Non-volant small mammals (Rodentia and Didelphimorphia) diversity in an isolated area of the Serra da Mantiqueira, Minas Gerais state, Brazil

Diversidade de pequenos mamíferos não voadores (Rodentia e Didelphimorphia) em uma área isolada da Serra da Mantiqueira, Minas Gerais, Brasil

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Abstract: The southeastern Atlantic Forest of the *Zona da Mata Mineira* is located in the extreme north of Minas Gerais state, Brazil. The Serra da Mantiqueira, is a mountainous region with a high diversity of small non-volant mammal species, several of which are rare species or endemic lineages. The presence of cryptic species in small mammals makes the karyotype an excellent tool for identification and detection of new lineages. We analyzed the karyotype of 14 species: 11 rodents – *Abrawayaomys ruschii*, *Akodon cursor*, *Blarinomys breviceps*, *Delomys sublineatus*, *Juliomys ossitenuis*, *Oligoryzomys nigripes*, *Oligoryzomys flavescens*, *Oxymycterus dasytrichus*, *Rhipidomys tribei*, *Sooretamys angouya*, and *Thaptomys nigrita*; and three marsupials – *Monodelphis scalops*, *Philander quica*, and *Marmosops incanus*. We described for the first time the fundamental autosomal number and the morphology of sex chromosomes of *Abrawayaomys ruschii*, a rare sigmodontine species, and described a new karyotype for *Blarinomys*. The revision of published karyotypes of the species herein studied enabled the expansion of the geographic distribution of *Oligoryzomys nigripes* and *Delomys sublineatus*.

Keywords: Rodents. Marsupials. Chromosome complement. Geographic distribution.

Resumo: A Mata Atlântica do sudeste da Zona da Mata Mineira está situada no extremo norte da serra da Mantiqueira, estado de Minas Gerais, Brasil, sendo uma região de montanha, com alta diversidade de espécies de pequenos mamíferos não voadores, várias delas raras e endêmicas. A presença de espécies crípticas torna o cariótipo uma excelente ferramenta para identificação e detecção de novas linhagens. Foi analisado o cariótipo de 14 espécies de pequenos mamíferos não voadores: 11 roedores – *Abrawayaomys ruschii*, *Akodon cursor*, *Blarinomys breviceps*, *Delomys sublineatus*, *Juliomys ossitenuis*, *Oligoryzomys nigripes*, *Oligoryzomys flavescens*, *Oxymycterus dasytrichus*, *Rhipidomys tribei*, *Sooretamys angouya* e *Thaptomys nigrita*; e três marsupiais – *Monodelphis scalops*, *Philander quica* e *Marmosops incanus*. Descrevemos pela primeira vez o número fundamental autossômico e a morfologia dos cromossomos sexuais de *Abrawayaomys ruschii*, uma espécie rara de sigmodontíneos, e descrevemos um novo cariótipo para *Blarinomys*. A revisão de cariótipos publicados das espécies aqui estudadas permitiu ampliar a distribuição geográfica de *Oligoryzomys nigripes* e *Delomys sublineatus*.

Palavras-chave: Roedores. Marsupiais. Complemento cromossômico. Distribuição geográfica.

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INTRODUCTION

Within the Neotropics the Atlantic Forest, South America, is considering a hotspot region with several endemic mammalian species. The southeastern region of the Atlantic Forest is formed by a topographical complex of mountains such as the Serra do Mar and Serra da Mantiqueira mountains, which directly influence this biome's landscapes (Costa, L. *et al*., 2000). These subparallel mountain systems are composed of the coastal Serra do Mar and the inland Serra da Mantiqueira, distributed along the continental Atlantic border. The Serra da Mantiqueira mountain range, formed by a mountainous area of rainforest vegetation (Ab'Saber, 1977), is about 500 km long, and spans three Brazilian states: São Paulo (30%), Minas Gerais (60%), and Rio de Janeiro (10%). The Serra da Mantiqueira originated in the Paleocene, resulting in a complex of mountains with wide altitudinal variation (Almeida & Carneiro, 1998), and its importance is highlighted in studies of the biogeographical history of different groups of small mammals (Costa, L. *et al*., 2000; Costa, B. *et al*., 2011; Faria *et al*., 2016; Leite, 2003). These studies point to the importance of this mountain range in the diversification of fauna in the region.

