Distribution and karyotypic variation of Brazilian molossid bats (Chiroptera, Mammalia)

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Recent biogeographic studies have shown that geographically distant populations of different animal groups, including bats, can present genetic differentiation. Given this, the objective here was to study the composition of Molossidae species that occur in Brazil and investigate whether these species present karyotypic differences between populations from different biomes. A bibliographical survey was carried out, and the karyotypes found for each species were analyzed and compared in relation to the diploid number of chromosomes, the fundamental number of arms of the autosomal chromosomes, the centromeric position, and the presence of other structural chromosomal variations. The results showed that of the 32 species of Molossidae recorded for Brazil, 14 have their karyotypes described. Of these, only nine refer to specimens collected in Brazil. For *Molossus molossus*, karyotypic variations were observed between the Amazon and the Atlantic Forest, and within the Amazon, in regions separated by the Amazon River. *Molossops temminckii* showed variations from the Atlantic Forest. *Molossus rufus* showed variation between populations from the Atlantic Forest and *Caatinga*. The variations observed were structural in autosomal and sexual chromosomes between different populations. The species *Cynomops abrasus*, *Eumops glaucinus*, and *Nyctinomops laticaudatus* have studies only for the Atlantic Forest region, while *Eumops hansae* has karyotypic studies only for the Amazon region. This study showed the existence of karyotypic variation between different populations of five species of molossids. Furthermore, it highlighted the need for investment in knowledge of family karyology, which is scarce, with the aim of better understanding aspects of karyotypic evolution in this group.

Estudios biogeográficos recientes han demostrado que poblaciones geográficamente distantes de diferentes grupos de animales, incluidos los murciélagos, pueden presentar diferenciación genética. Ante esto, el objetivo fue estudiar la composición de las especies de Molossidae presentes en Brasil e investigar si estas especies presentan diferencias cariotípicas entre poblaciones de diferentes biomas. Se realizó un levantamiento bibliográfico y los cariotipos encontrados para cada especie fueron analizados y comparados en relación con el número diploide de cromosomas, el número fundamental de brazos de los cromosomas autosómicos, la posición centromérica y la presencia de otras variaciones cromosómicas estructurales. Los resultados mostraron que de las 32 especies de Molossidae registradas para Brasil, 14 tienen el cariotipo descrito. De ellos, sólo nueve se refieren a ejemplares recolectados en Brasil. Para *Molossus molossus*, se observaron variaciones cariotípicas entre la Amazonía y la Mata Atlántica, y dentro de la Amazonia, en regiones separadas por el río Amazonas. *Molossops temminckii* mostró variaciones entre poblaciones en el estado de Minas Gerais. *Eumops perotis y Cynomops planirostris* también mostraron diferenciación entre poblaciones de la Amazonía y la Mata Atlántica. *Molossus rufus* mostró variación entre poblaciones. Las especies *Cynomops abrasus, Eumops glaucinus* y *Nyctinomops laticaudatus* tienen estudios sólo para la región de la Mata Atlántica, mientras que *Eumops hansae* tiene estudios cariotípicos sólo para la región amazónica. Este estudio mostró la existencia de variación cariotípica entre diferentes poblacionnes de cinco especies de molósidos. Además, destacó la necesidad de invertir en el conocimiento de la cariología familiar, que es escaso, con el objetivo de comprender mejores aspectos de la evolución cariotípica en este grupo.

Keywords: Brazil; karyological variation; Free-tailed bats; Molossidae; Phytophysionomy.

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Introduction

Molossids are known as free-tailed bats, morphologically characterized by a tail that extends about one-third beyond the outer edge of the uropatagium and the presence of a "hairbrush" on the outer toes (Gregorin and Cirranello 2016). This family has fast and long-lasting flight, adapted to open areas, which is reflected in their aerodynamic design of head, ears, and wings, and the quadrupedal habit, rare in Chiroptera (Vaughan 1966). The Molossidae family

currently includes 23 genera and 132 described species, with a circumtropical distribution (Gregorin and Cirranello 2016; Simmons and Cirranello 2023; see Wilson and Mittermeier 2019). In Brazil, eight genera and 32 species are registered, widely distributed throughout the national territory (Garbino et al.20224).

Currently, many studies have been carried out on the processes that resulted in the current patterns of distribution and differentiation of organisms. These works have shown that climate fluctuations promoted cycles of expansion and contraction of different vegetation formations in Brazil (Costa 2003; Werneck *et al.* 2012; Batalha-Filho *et al.* 2013). These events would have influenced the dispersal, genetic differentiation of populations, and speciation of different organisms, such as birds (Beven *et al.* 1984; Batalha-Filho *et al.* 2013), reptiles (Werneck *et al.* 2012), rodents (Costa 2003), and bats (Martins et al. 2009; Pavan *et al.* 2011; Silva *et al.* 2023).

Dealing with Chiroptera in more detail, a study carried out by Martins et al. (2009)) on Desmodus rotundus (Phyllostomidae) indicated through molecular analyses that there is a genetic structuring of species populations coinciding with the division of biomes in South America. Pavan et al., (2011) showed that Carollia perspicillata and C. brevicauda also exhibited genetic structuring of their populations throughout their distribution, coinciding with phytogeographic variation. A study carried out by Loureiro et al. (2020a) showed that some species of the genus Molossus presented structuring between populations distributed both on the continent and on islands suggesting a certain degree of genetic differentiation between populations of different species of the genus north and south of the Amazon River. Therefore, it is possible that other species in the family have been influenced by geoclimatic processes, such as the separation between humid forests, affecting gene flow and leading to the accumulation of distinct characteristics between populations.

