Chromosomal Mechanisms in the Evolution of Chiropteran Karyotype Chromosomal Tables of Chiroptera. **Caryologia 23**: 79-111.

Casado, F.; Bonvicino, C.R.; Nagle, C; Comas, B.; Manzur, T.D.; Lahoz, M.M. & Seuánez, H.N. 2010. Mitochondrial divergence between 2 populations of the Hooded Capuchin, *Cebus* (*Sapajus*) cay (Platyrrhini, Primates). **Journal of Heredity 101**: 261–269.

Cassens, I.; Vicario, S.; Waddell, V.G.; Balchowsky, H.; Van Belle, D.; Ding, W.; Fan, C;; Lal Mohan, R.S.; Simões-Lopesi, P.C. & Bastida, R. 2000. Independent adaptation to riverine habitats allowed survival of ancient cetacean lineages. **Proceedings of the National Academy of Sciences 97**: 11343–11347.

Dias, D. & Peracchi, A.L. 2007. Primeiro registro de *Myotis riparius* Handley (Mammalia, Chiroptera, Vespertilionidae) no estado do Rio de Janeiro, sudeste do Brasil. **Revista Brasileira de Zoologia 24**: 508-511.

Dias, D.; Carvalho, W. D.; Teixeira, T.S.M.; Tavares D.; Xavier B.S.; Valle, E.L.V. & Esbérard C.E.L. 2015. First record of *Myotis izecksohni* (Chiroptera, Vespertilionidae) for the Atlantic Forest of Minas Gerais, Southeastern Brazil. **Mastozoologia Neotropical 22**(1): 149-153.

Faria, M.B.; Ribeiro, M.C.S.; Oliveira, M.E. & Ferraz, D.S. Estudo da quiropterofauna (Mammalia: Chiroptera) em duas Reservas Particulares do Patrimônio Natural da Mata Atlântica, Minas Gerais, Brasil. 2017. **Boletim da Sociedade Brasileira de Mastozoologia 77**: 117-123.

Findley, J.S. 1972. Phenetic relationships among bats of the genus Myotis. **Systematic Zoology 21**: 31-52.

Gannon, W.L.; Sherwin, R.E.; DeCarvalho, T.N. & O'Farell, M.J. 2001. Pinnae and echolocation call differences between *Myotis californicus* and *M. ciliolabrum* (Chiroptera: Vespertilionidae). **Acta Chiropterologica 3**: 77-91.

Guindon, S. & Gascuel, O. 2003. A simple, fast and accurate algorithm to estimate larges phylogenies by maximum likelihood. **Systematic Biology 52**: 696-704.

Guindon, S.; Dufayard, J.F.; Lefort, V.; Anisimova, M. & Hordijk, W. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. **Systematic Biology 59**: 307-321.

Hasegawa, M.; Kishino, H. & Yano, T.A. 1985. Dating of the human ape splitting by a molecular clock of mitochondrial-DNA. **Journal of Molecular Evolution 22**: 160–174.

Hoffmann, F.G. & Baker, R.J. 2001. Systematics of bats of the genus *Glosssophaga* (Chiroptera: Phyllostomidae) and phylogeography in *G. soricina* based on the cytochrome-b gene. **Journal of Mammalogy 82**: 1092–

1101.

Irwin, D.M.; Kocher, T.D. & Wilson, A.C. 1991. Evolution of the Cytochrome b gene of mammals. **Journal of Molecular Evolution 32**: 128–144.

Keane, T.M.; Creevery, C.J.; Pentony, M.M.; Naughton, T.J. & Mclnerney, J.O. 2006. Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified. **BMC Evolutionary Biology 6**: 1-29.

Koopman, K.F. 1993. Order Chiroptera, pp. 137-241. In: Wilson D.E & Reeder, D.M. **Mammals species of the world: a taxonomic and geographic reference**. Johns Hopkins University Press, Washington, 2142p.

Lack, J.B.; Roehrs, Z.P.; Stanley Jr, C.E.; Ruedi, M. & Van den Bussche, R.A. 2010. Molecular phylogenetics of *Myotis* indicate familial-level divergence for the genus Cistugo (Chiroptera). **Journal of Mammalogy 91**: 976-992.

LARSEN, R.J.; KNAPP, M.C.; GENOWAYS, H.H.; KHAN, F.A.A.; LARSEN, P.A. WILSON, D.E. & BAKER, R.J. 2012. Genetic Diversity of Neotropical *Myotis* (Chiroptera: Vespertilionidae) with an Emphasis on South American Species. **PLoS ONE 7**(10): e46578.

LaVal, R. 1973. A revision of the neotropical bats of the genus *Myotis*. **Natural History Museum Los Angeles Country 15**: 1-54.

López-González, C.; Presley, S.J.; Owen, R.D. & Willig, M.R. 2001. Taxonomic status of *Myotis* (Chiroptera: Vespertilionidae) in Paraguay. **Journal of Mammalogy 82**: 138-160.