Non-volant small mammals, rodents belonging to the families of Cricetidae and Echimyidae and the marsupials of the family Didelphidae, account for 80% of the Atlantic Forest's endemic species, and are usually are the elements responsible for the regionalism of Neotropical mammal communities (Costa, L. *et al*., 2000). The subfamily Sigmodontinae encompasses a wide variety of forms and adaptive diversity (Patton *et al*., 2015), as well as the family Didelphidae (Gardner, 2008 [2007]).

Unfortunately, the levels of taxonomic differentiation of small mammal populations in the Atlantic Forest are still largely unknown to the majority of the genera, especially in the case of sigmodontine rodents. Although the subfamily Sigmodontinae has been studied for a long time (Hershkovitz, 1987), several species and genera have few specimens in scientific collections, *e.g*., *Pseudoryzomys simplex*, *Phaenomys ferrugineus*, *Phyllomys lundi* (Bonvicino *et al*., 2001b; Faria *et al*., 2016; Wolf *et al*., 2016), thus preventing important taxonomic, phylogenetic and biogeographic inferences. This situation is particularly worrying in view of the speed of destruction of unique environments in the Atlantic Forest, with 95% of its original area having now become the most populous region of South America (Galindo-Leal & Câmara, 2003). This high species diversity in the Neotropics can be attributed to forest refuges formed during the Pleistocene period (Haffer, 1969; Vanzolini & Williams, 1970), with this forest refuge hypothesis explaining the Atlantic Forest's biodiversity and species endemism (Ab'Saber, 1977; Carnaval & Moritz, 2008; Carnaval *et al*., 2009; Porto *et al*., 2012).

Among sigmodontine rodents this high diversity is underestimated due to the presence of morphologically similar species (Yazbeck *et al*., 2011). Although some species have chromosomal polymorphisms or variations (*e.g*., *Holochilus chacarius* in Nachman & Myers, 1989, and *Oligoryzomys nigripes* in Bonvicino *et al*., 2001a), most of them are characterized by an essential constant chromosomal complement (*e.g*., species of the genera *Calomys* in Bonvicino *et al*., 2003, *Juliomys* in Paresque *et al*., 2009, *Brucepattersonius* and *Oxymycterus* in Bonvicino, 2011), thus making the karyotype an excellent tool for identifying species, especially small rodents where the presence of cryptic species is constant, in addition to undescribed species (Bonvicino *et al*., 2003; Paresque *et al*., 2009). Studies that integrate morphology, karyotype and DNA sequence data lead to an advance in knowledge about sigmodontinae rodents and Didelphimorphia marsupials, with a substantial increase in the number of recognized species (Gonçalves *et al*., 2005; Jansa & Voss, 2000; Patton *et al*., 2000; Voss *et al*., 2005; Voss & Jansa, 2009). However, few karyotypic studies integrate morphology and geographic distribution data into their analyses (Bonvicino *et al*., 2013; Weksler *et al*., 2017), and this has led to misidentification in the past. For example, the lack of integrated morphology and karyotype studies, led to the association of the same diploid number (2n) of 62 and fundamental autosomal number (FNa) of 64 with different co-generic species, *Oligoryzomys eliurus* and *Oligoryzomys fornesi* (Andrades-Miranda *et al*., 2001; Bonvicino & Weksler, 1998), thus showing the importance of morphologic documentation in karyologic studies. In contrast to morphologic and molecular data, it is impossible to perform a retrospective analysis of karyotype, which also hampers karyological studies.

This study therefore aims to evaluate the chromosomal features and contribute to the debate on the species taxonomy and distribution of rodents and marsupials, including endemic species of the *Zona da Mata Mineira*, a hotspot mountain region in the Atlantic Forest biome. Herein, we karyotyped small mammals, and we discuss the karyotype variation of these species, and comment on the geographic distributions of the species surveyed.