Among the several ways of accessing the evolutionary differences between different taxa is cytogenetics, a field of study in biology that, based on the use of different techniques, allows the observation of numerical and structural characteristics of the chromosomes of distinct organisms (Varella-Garcia and Taddei 1989). Cytogenetics is, therefore, a method of studying karyotypic diversity and the variations that exist between different individuals, populations, species, and biological groups.

This field of research has been aiding in the process of identifying species, especially in groups where there may be taxonomic controversies, such as Rodentia (Bonvicino and Weksler 1998; Christoff et al. 2000) and Chiroptera (Eick et al. 2007; Ao et al. 2006; Moratelli et al. 2007; de Lemos Pinto et al. 2012). Furthermore, this tool can be very important for understanding the biogeography of some groups, such as the African murids studied by Granjon and Dobigny (2003), the Neotropical cichlids studied by Thompson (1979), and the species Rhinophylla fischerae from Phyllostomidae, which may represent more than one species, according to geographic variation in the karyotype (Gomes et al. 2010). For molossids, however, studies have generally focused on describing the karyotypes of the species without aiming to cytogenetically compare species collected in different regions or biomes of Brazil. The studies by Morielle-Versute et al. (1996) and Corrêa (2016), for example, described the karyotype of the species Cynomops planirostris and Molossus molossus for the Atlantic Forest region and the Amazon, respectively. However, no studies regarding possible karyotypic variations between populations of these species have been conducted. Seeking this information is of great value from a conservation perspective, which aims to protect the diversity and uniqueness of species and their populations (<u>Palacios-Mosquera *et al.* 2020</u>), as well as from an evolutionary perspective, which aims to understand the biological processes that led to current diversity (<u>Santos *et al.* 2019</u>).

Given this, the central objective of the present study was to conduct a survey of Molossidae species that occur in Brazil and investigate whether they exhibit structural and/ or numerical karyotypic differences between their populations. In more detail, the objectives were: (i) to map the distribution of molossid species in Brazil, identifying their distribution across different biomes; (ii) to carry out a karyotypic survey of these species; and (iii) to comparatively analyze the karyotypes obtained from each species, aiming to identify karyotypic variations between populations from different biomes.

Materials and methods

The survey of the karyotypic descriptions of the species and their occurrence records was carried out by consulting the online databases Google Scholar, SCIELO (The Scientific Electronic Library Online), BHL (Biodiversity Heritage Library), GBIF (Global Biodiversity Information Facility), and ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade). The nomenclature of bat species follows <u>Garbino et al. (2022)</u>.

A compilation of occurrence records obtained from bibliographic research was carried out to understand the distribution of species across different biomes. The locations of occurrence records for each species were plotted on distribution maps, created using QGIS software. Additionally, the locations of the karyotypes described in the literature were also indicated on maps.

The karyotypes obtained through bibliographic research were reorganized according to the morphology and position of the centromeres, following Levan *et al.* (1964). The karyological data for each species were analyzed in relation to the diploid number of chromosomes (2n), the fundamental number of autosomal chromosome arms (FN), the centromeric position, and the presence of other structural chromosomal variations. Subsequently, comparisons were made between the different karyotypes described in the literature for each species.

Results

Distribution of species by Biome. Currently, there are records in Brazil for eight genera and 32 species of molossids (<u>Garbino et al. 20234</u>), which are widely distributed across Brazilian biomes. The species distribution data in the surveyed biomes are organized and summarized in Table 1. **Table 1.** Species of Molossidae recorded in Brazil and the respective biomes in which they occur. Amazon (Am); Atlantic Forest (AF); Cerrado (Ce); Caatinga (Ca); Pantanal (Pt); Pampa (Pp).

Species	Biomes	References	
Cynomops abrasus	Am, AF, Ce, Ca, Pt	Paglia <i>et al.</i> 2012	
Cynomops greenhalli	Am, Ca	Paglia <i>et al.</i> 2012	
Cynomops milleri	Am	Moras et al. 2018	
Cynomops mastivus	Am	Moras et al. 2016	
Cynomops planirostris	Am, AF, Ce, Ca, Pt	Santos <i>et al.</i> 2015; Mendes <i>et al.</i> 2020	
Eumops auripendulus	Am, AF, Ce, Ca, Pt	Eger 1977, 2008	
Eumops bonariensis	AF, Ce, Pp	Eger 2008; Bordignon, 2006; Bernardi <i>et al.</i> 2009	
Eumops chimaera	AF	Gregorin <i>et al.</i> 2016	
Eumops dabbenei	Pt	Fischer <i>et al.</i> 2015	
Eumops delticus	Am, AF, Ce	Eger 2008; Silva <i>et al</i> . 2013	
Eumops glaucinus	Am, AF, Ce, Ca, Pt	Paglia <i>et al.</i> 2012	
Eumops hansae	Am, AF, Ce	Paglia <i>et al</i> . 2012	
Eumops maurus	Am, AF, Ce	Eger 2008; Sodré <i>et al</i> . 2008; Díaz, 2011	
Eumops patagonicus	AF, Pp, Pt	Bernardi <i>et al.</i> 2009; Bordignon <i>et al.</i> 2011; Carvalho <i>et al.</i> 2017	
Eumops perotis	Am, AF, Ce, Ca, Pt	Torres et al. 2020	
Eumops trumbulli	Am	Paglia <i>et al.</i> 2012	
Molossops neglectus	Am, AF	Althoff et al. 2018	
Molossops temminckii	Am, AF, Ce, Ca, Pt	Nunes <i>et al.</i> 2013	
Molossus aztecus	Am, AF, Ce, Ca, Pt	Loureiro <i>et al.</i> 2018	
Molossus coibensis	Am, AF, Ce, Ca	Loureiro <i>et al.</i> 2018	
Molossus currentium	Am, Pt	Paglia <i>et al.</i> 2012; Loureiro <i>et al</i> . 2018	
Molossus fluminensis	AF, Ce, Pt	Loureiro <i>et al</i> . 2020a	
Molossus molossus	Am, AF, Ce, Ca, Pt, Pp	Rocha <i>et al</i> . 2010; Barros <i>et al</i> . 2014; Loureiro <i>et al</i> . 2018	
Molossus pretiosus	Ce, Pt	Loureiro <i>et al</i> . 2018	
Molossus rufus	Am, AF, Ce, Ca, Pt	Souza <i>et al.</i> 2016; Loureiro <i>et al</i> . 2018	
Neoplatymops mattogrossensis	Am, AF, Ce, Ca	Novaes et al. 2013	
Nyctinomops aurispinosus	AF, Ce, Ca	Oliveria <i>et al.</i> 2019	
Nyctinomops laticaudatus	Am, AF, Ce, Ca, Pt, Pp	Paglia <i>et al.</i> 2012	
Nyctinomops macrotis	Am, AF, Ce, Ca, Pt	Rocha <i>et al</i> . 2015	
Promops centralis	Am, AF, Ce, Ca, Pt	Hintze, <i>et al</i> . 2020	
Promops nasutus	Am, AF, Ce, Ca, Pt, Pp	Paglia <i>et al</i> . 2012	
Tadarida brasiliensis	Am, AF, Ce, Ca, Pt, Pp	Tavares et al. 2008	