Maas, A.C.S.; Dias, D.; Pol, A.; Martins, M.A.; Araújo, R.M.; Gil, B.B.; Schutte, M. & Peracchi, A.L. 2013. New records of bats for the state of Piauí, northeastern Brazil (Mammalia, Chiroptera). **Check List 9**(2): 445-449.

Menezes, A.N.; Bonvicino, C.R. & Seuánez, H.N. 2010. Identification, classification and evolution of Owl Monkeys (*Aotus,* Illiger 1811). **BMC Evolutionary Biology 10**: 248.

Miranda, J.M.D.; Kaku-Oliveira, N.Y.; Munster, L.C.; Bernardi I.P., Moro-Rios, R.F. & Passos, F.C. 2010. Primeiros dados de uma colônia reprodutiva de *Myotis levis* (I. Geoffroy, 1824), nos campos de Palmas, Paraná, Brasil (Vespertilionidae). **Chiroptera Neotropical 16**(2): 762-768.

Miranda, J.M.D.; Pulchério-Leite, A.; Bernardi, I.P. & Passos, F.C. 2013. First record of *Myotis albescens* (É. Geoffroy) from Paraná State, Brazil (Chiroptera: Vespertilionidae). **Biota Neotropica 7**: 231-233.

Moratelli, R. & Morielle-Versute, E. 2007. Métodos e aplicações da citogenética na taxonomia de morcegos brasileiros, pp. 197-218. In: Reis N.R., Peracchi A.L., Pedro W.A. & *Lima* I.P. **Morcegos do Brasil**.

Londrina, Paraná.

Moratelli, R. & Oliveira, J.A. 2011. Morphometric and Morphological variation in South American populations of *Myotis albescens* (Chiroptera: Vespertilionidae). **Zoologia 28**(6): 789-802.

Moratelli, R. & Peracchi, A.L. 2007. Morcegos (Mammalia: Chiroptera) do Parque Nacional da Serra dos Órgãos. **Ciência e Conservação da Serra dos Órgãos**: 193-209.

Moratelli, R. & Wilson, D.E. 2011. The identity of *Myotis punensis* (Chiroptera: Vespertilionidae). **Zoologia 28**(1): 115-121.

Moratelli, R. & Wilson, D.E. 2013. Distribuition and natural history of *Myotis lavali* (Chiroptera, Vespertilionidae). **Journal of Mammalogy 94**(3): 650-656.

Moratelli, R. 2012. *Myotis simus* (Chiroptera: Vespertilionidae). **Mammalian Species 44**(1): 26-32.

Moratelli, R.; Andreazzi, C.S.; Oliveira, J.A. & Cordeiro, J.L.P. 2011a. Current and potential distribuition of *Myotis simus* Thomas (Chiroptera: Vespertilionidae). **Mammalia 75**: 227-234.

Moratelli, R.; Gardner, A.L.; Oliveira, J.A. & Wilson, D.E. 2013. Review of Myotis (Chiroptera, Vespertillionidae) from northern South America, including description of a new species. **American Museum Novitates 3780**: 1-36.

Moratelli, R.; Peracchi, A.L.; Dias, D. & Oliveira, J.A. 2011b. Geographic variation in South American populations of Myotis nigricans (Schinz, 1821) (Chiroptera, Vespertilionidae), with the description of two new species. **Mammalian Biology 76**: 592-607.

Moratelli, R.; Wilson, D. E.; Gardner, A. L.; FisherR, R.; D. & Gutiérrez, E.E. 2016. A new species of *Myotis* from Suriname (Chiroptera, Vespertilionidae). **Occasional Papers, Museum of Texas Tech University 65** : 49–63.

Moratelli, R.; Wilson, D.E.; Novaes, R.L.M.; Helgen, K.M. & Gutiérrez, E.E. 2017. Caribbean *Myotis* (Chiroptera, Vespertilionidae), with description of a new species from Trinidad and Tobago. **Journal of Mammalogy xx** (x):1–15.

Nogueira, M.R.; Lima, I.P.; Moratelli, R.; Tavares, V.C.; Gregorin, R. & Peracchi, A.L. 2014. Checklist of Brazilian bats, with comments on original records. **Check List 10**(4): 808-821.

NOVAES, R.L.A.; SOUZA, R.F. & MORATELLI, R. 2017. *Myotis riparius* (Chiroptera: Vespertilionidae). **Mammalian Species 49**(946): 51-56.

Paglia, A.P.; da Fonseca, G.A.B.; Rylands, A.B.; Herrmann, G.; Aguiar, L.M.S.; Chiarello, A.G.; Leite, Y.L.R.; Costa, L.P.; Siliciano, S.; Kierulff, M.C.M.; Mendes, S.L.; Tavares, V.C.; Mittermeier, R.A. & Patton, J.L. 2012.