MATERIAL AND METHODS

The study area is located in the municipality of Alto Jequitibá, Minas Gerais state, in the Atlantic Forest biome (Figure 1), and included two protected areas, classified as *Reserva Particular de Patrimônio Natural* (RPPN) category, in the *Fazenda Harmonia* (farm Harmonia). We carried out six field expeditions during one year in both areas, RPPN *Santuário Ecológico Mata dos Jacus* (-20.483404 S, -42.046899 W) and RPPN *Refúgio dos Sauás* (-20.407001 S, -42.041710 W), both with semi-deciduous mountain vegetation (Veloso *et al*., 1991). In order to conduct inventories for the non-volant small mammals we placed Sherman and tomahawk live traps and one linear transect with 12 buckets (60 l), 10 meters apart, in each locality (Table 1). The study was conducted under permit number 41959-1 from the *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio).

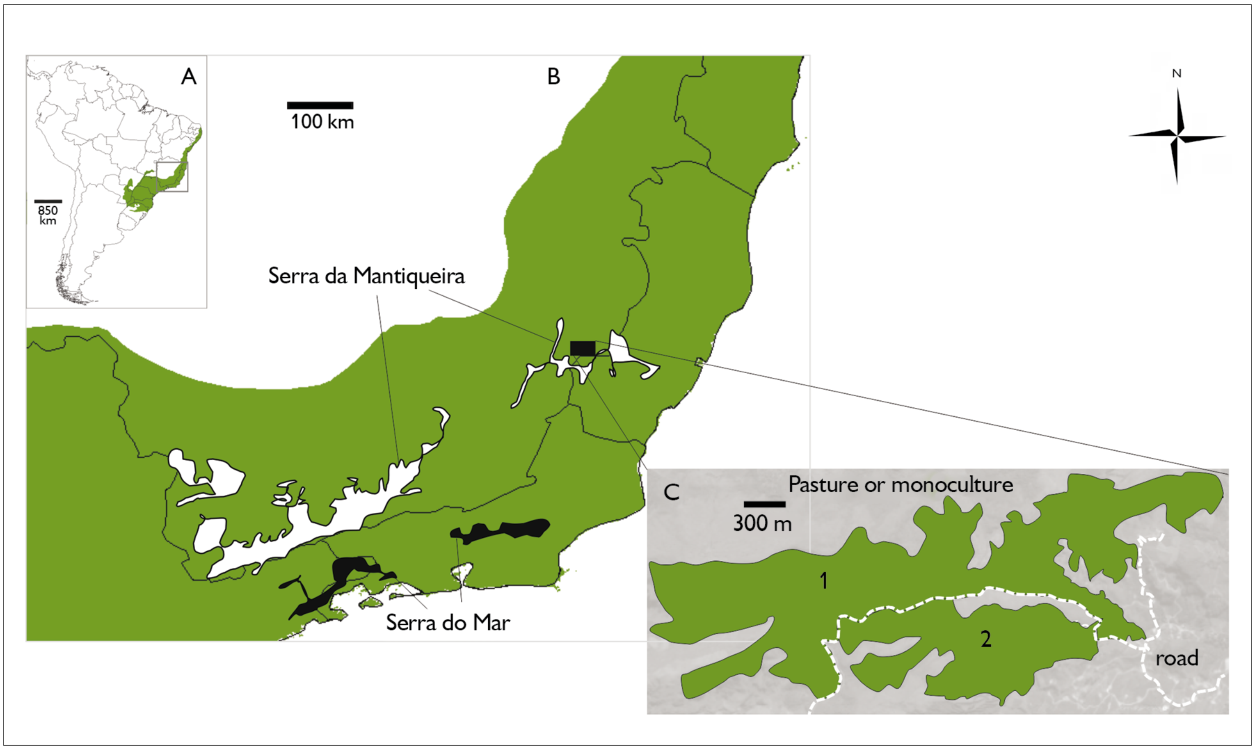

Figure 1. Locality of sampled area: A) South America map with Atlantic forest in green; B) Southeastern Brazil with Atlantic Forest (green), collecting locality (black rectangle), the highlands of the mountain range of Serra da Mantiqueira (white patches) and Serra do Mar (black patches); C) Collecting localities with RPPN *Refúgio dos Jacus* (1) and the RPPN *Refúgio dos Sauás* (2), Minas Gerais state, Brazil.

Table 1. List of studied areas in the municipality of Alto Jequitibá, Minas Gerais state, specifying the collecting data, trap type and total effort per area.