Based on the bibliographic survey of distribution data carried out in this study, it was observed that three of the molossid species recorded in Brazil (*Cynomops mastivus*, *Cynomops milleri* and *Eumops trumbulli*) occur only in the Amazon region. Two species (*Eumops chimaera* and *Molossus fluminensis*) have records for the Atlantic Forest in Brazil, both with a probable distribution in the Cerrado and Pantanal, since they are also recorded in Bolivia (Taylor et al. 2019). Molossops neglectus occurs only in the Amazon and the Atlantic Forest. *Cynomops greenhalli* is found in the Amazon and Caatinga, while *Molossus currentium* has

records in the Amazon and Pantanal. *Eumops bonariensis* is concentrated in the Atlantic Forest, Cerrado, and Pampas; *Eumops patagonicus* is found in the Atlantic Forest, Pantanal, and Pampas; and *Nyctinomops aurispinosus* occurs in the Atlantic Forest, Caatinga, and Cerrado. *Molossus pretiosus* is recorded only in the Cerrado, Pantanal, and NW Amazon. The remaining 20 species have broader occurrences in Brazilian biomes (Taylor et al. 2019).

Karyotypic variation in Brazilian species. Of the 32 species of Molossidae recorded for Brazil, 14 have published karyotypic descriptions, with only nine referring to specimens collected in Brazil (Table 2). *Cynomops planirostris, Eumops perotis,* and *Molossus molossus* have karyotypic studies for both the Amazon and Atlantic Forest regions. *Molossus rufus* has karyotype studies in the Atlantic Forest, Cerrado, and Caatinga regions. *Cynomops abrasus, Eumops glaucinus, Molossops temminckii,* and *Nyctinomops laticaudatus* have studies only for the Atlantic Forest region, while *Eumops hansae* has a karyotypic study only for the Amazon region (Table 2).

The next section details the distribution of species with available karyotypic studies available in the literature and describes the karyotypic studies identified in the bibliographic survey.

Distribution and description of the karyotypic variation of the species

Molossus E. Geoffroy, 1805

Molossus molossus (Pallas, 1766)

Type locality: Martinique, Lesser Antilles.

Distribution: The distribution of *Molossus molossus* is extensive, covering southern North America, Central America, and South America. Records indicate its occurrence in all Brazilian biomes (<u>Peracchi et al. 2011; Barros et al. 2014;</u> Loureiro et al. 2018; Figure 1).

Karyology: The karyotypes described for *Molossus* molossus by Lopes (1978) for eastern Pernambuco and by Cristoff and Freitas (1987) for Rio Grande do Sul, according to Varella-Garcia et al. (1989), presented 2n = 48 and FN = 56. However, other karyotypes described for regions in the Amazon (Corrêa and Bonvicino 2016) and Atlantic Forest (Morielle-Versute et al. 1996) have 2n = 48 and FN = 64. Karyotypic studies for *Molossus molossus* in Brazil generally describe the species' autosomal chromosomes as consisting of one pair of very large submetacentrics, three pairs of medium submetacentrics, five pairs of medium to small subtelocentrics and 14 pairs of medium to small acrocentrics (Morielle-Versute et al. 1996; Faria 2003; Brandão 2015). Leite-Silva et al. (2003) describe a large submetacentric pair, eight pairs of medium-sized two-armed chromosomes, and 14 medium-to-small-sized acrocentric pairs. Corrêa (2016) describes a pair of large metacentric chromosomes, eight pairs of metacentric or submetacentric chromosomes, ranging from large to medium, and 14 pairs of acrocentric chromosomes, ranging from large to small.

Regarding sex chromosomes, <u>Morielle-Versute et al.</u> (1996) and <u>Leite-Silva et al.</u> (2003) describe the X chromosome as a medium-sized submetacentric and the Y chromosome as a small subtelocentric. <u>Faria</u> (2003) described the X chromosome as a medium-sized submetacentric and the Y chromosome as a small acrocentric. <u>Corrêa and Bonvicino</u> (2016) described the X chromosome as a large metacentric and the Y chromosome as a small acrocentric.



