

NATURAL HISTORY NOTE

Karyotype of *Myotis lavalii* (Chiroptera, Vespertilionidae)

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Myotis lavalii Moratelli et al. 2011 is an insectivorous bat that was recently described from the *M. nigricans* complex based on morphological data from museum specimens of the Brazilian Caatinga (Moratelli et al. 2011). Subsequently, its distribution was extended and the species occurs throughout the South American dry diagonal, including Brazilian Cerrado and Pantanal, Paraguayan Chaco and Argentinean Yungas, in addition to peripheral records in adjacent Atlantic Forest localities in Brazil (Moratelli & Wilson 2013, Weber et al. 2019). Although widely distributed in South America, little biological information on *M. lavalii* is available (Moratelli et al. 2019). Here we describe the karyotype of this species, and discuss the taxonomic and evolutionary implications.

The study area is located in Minas Gerais (Southeastern Brazil) at the Vale do Jequitinhonha region. Bats were sampled in March 2011 and March 2012, from two municipalities, Diamantina (Fazenda Santa Cruz; 18°16'16" S, 43°23'18" W) and Padre Paraíso (Fazenda Palmares; 17°07'18" S, 41°36'48" W). We used 13 to 15 mist-nets (7 × 2.5 m, 19 mm mesh), which remained open for the first six hours after sunset in each locality per campaign. These localities are within the Cerrado domain, and the vegetation is classified as Grassy-Woody Savanna with Deciduous Forest enclaves (IBGE 2012). Mist-nets were set in the grasslands with rocky outcrops, in gallery forests and near human

ABSTRACT

Myotis lavalii is an insectivorous bat that occurs along the South American Dry Diagonal, extending from the Brazilian Caatinga southward to Paraguayan Alto Chaco. This species was described recently and there is little information about its biology. Herein, we describe the conventional karyotype from three males captured in an arboreal savanna from Vale do Jequitinhonha, State of Minas Gerais, Brazil. The diploid number (2n) and the fundamental number of autosomes (FNa) were 44 and 50, respectively. Its karyotype, in Giemsa staining, is similar to others described for Neotropical *Myotis*, and it is not useful to identify specimens.

buildings. A more detailed description of the study area is available in Geise et al. (2017).

Nine adult individuals of *M. lavalii* were collected, consisting of four males and five females. Karyotyping was performed in the field from three males, two from Fazenda Santa Cruz and one from Fazenda Palmares. Vouchers are in the Mammals Collection of the Museu Nacional, Universidade Federal do Rio de Janeiro (MN 79988, 79989, 79990, 79991*, 79992, 79993, 79994*, 79995, 80003*; vouchers karyotyped are marked with asterisk).

Metaphasic chromosomes were obtained through in vitro bone marrow culture grown in a solution of Dulbecco's MEM, supplemented with 10% fetal bovine serum and colchicine for 2 hours, following by an incubation in KCl 0.075M solution at 37 °C for 30 minutes, centrifugation, fixation in Carnoy solution (methanol:acetic acid, 3:1 v/v), as described by Geise (2014). The fixation step was repeated three times. Preparation was done by dropping one drop by distance onto clean microscope slides and air-dried. Conventional staining with Giemsa 5% buffered solution was used to describe diploid number (2n), fundamental number of autosomes (FNa; which does not include the count of arms of sex chromosomes), and chromosome morphology and size. This analysis was carried out using an optic