Study area	Collecting dates	Trap types	Total effort
RPPN *Santuário Ecológico Mata dos Jacus*	November 14-18, 2013 January 28 to February 01, March 13-17, May 14-18, August 05-09, October 18-22, 2014	40 Sherman 40 Tomahawk 11 pitfalls	2,730 trap.nights
RPPN *Refúgio dos Sauás*	November 14-18, 2013 January 28 to February 01, March 13-17, May 14-18, August 05-09, October 18-22, 2014	11 pitfalls 40 Sherman 40 Tomahawk	2,730 trap.nights
Total	30 days	182 traps	5,460 trap.nights

To confirm species identifications, specimens were karyotyped in the field using Andrade & Bonvicino's (2003) methodology. In addition, we identified the specimens based on morphologic characters (Gardner, 2008 [2007]; Patton *et al*., 2015), and they were deposited in the mammal collection of the *Museu de Zoologia Newton Baião de Azevedo* (MZNB), Carangola, Minas Gerais State, Brazil. In order of associate species and karyotypes, the skull of one specimen of each species was photographed and composed together the respective species karyotype, except for *Thaptomys nigrita*. All specimens collected alive were karyotyped.

Chromosome preparations were obtained from short bone marrow cultures in RPMI 1640 medium supplemented with 20% fetal calf serum and colchicine (10^{-6} M) for two hours (Andrade *et al*., 2004). Chromosomes were ordered according to morphology and size. Conventional staining was performed with 5% Giemsa solution (phosphate buffer, pH 6.8) and G-banding patterns were carried out according to Seabright (1971). For each cell suspension sample, several metaphases were captured and subjected to microscopic analysis, and five metaphases were mounted. Fundamental number (FNa) refers only to autosome complement. An extensive inventory was carried out in several scientific data bases available online, such as Google Scholar (s. d.) and Web of Science (s. d.) for identifying the localities of karyotyped individuals for each analyzed species. Geographical distributions of each analyzed species were based on Patton *et al.* (2015) for rodent species and on Gardner (2008 [2007]) for Didelphimorphia species.

RESULTS

We karyotyped 14 species, 11 rodents and three marsupials (Figures 2 to 6), as bellow described.

Order Rodentia: *Abrawayaomys ruschii* Cunha & Cruz, 1979 male MZNB 167 and female MZNB 176, showed 2n = 58 and FNa = 58 (Figure 2). The autosome complement showed 28 chromosome pairs, one small metacentric and 27 acrocentric pairs varying in size from large to small. The sexual X chromosome is the largest acrocentric chromosome of the complement, and the Y a median size acrocentric. The G-band karyotype was also obtained (Figure 2, bottom left). The autosome complement showed 20 chromosome pairs, 15 acrocentric pairs varying in size from medium to small, and five large biarmed pairs; the sexual X chromosome is a median sized acrocentric and Y a small acrocentric.

Akodon cursor (Winge, 1887), seven males and four females, showed 2n = 14 (Figure 3A) with different fundamental autosomal numbers, FNa = 18 in MZNB 209, MZNB 192, MZNB 234, MZNB 245, FNa = 19 in MZNB 184, MZNB 232, MZNB 256, FNa = 20 in MZNB 256, MZNB 208, MZNB 248, and FNa = 21 in MZNB 164 (Figure 3A), with a small acrocentric X chromosome and a very small Y chromosome. Polymorphisms in fundamental numbers are due to pericentric inversions in the second, third and fourth pairs. *Blarinomys breviceps* (Winge, 1887)