Figure 1. Map of the distribution and location of karyotypic studies of *Molossus* molossus. Black dots: occurrence records. Blue Crosses: records that have karyotypic data described.

Molossus rufus E. Geoffroy, 1805

Type locality: Caiene, French Guiana.

Distribution: *Molossus rufus* is widely distributed in South America. However, Loureiro et al. (2020b) recently revalidated *Molossus fluminensis* as a distinct species, with type locality at Rio de Janeiro, and previously considered a junior synonym of *Molossus rufus*. Loureiro et al. (2020b) describe the distribution of *M. fluminensis* as being in southeastern Brazil, Bolivia, Paraguay, and Argentina, and the distribution of *M. rufus* as being in central and northern Brazil, Bolivia, Ecuador, French Guiana, Guyana, Peru, Suriname, Trinidad and Tobago, and Venezuela. In Brazil, *M. rufus* is recorded in the Amazon, Caatinga, Cerrado, Atlantic Forest. *M. fluminensis* is recorded in Atlantic Forest, Cerrado, Pantanal, and Pampa (Souza et al. 2016; Loureiro et al. 2018; Loureiro et al. 2020b; Figure 2).

Karyology: In studies carried out by <u>Morielle-Versute</u> <u>et al. (1996)</u>, <u>Faria (2003)</u>, and <u>Leite-Silva et al. (2003)</u> with specimens collected in the Atlantic Forest, the species *Molossus rufus* presented 2n = 48 and FN = 64. In the study by <u>Leal (2012)</u> for the Caatinga, 2n = 48 and FN = 62 was found. <u>Leal (2012)</u> also mentions that Dantas (2004) and Sousa (2007) also found 2n = 48 and FN = 62 in their studies with specimens collected in Pará and Piauí, respectively.

The karyotypic studies of *Molossus rufus* conducted by <u>Morielle-Versute *et al.* (1996)</u> and <u>Faria (2003)</u> describe the

species' autosomal chromosomes as consisting of one pair of large submetacentrics, three pairs of medium submetacentrics, five pairs of medium to small subtelocentrics, and 14 pairs of medium to small acrocentrics. The study by Leite-Silva et al. (2003) describes the karyotype as having a large submetacentric pair, eight pairs of medium-sized two-armed chromosomes, and 14 medium- to small-sized acrocentric pairs. The study by Leal (2012) reports eight pairs of metacentric and submetacentric chromosomes, one of which is large and the others medium in size, along with 15 acrocentric pairs that range from medium to small. Sex chromosomes were characterized by Morielle-Versute et al. (1996) and Leite-Silva et al. (2003) as having an X chromosome that is medium-sized and submetacentric, and a Y chromosome that is small and subtelocentric. According to Faria (2003) and Leal (2012), the X chromosome is mediumsized and submetacentric, while the Y chromosome is small and acrocentric.



Figure 2. Map of the distribution and location of karyotypic studies of *Molossus rufus* and *M. fluminensis*. Black dots: occurrence records of *M. rufus*. Blue Crosses: records that have karyotypic data described for *M. rufus*. Gray triangle: potential occurrence records of *M. fluminensis*. Orange Crosses: records that have karyotypic data described, potentially, for *M. fluminensis*.

Molossops Peters, 1866

Molossops temminckii (Burmeister, 1854)

Type locality: Lagoa Santa, Minas Gerais, Brazil.

Distribution: *Molossops temminckii* occurs in South America, with records in Argentina, Bolivia, Brazil, Colombia, Ecuador, Guyana, Paraguay, Peru, Uruguay, and Venezuela (Eger 2008). In Brazil, it has been recorded in the Amazon, Caatinga, Cerrado, Atlantic Forest, and Pantanal (Nunes et al. 2013; Figure 3).

Karyology: The karyotypic studies of *Molossops tem*minckii conducted by <u>Morielle-Versute et al. (1996)</u> and by <u>Santos (2013)</u> observed 2n = 48 and FN = 68. The karyotypic morphology of autosomal chromosomes was described by Morielle-Versute *et al.* (1996) as one large submetacentric pair, two medium-sized submetacentric pairs, eight subtelocentric pairs, and 12 medium-to-small acrocentric pairs. Santos (2013) described three metacentric, four submetacentric, five subtelocentric, and 10 acrocentric pairs. It is noted that there may be an error in chromosome counting in Santos (2013), with possibly 11 pairs of acrocentric chromosomes instead of 10. Morielle-Versute *et al.* (1996) described the sex chromosomes with the X as a medium-sized subtelocentric and the Y as a small subtelocentric. Santos (2013) only analyzed the karyotype of a female, thus describing the X chromosome as subtelocentric.



Figure 3. Map of the distribution and location of karyotypic studies of *Molossops temminckii*. Black dots: occurrence records. Blue Crosses: records that have karyotypic data described.

Eumops Miller, 1906

Eumops perotis (Schinz, 1821)

Type locality: Campos dos Goytacazes, Rio de Janeiro, Brazil.

Distribution: According to Eger (1977), Eumops perotis has two disjoint populations: one in North America, identified as Eumops perotis californicus, and another in South America, recognized as Eumops perotis perotis. In Brazil, it has been recorded in the Amazon, Caatinga, Cerrado, Atlantic Forest, and Pantanal (Torres et al. 2020; Figure 4).