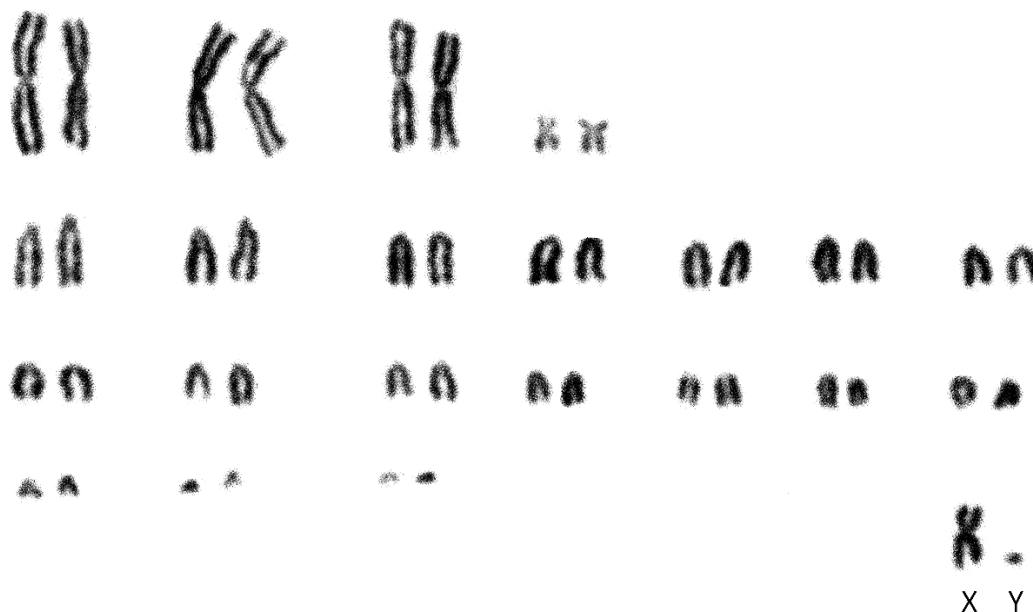


Fig. 1 - Conventional staining karyotype of an adult male of *Myotis lavalii* (MN 79994; $2n = 44$, $NF = 50$) from Diamantina, State of Minas Gerais, Brazil.

photomicroscope (Nikon Eclipse 50i), with a 1,000 increase—lens of 100 plus 10 ocular lenses. Fieldwork was conducted under IBAMA (1785/89-IBAMA) and SISBIO (4156/95-46) permits.

All three specimens showed the same karyotype. The diploid number ($2n$) and the fundamental autosomal number (FN) were 44 and 50, respectively. The karyotype is composed by four metacentric and 17 acrocentric pairs. The X is a medium metacentric, and the Y is a small acrocentric (Fig. 1). This karyotype is similar to others described for New and Old-World species of *Myotis* (i.e. Baker & Jordan 1970, Moratelli & Morielle-Versute 2007, Moratelli et al. 2019), and it is not useful in species delimitation or to identify specimens.

Karyotypes have been used to establish phylogenetic relationships and as a taxonomic tool to confirm species identities (Baker 1970, Baker et al. 1985, Bickham 1979, Volleth & Heller 1994, Ribeiro et al. 2003). However, the cytogenetic information does not have the same resolution power for all taxa. Although the most Vespertilionidae comprises the richest and widest distributed bat group, the karyotype remodeling did not participate in the diversification processes of this group (Sotero-Caio et al. 2017). In general, vespertilionids present very conserved karyotypes in the generic level, except for few genera (Baker & Bickham 1980, Baker et al. 1982). Bickham (1979) proposed $2n = 44$, $NF = 50$ as a primitive condition for vespertilionids. Thus, *Myotis* karyotype would be close and very conserved when compared to the primitive condition, since this is the karyotype found in virtually all congeners (Baker & Jordan 1970, Bickham 1979, Bickham et al. 1986, Varella-Garcia et al. 1989, Volleth & Heller 2012). Currently 127 species of *Myotis* are recognized worldwide (Moratelli et al. 2019) and the karyotype has been described for just over half of them (ca. 70 species; Baker & Jordan 1970, Harada & Yosida 1978,

Bickham 1979, Moratelli & Morielle-Versute 2007, Volleth & Heller 2012). Except for *Myotis annectans* Dobson, 1871 from Thailand ($2n = 46$, $NF = 54$; Bickham et al. 1986) and *Myotis laniger* (Peters, 1871) from China ($2n = 48$, $NF = 54$; Zhang 1984), the other *Myotis* species—for which the karyotype is known—have karyotype with 44 chromosomes.