Lista anotada dos mamíferos do Brasil/ Annotated checklist of Brazilian mamals. Occasional Papers in Conservation Biology. Arlington, VA.

PASSOS, F.C.; MIRANDA, J.M.D.; BERNARDI, I.P.; KAKU-OLIVEIRA, N.Y. & MUNSTER, L.C. 2010. Morcegos da região Sul do Brasil: análise comparativa da riqueza de espécies, novos registros e atualizações nomeclaturais (Mammalia, Chiroptera). **Iheringia 100**(1): 25-34.

PERACCHI, A.L.; LIMA, I.P.; REIS, N.R.; NOGUEIRA, M.R. & ORTÊNCIO-FILHO, H. 2011. Ordem Chiroptera, pp. 155-234. In: REIS N.R.; PERACCHI A.L.; PEDRO W.A. & LIMA I.P. **Morcegos do Brasil**. Londrina, Paraná.

PORTER, C.A. & BAKER, R.J. 2004. Systematis of vampyressa and related genera of phyllostomid bats as determined by cytochrome-b sequences. **Journal of Mammalogy 85**(1): 126–132.

POSADA, D. & CRANDALL, K.A. 2001. Selecting the best-fit model of nucleotide substitution. **Systematic Biology 50**(4): 580-601.

RUEDI, M. & MAYER, F. 2001. Molecular systematics of bats of the genus Myotis (Vespertilionidae) suggests deterministic ecomorphological convergences. **Molecular Phylogenetics and Evolution 21**(3): 436-448.

RUEDI, M.; ARLETTAZ R. & MADDALENA T. 1990. Distinction morphologique et biochimique de deux espèces jumelles de chauves-souris: *Myotis myotis* (Bork.) et *Myotis blythi* (Tomes) (Mammalia; Vespertilionidae). **Mammalia 54**: 415-429.

RUEDI, M.; STADELMANN, B.; GAGER, Y.; DOUZERY, E.J.P.; FRANCIS, C.M.; LIN, L.K.; GUILLÉN-SERVENT, A. & CIBOIS, A. 2013. Molecular phylogenetic reconstructions identify East Asia as the cradle for the evolution of the cosmopolitan genus *Myotis* (Mammalia, Chiroptera). **Molecular Phylogenetics and Evolution 69**: 437-449.

SAMBROOK, J.; FRITSCHI, E.F. & MANIATIS, T. 1989. **Molecular Cloning: A Laboratorymanual**. New York, Cold Spring Harbor Laboratory Press, 1626p.

SIMMONS, N. 2005. Order Chiroptera, pp. 312–529. In: WILSON, D.E. and REEDER, D.M. **Mammal species of the world: a taxonomic and geographic reference**. Johns Hopkins University Press, Baltimore, Maryland, 1626p.

SMITH, M.F. & PATTON, J.L. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. **Biological Journal of the Linnean Society 50**: 149–177.

SOTERO-CAIO, C.G.; BAKER, R.J. & VOLLETH. 2017. Chromosomal Evolution in Chiroptera. **Genes 8**: 1-58.

STADELMAN, B.; LIN, L.K.; KUNZ, T.H. & RUEDI, M.

2007. Molecular phylogeny of New World (Chiroptera, Vespertilionidae) inferred from mitochondrial and nuclear DNA genes. **Molecular Phylogenetics and Evolution 43**: 32-48.

Stadelmann, B.; Herrera, L.G.; Arroyo-Cabrales, J.; Flores-Martínez, J.J.; May, B.P. & Ruedi, M. 2004. Molecular systematics of the fishing bat Myotis vivesi. **Journal of Mammalogy 85**: 133-139.

Strelkov, P. P. & Volobluev, V. T. 1969. Identićnost' kariotipov v rode *Myotis*. **Ibid**., 14-15.

Tamura, K.; Dudley, J.; Nei, M.; Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. **Molecular Biology and Evolution 24**:1596–1599.

Tate, G.H. 1941. A review of the genus *Myotis* (Chiroptera) of Eurasia, with special reference to species occurring in the East Indies. **Bulletin of the American Museum of Natural History 78**: 573-565.

Tavares, V.C.; Gregorin, R. & Peracchi, A.L. 2008. A diversidade de morcegos no Brasil: Lista atualizada com comentários sobre distribuição e taxonomia, pp. 25-58. In: Pacheco, S.M.; Marques, R.V.; Esbérard, C.E.L. **Morcegos do Brasil: biologia, sistemática, ecologia e conservação**. Armazém digital, Porto Alegre.

Weber, M.M.; Terrible, L.C. & Carceres, N.C. 2010. Potential geographic distribuition of *Myotis ruber* (Chiroptera, Vespertilionidae), a threatened Neotropical bat species. **Mammalia 74**: 333-338.

Wilson, D.E. 2008. Genus Myotis Kaup 1829, pp. 468-481. In: Gardner, A.L. **Mammals of South America**. University of Chicago Press.

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