Karyology: According to <u>Varella-Garcia (1989)</u>, Toledo (1973) describes 2n = 48 and FN = 54. Studies by <u>Corrêa</u> (2016) for the Amazon and by <u>Morielle-Versute *et al.* (1996)</u> and <u>Okumura Finato *et al.* (2000) for the Atlantic Forest presented 2n = 48 and FN = 58. <u>Morielle-Versute *et al.* (1996)</u> described autosomal chromosomes as one pair of large submetacentric chromosomes, three pairs of medium submetacentric chromosomes, two pairs of subtelocentric chromosomes, and 17 pairs of acrocentric chromosomes, ranging from medium to small. <u>Corrêa (2016)</u> describes a pair of large metacentric chromosomes, five pairs of</u>

medium metacentric or submetacentric chromosomes, and 17 pairs of acrocentric chromosomes, ranging from medium to small. In the study by <u>Morielle-Versute *et al.* (1996)</u>, the X chromosome is described as medium submetacentric and the Y chromosome as small acrocentric. In <u>Corrêa (2016)</u>, the X chromosome may have been mistakenly described as a medium metacentric, as only females were analyzed, so it is not possible to rule out that it is the same.



Figure 4. Map of the distribution and location of karyotypic studies of *Eumops perotis*. Black dots: occurrence records. Blue Crosses: records that have karyotypic data described.

Cynomops Thomas, 1920

Cynomops planirostris (Peters, 1866)

Type locality: Caiene, French Guiana.

Distribution: *Cynomops planirostris* is known from Venezuela, Guianas, Suriname, eastern Colombia, Peru, Bolivia, Paraguay, Argentina, and Brazil (Eger 2008). In Brazil, it has been recorded in all biomes except the Pampa (<u>Santos *et al.*</u> 2015; Mendes *et al.* 2020; Figure 5).

Karyology: The karyotype study by Santos (2013), carried out with specimens collected in Itinga (MG) in the central region of the Atlantic Forest, showed 2n = 34 and FN = 64. The autosomal chromosomes were described as 13 metaor submetacentric pairs, two subtelocentric pairs, and one acrocentric pair. The studies by Leite-Silva et al. (2003), for the northern region of the Atlantic Forest and Corrêa (2016) for the Amazon presented 2n = 34 and FN = 60, with autosomal chromosomes described as 14 pairs of metacentric or submetacentric chromosomes, ranging from large to small, and two pairs of acrocentric chromosomes. The X chromosome in was described as a large metacentric, in Leite-Silva et al. (2003) as a medium submetacentric, and in Corrêa (2016) as a medium metacentric. The Y chromosome in was described as a small metacentric, while in Leite-Silva et al. (2003) and Corrêa (2016) it was described as a small acrocentric.



Figure 5. Map of the distribution and location of karyotypic studies of *Cynomops planirostris*. Black dots: occurrence records. Blue Crosses: records that have karyotypic data described.

Discussion

Status of cytogenetic knowledge of molossids in Brazil. Our results showed that although the Molossidae family has 32 species in Brazil, there is limited knowledge about their karyology. Through our bibliographical research, we identified a total of 25 karyotypic studies that included molossid species occurring in Brazil. Of these, 11 studies were carried out with specimens collected within the country (Table 2). It is worth noting that, in general, the studies found were focused on describing the karyotypes of the species but did not aim to investigate karyotypic variations between populations from different phytogeographic regions in Brazil, as is the focus of the present study.

As highlighted in our results, only 14 molossid species have a karyotypic description. When we restrict this number to karyotypes from specimens collected in Brazil, only nine species have been studied (Table 2). Furthermore, even the species that have been studied do not have karyotype descriptions for all the different regions in which they occur in the country. For example, *Cynomops abrasus*, *Eumops glaucinus*, *Molossops temminckii*, and *Nyctinomops laticaudatus* have a wide distribution but only have karyotypic studies within the Atlantic Forest (tables 1 and 2).

The work carried out by <u>Morielle-Versute *et al.* (1996)</u> was one of the first studies on Brazilian molossids, presenting the karyotypes of seven species. This study was of great importance for understanding the karyotypic variation within the family in Brazil. Since then, other studies have been conducted; however, the karyology of populations of 23 species remains unknown and needs to be investigated.

According to <u>Sotero-Caio et al. (2017</u>), approximately 50 % of molossid bat species have been studied using conventional staining (Giemsa) worldwide, 11 species have been analyzed with G-banding, and four species have been analyzed with the Zoo-FISH technique. Of the species stud-

ied, 41 had 2n = 48, with the FN varying between 54 and 66. In nine species, the diploid number varied widely, ranging from 34 to 52 (Tables 2). The authors highlight the challenges in determining the number of chromosomal arms in Molossidae, as many species have numerous subtelocentric chromosomes. These difficulties are also related to the level of chromosome condensation and the quality of chromosome preparation. Nevertheless, accurate identification of chromosomal arms is considered crucial for understanding the karyotypic evolution of the family.

Due to the limited variation in diploid and fundamental numbers found in the literature, the most accepted hypothesis is that karyotypic conservatism characterizes the evolution of Molossidae. This conservatism is primarily attributed to pericentric inversions, Robertsonian rearrangements, and translocations (Morielle-Versute et al. 1996; Sotero-Caio et al. 2017). According to Sotero-Caio et <u>al. (2017)</u>, although chromosomal evolution in Molossidae is generally conservative, intrageneric variations were identified within Cynomops and between the genera Cynomops and Molossops, as reported by Leite-Silva et al. (2003). Intraspecific variations were also observed in Eumops glaucinus, as documented by Warner et al. (1974), with differences found between specimens from Colombia (2n = 40, NF =64) and those from Mexico and Costa Rica (2n = 38, NF =64). These variations may have been influenced by geographic factors.