Although karyotype descriptions are informative for other systematic rearrangements (e.g. Parlos et al. 2014), has low applicability for the *Myotis* taxonomy, so that, the intrageneric comparison of *Myotis* using chromosome painting has revealed total conservation of syntenic blocks (Ao et al. 2006). However, the use of comparative genomic mapping using whole chromosome probes will be more informative about karyotypic evolution and differentiation among species of the genus. For such purposes we will need new captures and chromosomal preparations. Finally, the description of the karyotypes consists of basic information about the genetic diagnosis of species and is important for describing biodiversity and evolutionary history of taxa. Therefore, new karyotype descriptions and cataloging chromosome numbers are relevant data to understand bat diversity, including New World *Myotis*.

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REFERENCES

- AO, L., GU, X., FENG, Q., WANG, J., O'BRIEN, P. C., FU, B., MAO, X., SU, W., WANG, Y., VOLLETH, M., et al. (2006). Karyotype relationships of six bat species (Chiroptera, Vespertilionidae) from China revealed by chromosome painting and G-banding comparison. *Cytogenet Genome Res*, 115(2): 145-53. <https://doi.org/10.1159/000095235>
- BAKER, R. J. (1970). Karyotypic trends in bats. In: *Biology of bats*. ed.: Academic Press, INC. New York, USA, p.65-97.
- BAKER, R. J. & JORDAN, R. G. (1970). Chromosomal Studies of some Neotropical Bats of the Families Emballonuridae, Noctilionidae, Natalidae and Vespertilionidae. *Caryologia*, 23(4): 595-604. <https://doi.org/10.1080/0087114.1970.10796397>
- BAKER, R. J. & BICKHAM, J. W. (1980). Karyotypic evolution in bats: evidence of extensive and conservative chromosomal evolution in closely related taxa. *Syst Zool*, 29(3): 239-253. <https://doi.org/10.2307/2412660>
- BAKER, R. J., HAIDUK, M. W., ROBBINS, L. W., CADENA, A. & KOOP, B. F. (1982). Chromosomal studies of South American bats and their systematic implications. In: *Mammalian Biology South America*. ed.: Pymatuning Lab. Ecol. Linesville, Pennsylvania, USA, p.303-327.
- BAKER, R., BICKHAM, J. & ARNOLD, M. (1985). Chromosomal Evolution in Rhogeessa (Chiroptera: Vespertilionidae): Possible Speciation by Centric Fusions. *Evolution*, 39(2): 233-243. <https://doi.org/10.2307/2408359>
- BICKHAM, J. W. (1979). Chromosomal Variation and Evolutionary Relationships of Vespertilionid Bats. *J Mammal*, 60(2): 350-363. <https://doi.org/10.2307/1379807>
- BICKHAM, J., MCBEE, K. & SCHLITTER, D. (1986). Chromosomal Variation among Seven Species of *Myotis* (Chiroptera: Vespertilionidae). *J Mammal*, 67(4): 746-750. <https://doi.org/10.2307/1381139>
- GEISE, L. (2014). Procedimentos Genéticos Iniciais na Captura e Preparação de Mamíferos. In: *Técnicas de Estudos Aplicadas aos Mamíferos Silvestres Brasileiros*. ed.: Technical Books. Rio de Janeiro, Brazil, p.221-235.
- GEISE, L., PEREIRA, L. G., ASTÚA, D., AGUIEIRAS, M., LESSA, L. G., ASFORA, P. H., DOURADO, F. & ESBÉRARD, C. E. L. (2017). Terrestrial mammals of the Jequitinhonha River Basin, Brazil: a transition area between Atlantic Forest and Cerrado. *Mastozoología Neotropical*, 24(1): 95-119.