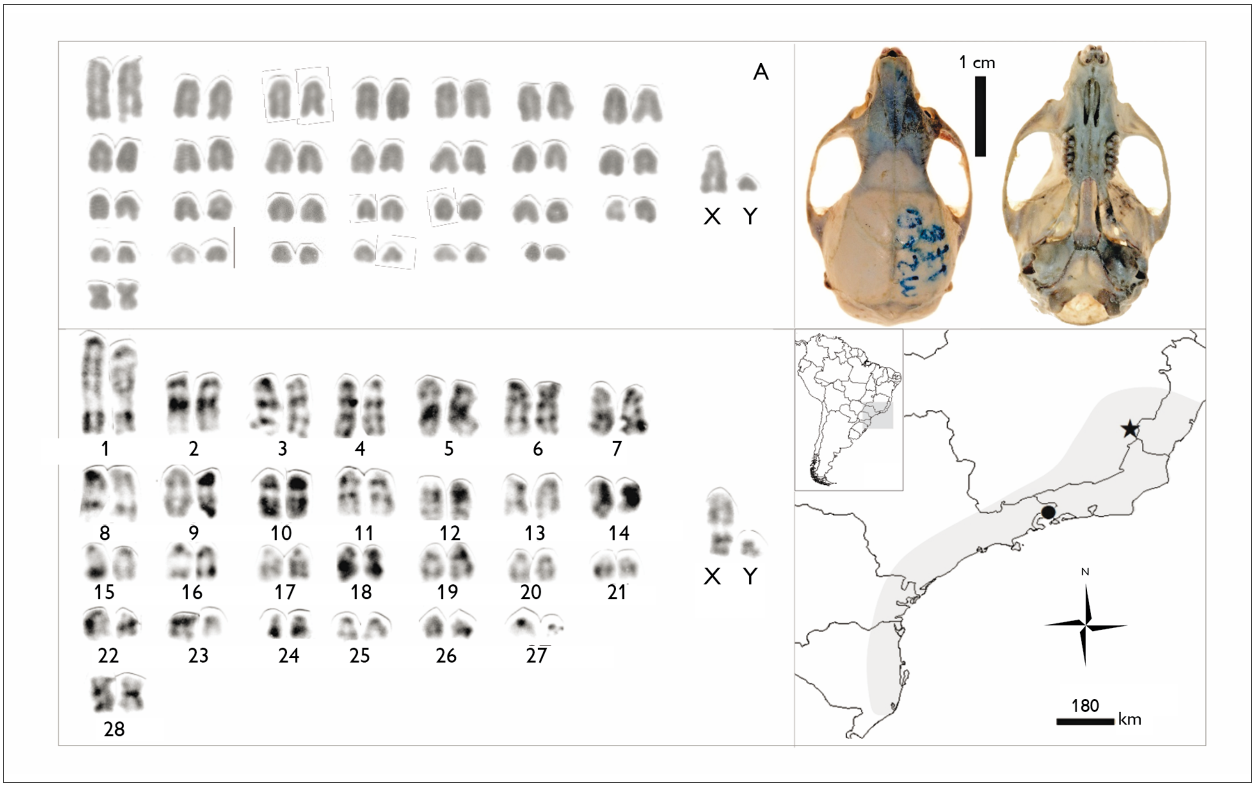

Figure 2. *Abrawayaomys ruschii* male MZNB 167. Giemsa staining (top lef) and G-banding karyotypes with 2n = 58 and FNa = 58 (bottom left), dorsal and ventral views of the skull (top right), and map of species distribution in gray (according to Patton *et al.*, 2015), with locality of this study (black star), and localities of karyotyped specimens (black circle, for localities list see Table 2) (bottom right).

male MZNB 268 showed 2n = 42 and FNa = 50 (Figure 3B). The autosome complement showed 20 autosomes pairs, five large sized biarmed pairs and 15 acrocentric pairs varying in size from median to small. The X chromosome is a median sized acrocentric and the Y chromosome a small acrocentric (Figure 3B). *Oxymycterus dasytrichus* (Schinz, 1821), two males (MZNB 171 and MZNB 279), showed 2n = 54 and FN = 64 (Figure 3C), with submetacentric X and Y chromosomes. *Thaptomys nigrita* (Lichtenstein, 1829) female MZNB 275 showed 2n = 52 and FN = 52 (Figure 3C). In this species the acrocentric chromosomes are very similar in size to sexual pair (Colombi, 2013; Ventura *et al.*, 2004). So, since we used only Giemsa staining for the karyotype analysis of this sample, we not indicated the sexual pair.