Chromosomal variations in Brazilian molossids. In this section, we will individually discuss the variations found between the karyotypic studies carried out for each species.

Molossus molossus. According to <u>Morielle-Versute *et al.*</u> (1996), the differences in FN observed between the studies by Lopes (1978) and Cristoff and Freitas (1987) compared to others are attributed to the observation of less condensed metaphases obtained from fibroblast cultures. Although the most recent studies do not show numerical chromosomal variations, they do reveal structural differences in autosomal and sexual chromosomes.

In general, the karyotypic descriptions by Morielle-Versute et al. (1996), Faria (2003), and Brandão (2015) are more like each other compared to the description by Corrêa (2016). The latter study not only showed greater differences in autosomal chromosomes but also in the X chromosome. This differentiation aligns with findings by Loureiro et al. (2020a), which indicated genetic differentiation between molossid populations separated by the Amazon River. Corrêa's study was carried out with specimens collected in Barcelos (AM) and Caracaraí (RR), north of the Amazon River, while the other studies focused on populations south of the river. However, further cytogenetic and molecular studies are needed to explore this hypothesis, including research across other South American biomes.

Regarding the description of the Y chromosome, no direct relationship can be identified concerning population differentiation between the Amazon and Atlantic For

 Table 2. Karyotypic data of fourteen species of molossids that occur in Brazil. Diploid number (2n); Fundamental Number (FN); synthetic nucleotide 5-bromo-2-deoxyuridine (BrdU); Band C (C); G Band (G); conventional staining (Giemsa-); Nucleolus organizing regions (NOR); Fluorescence in situ hybridization (FISH). Biomes: Amazon (Am); Atlantic Forest (AF); Cerrado (Ce); Caatinga (Ca); Pantanal (Pt); Pampa (Pp).

Species	2n	Fn	Method	Biome	References
Cynomops abrasus	34	60	Giemsa	-	27
	34	64	C and G	FA	20
Cynomops greenhalli	34	60	Giemsa	-	2, 9, 16, 27
Cynomops planirostris	34	64	Giemsa	FA	22
	34	60	G, C, NOR and FISH	FA	14,15
	34	60	C	Am	5
Eumops auripendulus	42	60	Giemsa	-	27
	42	62	Giemsa	-	25
Eumops glaucinus	40	64	G, C and NOR	FA	12, 18, 20,
	40	64	Giemsa	-	27
	38	64	Giemsa	-	27
Eumops hansae	48	58	Giemsa and FISH	Am	5
Eumops perotis	48	54	Giemsa	-	24
	48	56	Giemsa	-	1, 26
	48	58	C and G	FA	12, 20
	48	58	С	Am	5
Molossops temminckii	42	56	Giemsa	-	9
	42	56	Giemsa	FA	22
	48	68	C and G	FA	10
Molossus molossus	48	56	C, G and NOR	Pa, FA	3, 7, 17
	48	58	Giemsa	-	27
	48	64	G, C, RON, BrdU and FISH	FA	10, 15, 20, 21, 18
	48	64	C, G and RON	Am	4, 5, 6
Molossus rufus	48	58	Giemsa	-	27
	48	60	Giemsa	FA	17, 25
	48	62	Giemsa	Ce, Ca	8, 13, 23
	48	64	G, C, NOR, BrdU and FISH	FA	10, 11, 15, 20, 21
Nyctinomops laticaudatus	48	64	C and G	FA	20
Promops centralis	48	58	Giemsa	-	27
Promops nasutus	40	54	Giemsa	-	26
Tadarida brasiliensis	48	56	G	-	2, 27
	48	58	Giemsa	-	27

References: 1. <u>Baker (1970)</u>; 2. Baker et al. (1982)a; 3. Baker and Lopez (1970); 4. <u>Brandão (2015)</u>; 5. <u>Corrêa (2016)</u>; 6. <u>Corrêa and Bovicino (2016)</u>; 7. Cristoff and Freitas (1987)a; 8. Dantas (2004)c; 9. Gardner (1977)b; 10. <u>Faria (2003)</u>; 11. <u>Faria and Morielle-Versute (2006)</u>; 12. Finato (2000); 13. <u>Leal (2012)</u>; 14. Leite-Silva *et al.* (2000) b; 15. <u>Leite-Silva et al.</u> (2003); 16. Linares and Kiblisky (1969)b; 17. Lopes (1978)a; 18. Moratelli et al. (2000) b; 19. Morielle et al.(1988)a; 20. <u>Morielle-Versute et al.</u> (1996); 21. Morielle-Versute and Varella-Garcia (1994); 22. <u>Santos (2013)</u>; 23. Sousa (2007)c; 24. Painter (1925)b; 25. Toledo (1973)a; 26. Wainberg (1966)b; 27. <u>Warner *et al.* (1974)</u>. The citation's final letters indicate the cited author and data source: a - <u>Varella-Garcia and Taddei (1989)</u>; b - <u>Moratelli *et al.* (2007)); c - <u>Leal (2012)</u>.</u>

est biomes or within the Atlantic Forest biome itself. Both the study by <u>Morielle-Versute *et al.* (1996)</u>, which described the Y chromosome as subtelocentric, and the study by <u>Faria</u> (2003), which described it as acrocentric, were conducted with specimens collected in the Atlantic Forest region of São Paulo. Additionally, the study by <u>Leite-Silva *et al.* (2003)</u>,

which also identified the Y chromosome as subtelocentric, was based on specimens from Pernambuco. Therefore, variations in the Y chromosome of *Molossus molossus* require further investigation.