- HARADA, M. & YOSIDA, T. H. (1978). Karyological study of four Japanese *Myotis* bats (Chiroptera, Mammalia). *Chromosoma*, 65: 283-291 (1978). <https://doi.org/10.1007/BF00327623>
- IBGE. (2012). Manual técnico da vegetação brasileira. Second Edition. ed.: Instituto Brasileiro de Geografia e Estatística. Rio de Janeiro, Brazil, 274 pp.
- MORATELLI, R. & MORIELLE-VERSUTE, E. (2007). Métodos e aplicações da citogenética na taxonomia de morcegos brasileiros. In: *Morcegos do Brasil*. ed.: Editora da Universidade Estadual de Londrina. Londrina, Brazil, p.197-218.
- MORATELLI, R., PERACCHI, A. L., DIAS, D. & OLIVEIRA, J. A. (2011). Geographic variation in South American populations of *Myotis nigricans* (Schinz, 1821) (Chiroptera, Vespertilionidae), with the description of two new species. *Mamm Biol*, 76: 592-607. <https://doi.org/10.1016/j.mambio.2011.01.003>
- MORATELLI, R. & WILSON, D. E. (2013). Distribution and natural history of *Myotis lavalii* (Chiroptera, Vespertilionidae). *J Mammal*, 94(3): 650-656. <https://doi.org/10.1644/1545-1542-94.4.962>
- MORATELLI, R., BURGÍN, C. J., CLÁUDIO, V. C., NOVAES, R. L. M., LÓPEZ-BAUCELLS, A. & HASLAUER, R. (2019). Family Vespertilionidae (Vesper Bats). In: *Handbook of the Mammals of the World, Bats*, Vol. 9. ed.: Lynx Editions. Barcelona, Spain, p.716-981.
- PARLOS, J. A., TIMM, R. M., SWIER, V. J., ZEBALLOS, H. & BAKER, R. J. (2014). Evaluation of the paraphyletic assemblage within Lonchophyllinae, with description of a new tribe and genus. *Occasional Papers, Museum of Texas Tech University*, 320(1): 1-23.
- RIBEIRO, N. A. B., NAGAMACHI, C. Y., PIECZARKA, J. C., RISSINO, J. D., NEVES, A. C. B., GONÇALVES, A. C. O., MARQUES-AGUIAR, S., ASSIS, M. F. L. & BARROS, R. M. S. (2003). Cytogenetic analysis in species of the Subfamily Glossophaginae (Phyllostomidae, Chiroptera) supports a polyphyletic origin. *Caryologia*, 56(1): 85-96. <https://doi.org/10.1080/00087114.2003.10589311>
- SOTERO-CAIO, C. G., BAKER, R. J. & VOLLETH, M. (2017). Chromosomal evolution in Chiroptera. *Genes*, 8(272): 1-25. <https://doi.org/10.3390/genes8100272>
- VARELLA-GARCIA, M., MORIELLE-VERSUTE, E. & TADDEI, V. A. (1989). A survey of cytogenetic data on Brazilian bats. *Rev Bras Genet*, 12(4): 761-793.
- VOLLETH, M. & HELLER, K. -G. (1994). Phylogenetic relationships of vespertilionid genera (Mammalia: Chiroptera) as revealed by karyological analysis. *J Zool Syst Evol Res*, 32(1): 11-34. <https://doi.org/10.1111/j.1439-0469.1994.tb00467.x>
- VOLLETH, M. & HELLER, K. -G. (2012). Variations on a theme: karyotype comparison in Eurasian *Myotis* species and implications for phylogeny. *Vespertilio*, 16: 329-350.
- WEBER, M. M., NOVAES, R. L. M., DELGADO-JARAMILLO, M., BARBIER, E., CLÁUDIO, V. C., BERNARD, E. & MORATELLI, R. (2019). Is *Myotis lavalii* (Chiroptera, Vespertilionidae) endemic to the South American dry diagonal? *J Mammal*, 100(6): 1879-1888. <https://doi.org/10.1093/jmammal/gyz141>
- ZHANG, W. (1984). Chromosome analysis of *Myotis chinensis* Tomes and *Myotis laniger* Peters. *Journal of Anhui Normal University - Natural Science Edition*, 84: 42-47