Seven *Oligoryzomys nigripes* (Olfers, 1818) specimens (MZNB 173, MZNB 175, MZNB 179, MZNB 206, MZNB 253, MZNB 255, and MZNB 258) showed 2n = 62 and FNa = 80-82 (Figure 4A), with a large sized submetacentric X chromosome and a small acrocentric Y. In four specimens, the third and fourth metacentric pairs were heteromorphic, with one biarmed and one acrocentric chromosome. *Oligoryzomys flavescens* (Waterhouse, 1837) female MZNB 183 showed 2n = 64 and FNa = 66 (Figure 4B), with a median size submetacentric X chromosome. *Sooretamys angouya* (G. Fischer 1814), males MZNB 197 and MZNB 201, showed 2n = 58 and FNa = 60 (Figure 4C), with a large acrocentric X chromosome and an acrocentric Y chromosome. *Rhipidomys tribei* Costa, Geise, Pereira & Costa, 2011 male MZNB 169 showed 2n = 44 and FNa = 50 (Figure 4D), with a medium sized acrocentric X chromosome and a small acrocentric Y chromosome.

Table 2. List of species found in the study area with karyotypic data, including diploid (2n) and fundamental autosomal number (FNa), locality, and source. Number between parentheses after locality name refers to the number of specimens herein karyotyped. Legends: Brazilian federative unities - BA (Bahia), CE (Ceará), DF (Federal District), ES (Espírito Santo), GO (Goiás), MG (Minas Gerais), MS (Mato Grosso do Sul), PR (Paraná), RJ (Rio de Janeiro), RS (Rio Grande do Sul), SC (Santa Catarina), SP (São Paulo). Source: 1 = Sbalqueiro & Nascimento (1996); 2 = Geise (1995); 3 = Geise *et al.* (1998); 4= Moreira *et al*. (2009); 5 = Yonenaga (1972); 6 = Paresque *et al.* (2004); 7 = Yonenaga *et al.* (1975); 8 = Yonenaga-Yassuda (1979); 9 = Yonenaga-Yassuda *et al*. (1983); 10 = Fagundes *et al.* (1998); 11 = Araújo (2014); 12 = Mochi (2014); 13 = Geise *et al*. (2004); 14 = Delciellos *et al.* (2012); 15 = Ventura *et al.* (2012); 16 = Zanchin *et al.* (1992b); 17 = Gonçalves & Oliveira (2014); 18 = Costa, L. *et al.* (2007); 19 = Grazzini *et al.* (2015); 20 = Aguieiras *et al.* (2013); 21 = Paresque *et al.* (2009); 22= Sbalqueiro *et al*. (1991); 23= Pereira, N. *et al.* (2008); 24 = Di-Nizo *et al.* (2015); 25 = Di-Nizo *et al.* (2014); 26 = Almeida & Yonenaga-Yassuda (1991); 27 = Azevedo (2009); 28 = Almeida Vieira (2012); 29 = Andrades-Miranda *et al*. (2000); 30 = Araújo Soares (2014); 31 = Bonvicino *et al.* (2002); 32 = Bonvicino *et al.* (2014); 33 = Bonvicino *et al.* (2001a); 34 = Brum-Zorrilla *et al.* (1988); 35 = Bueno *et al*. (1987); 36 = Espinosa & Reig (1991); 37 = Gatto de Almeida (2015); 38 = Grazzini (2014); 39 = Myers & Carleton (1981); 40 = Svartman (1989); 41 = Tortato *et al.* (2014); 42 = Weksler & Bonvicino (2005); 43 = Zanchin (1988); 44 = Carvalho, A. (2017); 45 = Ventura *et al.* (2010); 46 = Colombi (2013); 47 = Moreira & Oliveira (2011); 48 = Pereira, N. *et al.* (2008); 49 = Hass *et al.* (2011); 50 = Carvalho, B. *et al.* (2002); 51 = Faria (2008); 52 = Pereira, L. & Geise (2007). (Continue)