Molossus rufus. Molossus rufus exhibited both numerical and structural chromosomal variations between studies conducted in the Atlantic Forest and those from the Amazon, Caatinga, and Cerrado. Studies from the southern Atlantic Forest provided consistent descriptions for autosomal chromosomes. However, the northern Atlantic Forest study could not adequately identify the centromeric positions of these chromosomes. Research in the Caatinga and Cerrado showed identical 2n and FN values but differed from other biomes in the centromeric positions of chromosomes. Regarding sex chromosomes, no variation was observed for the X chromosome. The Y chromosome did show variation among studies, but this variation does not appear to be related to the different biomes.

The karyotypic variation found among specimens collected in the Atlantic Forest and other biomes may be interspecific. Considering the distribution proposed by Loureiro et al. (2020b), in which M. fluminensis occurs in southeastern Brazil, Bolivia, Paraguay, and Argentina, and M. rufus in the central and northern regions of Brazil, Bolivia, Ecuador, French Guiana, Guyana, Peru, Suriname, Trinidad and Tobago, and Venezuela, it is possible that the karyotyped specimens from the Atlantic Forest actually correspond to M. fluminensis. However, there are questions regarding the actual identification of the specimens in relation to the karyotypic samples from the Cerrado, Caatinga, and Atlantic Forest in the northeast region, since the samples used by Loureiro et al. (2020b) that separate the two species molecularly are from the Guianas and southeastern Brazil. According to the distribution proposed by the author, the FN of M. rufus would vary from 58 in Trinidad and Venezuela (Warner et al. 1974) to 64 in Pernambuco, with 62 in Piauí. In this case, new karyotypic studies are necessary to confirm this variation and to determine whether the karyotype from Pernambuco is in fact from M. rufus or M. fluminensis, and thus to clarify the karyotypic variation of these two species.

Molossops temminckii. The karyotypic study carried out by <u>Santos (2013)</u>, based on specimens collected in Itinga (MG), described three pairs of metacentric chromosomes. In contrast, the study by <u>Morielle-Versute *et al.* (1996)</u> for Minas Gerais found no metacentric chromosomes. This discrepancy resulted in differences in the number of submetacentric, subtelocentric, and acrocentric chromosomes between the karyotypes. No variations were observed in the description of the X chromosome. Since <u>Santos (2013)</u> only analyzed a single female, it was not possible to assess the Y chromosome.

Thus, the karyotypes of *Molossops temminckii* exhibit variations in the morphology of autosomal chromosomes within the state of Minas Gerais. Since the karyotype described by <u>Morielle-Versute *et al.* (1996)</u> lacks a precise description of the collection location, it is not possible to

determine whether the variation observed relative to <u>Santos (2013)</u> is truly between the Cerrado and Atlantic Forest biomes, or merely within different areas of the Atlantic Forest in Minas Gerais.

Eumops perotis. Considering the studies by <u>Corrêa</u> (2016) and <u>Morielle-Versute *et al.* (1996)</u>, *Eumops perotis* exhibited subtle karyotypic variation between the Amazon and Atlantic Forest regions, particularly in the position of the centromere in five pairs of autosomal chromosomes. Since <u>Corrêa (2016)</u> karyotyped only females, it was not possible to draw conclusions about the X chromosome. Considering the lack of records for *Eumops perotis* in much of central Brazil, the populations in the Amazon and Atlantic Forest biomes may be undergoing isolation. This potential isolation is supported by genetic studies on birds and bats across their distribution in Brazil, which have indicated similar patterns of differentiation and isolation (<u>Martins et al. 2009; Pavan *et al.* 2011; Batalha-Filho *et al.* 2013).</u>

Cynomops planirostris. We observed numerical and structural karyotypic variations, both in autosomal and sexual chromosomes, among three different populations of *Cynomops planirostris* occurring in the Amazon and the Atlantic Forest. The karyotypes for the northern Atlantic Forest (Leite-Silva *et al.* 2003) and the Amazon (Corrêa 2016) differed from the karyotype for the central Atlantic Forest (Santos 2013) in relation to FN. This variation is largely attributed to differences in the morphology of two pairs of autosomal chromosomes across the studies of these three populations.

The Y chromosome also showed a closer relationship between the populations of the Amazon and the northern Atlantic Forest, differing morphologically from the Y chromosome described for the central region of the Atlantic Forest by <u>Santos (2013)</u>. Notably, the Y chromosome described by <u>Corrêa (2016)</u> for the Amazon has a completely heterochromatic short arm, whereas the Y chromosome described by <u>Leite-Silva *et al.* (2003)</u> for the northern Atlantic Forest lacks constitutive heterochromatin. This variation may indicate a degree of isolation between these populations.

The chromosomal variations observed among the karyotypic descriptions of molossids collected in Brazil may result from several chromosomal rearrangement processes, such as insertions/deletions or pericentric inversions. For instance, in *Eumops perotis*, the main differences between studies were related to the position of the centromeres and/or the size of the chromosome arms. Additionally, these variations may be due to Robertsonian translocations, as suggested by the differences found in *Molossops temminckii* and *Cynomops planirostris*.

However, the processes and origins of the variations observed require further investigation. Improved banding techniques and the use of chromosomal probes are essential for understanding the evolution of the molossid karyotype throughout their distribution in Brazil. <u>Sotero-Caio</u> <u>et al. (2017)</u> and <u>Leite-Silva et al. (2003)</u> highlighted that in Molossidae, determining the fundamental number is challenging because the short arms of the chromosomes can be extremely small, complicating the distinction between subtelocentric and acrocentric forms. However, according to these authors, the detection of these differences is important, as they portray the mode of chromosomal evolution of Molossidae, which appears to be based mainly on pericentric inversions.

Leite-Silva *et al.* (2003) highlight that various studies suggest nucleolus organizing regions (NOR's) are important markers for studies of chromosomal evolution in Chiroptera and emphasize their potential role in the chromosomal evolution of molossids. Analyzes carried out on *Cynomops abrasus, Cynomops planirostris,* and *Molossops temminckii,* revealed that none of these species share the same number of NOR-bearing chromosomes (Leite-Silva *et al.* 2003; Morielle-Versute *et al.* 1996).

Furthermore, *Cynomops abrasus* and *C. planirostris* exhibit variation in the number of chromosomes with constitutive heterochromatin. *Cynomops planirostris* shows constitutive heterochromatin on the short arm of the X chromosome and on five autosomal chromosomes, while *C. abrasus* and *M. temminckii* have constitutive heterochromatin on all autosomal chromosomes (Corrêa and Bonvicino 2016; Leite-Silva *et al.* 2003; Morielle-Versute *et al.* 1996). Previously, these three species were classified under the genus *Molossops*, given that the hypothesis of intrageneric variation considered by Leite-Silva *et al.* (2003) and Morielle-Versute *et al.* (1996) currently also extends to an intergeneric variation.

It is important to highlight that none of the karyotypes found in the literature refer to a type specimen or a specimen collected at the type locality of these species. This highlights the need for investment in karyotypic studies to establish a "type karyotype" for each species and to subsequently describe the diversity and karyotypic evolution among species.

Influences of geoclimatic changes on the karyotypic variation of Brazilian molossid bats. Different studies indicate that Brazilian biomes experienced a highly dynamic history during the Tertiary and Quaternary periods, shaped by various climatic events that led to complex processes of retraction and expansion over time. These events influenced Brazilian biomes and were crucial in shaping the current floristic and faunal composition of Brazil (Machado *et al.* 2018; <u>Silveira *et al.* 2019; Werneck *et al.* 2012).</u>

Phylogeographic studies based on molecular data with certain groups of rodents and marsupials, as well as suboscine birds occurring in the Amazon and the Atlantic Forest, have identified similar connection routes between these biomes over time. The oldest connections are dated to the middle to late Miocene through central Brazil and Chaco region. Most recent connections likely occurred through the Cerrado and Caatinga in the northeastern Brazil from the Pliocene to the Pleistocene, driven by Quaternary climate changes that facilitated the expansion of gallery forests through these areas (<u>Batalha-Filho *et al.* 2013; Costa 2003</u>).

According to <u>Werneck *et al.* (2012)</u>, during the Last Interglacial, around 120,000 years ago, the climate was hotter and drier, likely promoting an expansion of the Cerrado into areas including the northern Amazon and the eastern coast of South American. Between the Last Interglacial and the Last Glacial Maximum, the Cerrado underwent a process of retraction, reaching its smallest extent during this period. Following this, the Cerrado began to expand again until the Middle Holocene, approximately 6,000 years ago, when it started to stabilize into its current form.

According to a study using molecular data from the vampire bat *Desmodus rotundus*, which is widely distributed across all Brazilian biomes, genetically structured populations were found for the southern Atlantic Forest, northern Atlantic Forest, Amazon, Cerrado, Pantanal, and Central America. This study indicates that Atlantic Forest populations separated from those in the Amazon and Cerrado during the Pleistocene, which is consistent with the emergence of a dry strip separating the Atlantic Forest from the Amazon during that period (Martins et al. 2009). The authors considered that there are no identifiable physical barriers to dispersal and gene flow within this bat distribution range, suggesting that the population structuring may be influenced by ecological separation barriers.

The same type of study conducted by Pavan et al., (2011) suggested that *Carollia perspicillata* and *C. brevicauda*, from the Phyllostomidae, may have appeared in the Amazon region during the Pleistocene approximately 700,000 years ago. It was also observed that *C. perspicillata* has two main evolutionary lineages that may have diverged during the Pleistocene: one lineage extends from Bahia to Paraná in the southern Brazilian Atlantic Forest, while the other, more geo-graphically widespread, is distributed across the northern part of the Atlantic Forest, Cerrado, and Amazon, as well as in other biomes of South and Central America (Pavan *et al.* 2011).

In a comparative phylogeographic analysis of island and continental bat species of the genus *Molossus*, <u>Loureiro et al. (2020a)</u> demonstrated the influence of the Amazon River as a dispersal barrier, as well as the impact of ecological factors and vegetation formations on the genetic structuring of these species in South America.

Thus, recent studies indicate that, although bats are capable of true flight, they are still affected by geographic distance and ecological variants, such as the distinction of vegetation between biomes (Pavan et al. 2011; Morales et al. 2018; Loureiro et al.2020a).

The results of this study reveal that, despite the limited number of karyotypic studies on molossids, it was possible to observe variations in the species between karyotypes from different biomes, and even within the same biome. The Molossidae family, which dates back 50 to 31 million years, began its diversification into Neotropical clades approximately 20 million years (<u>Amador et al. 2016</u>). This study underscores the critical need for increased investment in karyotypic research on molossid bats to better understand interspecific and intraspecific variations and the evolutionary trajectory of their karyotypes. Above all, this investment is necessary due to the presence of population variations and cryptic species, as seen with *Molossus fluminensis* and *M. rufus*, so that conservation and management plans appropriate to the group can be carried out.

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