Taxa	2n	FNa	Locality	Source
Order Rodentia				
Abrawayaomys ruschii	58	58	MG: Alto Jequitibá (n = 2)	This study
Abrawayaomys ruschii	58	-	RJ: Angra dos Reis	23
Akodon cursor	14	18-21	MG: Alto Jequitibá (n = 13)	This study
Akodon cursor	14	18	PR: Guaraqueçaba, RJ: Mendes, Rio Bonito, Angra dos Reis, Sumidouro, Macaé, Casimiro de Abreu SP: Ubatuba, Guaratatuba MG: Simão Pereira, Viçosa, Parque Estadual do Brigadeiro ES: Santa Teresa, Cariacica	1, 2, 3, 4, 5, 6
Akodon cursor	14	19	RJ: Rio de Janeiro, Pirai, Maricá, Rio Bonito, Rio das Ostras, Resende, Penedo Maromba SP: São Paulo, Picinguaba, Salesópolis, Juquitiba, Sete Barras, Iporanga, Iguapé, Ariri, Ilha do Cardoso, Guatatuba PR: Guaraqueçaba MG: Conceição do Mato Dentro, Rio Pomba ES: Cariacica, Santa Teresa PR: Matinhos, Paranaguá	1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13
Akodon cursor	14	20	RJ: Rio de Janeiro, Nova Friburgo, Resende, Penedo, Maromba PR: Guaraqueçaba SP: Iguapé, São Paulo, Sete Barras, Ilha do Cardoso, Picinguaba, Salesópolis Juquitiba, Guatatuba BA: Una ES: Santa Teresa, Cariacica	1, 2, 3, 5, 6, 7, 9, 10, 11, 13, 14
Akodon cursor	14	21	SP: Iguapé, São Paulo, Picinguaba RJ: Mendes, Rio de Janeiro BA: Una PR: Guaraqueçaba	1, 2, 3, 7, 10, 11
Blarinomys breviceps	43	50	MG: Alto Jequitibá (n = 1)	This study
Blarinomys breviceps	43	50	SP: Parque Estadual da Serra da Cantareira	15
Delomys sublineatus	72	90	MG: Alto Jequitibá (n = 5)	This study
Delomys sublineatus	72	90	PR: Antonina ES: Fazenda Monte Verde MG: Parque Estadual Serra do Brigadeiro SP: Salesópolis, Caucaia do Alto	16, 17

Table 2. (Continue)

Taxa	2n	FNa	Locality	Source
Juliomys ossitenuis	20	36	MG: Alto Jequitibá (n = 1)	This study
Juliomys ossitenuis	20	36	MG: Fervedouro, Dores do Rio Preto SP: Bananal, Cotia, Piedade, Tapiraí, Ribeirão Grande PR: Pirai do Sul RJ: Teresópolis	18, 19, 20, 21
Oligoryzomys flavescens	64	66	MG: Alto Jequitibá (n = 1)	This study
Oligoryzomys flavescens	64	66	RS: Mostardas, Osório, Sapiranga Tramandaí, Taim, Alpestre, Rio dos índios, Erechim Horto Florestal, Passo Fundo, Chapecó PR: Ponta Grossa, Curitiba, Piraguara, Esmeralda, Torres, Curitiba SP: Iporó	12, 22, 24
Oligoryzomys nigripes	62	80 - 82	MG: Alto Jequitibá (n = 9)	This study
Oligoryzomys nigripes	62	78 - 82	DF: Brasília BA: Morro do Chapéu, Rio de uma CE: Pacoti ES: Cariacica, Estação Ecológica Santa Lúcia, Monte Verde, Santa Maria de Jetibá, Santa Teresa, Venda Nova dos Imigrantes GO: Caldas Novas, Corumbaíba, Ipameri MG: Alto Caparaó, Caxambu, Juramento, Peirópolis, Serra da Canastra, Parque Estadual da Serra Brigadeiro MS: Bodoquena PR: Iguaçu, FLONA de Piraí do Sul RJ: Itaguaí, Itatiaia, Nova Friburgo, Rio Claro, Sumidouro, Teresópolis RS: Alpestre, Aratiba, Caxias do Sul, Chapecó, Charqueadas, Erechim, Ivaí, PE do Turvo, Derrubadas, Estação Ecológica do Taim, Maquiné, Mostardas, Morro Alto, Muitos Capões, Osório, Picada Verão, Quintão, Reserva Biológica Estadual do Sassafrás, Riozinho, Rio dos Índios, São Francisco de Paula, Sapiranga, Tainhas, Tapes, Torres, Tramandaí, Tupanciretã SC: Concordia, Costa de entro, Ilha de Santa Catarina, São Francisco do Sul SP: Águas de Santa Bárbara, Araçariguama, Araraquara, Casa Grande, Guaratuba, Iguapé, Iporanga, Itapetininga, Intervales, Juquiá, Luiz Antônio, Pedreiras Pedro de Toledo, Parque Estadual Serra do Mar, Pilar do Sul, Santa Maria da Serra, Ribeirão Preto, Rio Claro, Santa Virgínia, Santo Antônio, Taubaté	4, 6, 13, 12, 21, 24, 25, 26, 28, 29, 30, 31, 32, 33a, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43
Oxymycterus dasytrichus	54	64	MG: Alto Jequitibá (n = 1)	This study
Oxymycterus dasytrichus	54	64	MG: Parque Estadual do Brigadeiro RJ: Itatiaia PR: Matinhas	4, 12, 13
Rhipidomys tribei	44	50	MG: Alto Jequitibá (n = 1)	This study
Rhipidomys tribei	44	50	ES: Castelo, Ibitirana, Muqui MG: Fervedouro, Santa Barbara	44
Sooretamys angouya	58	60	MG: Alto Jequitibá	This study

Table 2. (Conclusion)

Taxa	2n	FNa	Locality	Source
Sooretamys angouya	58	60	ES: Monte Verde RS: Sapiranga, Tramandaí, Mostardas, Faxinal, Torres, Tramandaí, Caxias do Sul, Quintão, Osorio, Doutor Pedrito SC: Florianópolis PR: Piraí do Sul MG: Parque Nacional do Caparaó SP: Santa Virgínia, Parque Estadual da Serra do Mar	20, 25, 32, 38, 41
Thaptomys nigrita	52	52	MG: Fervedouro. ES: Santa Teresa, Domingos Martins, Dores do Rio Preto, Luminárias. SP: São Bernardo do Campo, Biritiba Mirim, Pilar Do Sul, Iguapé, São João Batista da Boa Vista, Ibiúna, Piedade, Tapirai, Cotia, Capão Bonito, Piraquara, Santa Virginia PR: Ortigueira, Matinhos, Piraguara, Curitiba RS: Maniqué SC: Blumenau BA: Una	4, 6, 31, 37, 45, 46, 47, 49
Thaptomys nigrita	52	52	MG: Alto Jequitibá (n = 1)	This study
Order Didelphimorphia				
Monodelphis scalops	18		MG: Alto Jequitibá (n = 2)	This study
Monodelphis scalops	18	30	SP: Santa Virginia	12
Marmosops incanus	14	24	MG: Alto Jequitibá (n = 14)	This study
Marmosops incanus	14	24	BA: Pau-Brasil, Lençóis, Remanso MG: Santa Barbara, Pedra Dourada, Fervedouro, Tombos ES: Santa Teresa, Cariacica SP: Caucaia do Alto, Parque Estadual da Serra do Mar, Santa Virginia	6, 25, 27, 50, 51, 52
Philander quica	22		MG: Alto Jequitibá (n = 4)	This study
Philander quica	22	20	RJ: Angra dos Reis SP: Santa Virginia, Serra da Cantareira SC: Doutor Pedrito RS: Aratinga, Sapiranga, Osório ES: Santa Teresa	6, 25, 38, 48, 50

Two specimens of *Delomys sublineatus* (Thomas, 1903) were collected, the female MZNB 243 and the male MZNB 216 that showed 2n = 72 and FNa = 90 (Figure 5A), with a large sized submetacentric X chromosome and a small acrocentric Y chromosome. *Juliomys ossitenuis* Costa, Pavan, Leite & Fagundes, 2007, male MZNB 207, showed 2n = 20 and FNa = 36 (Figure 5B), with a large metacentric X chromosome and a medium submetacentric Y chromosome.

Order Didelphimoprhia: two *Monodelphis scalops* (Thomas, 1888) were collected, male MZNB 269, and male MZNB 180 that showed 2n = 18 (Figure 6A), with small acrocentric X and Y sexual chromosomes. Two *Philander quica* (Temminck, 1824) were collected, male MZNB 239, and male MZNB 165 that showed 2n = 22 (Figure 6B), with a medium acrocentric X chromosome and a small acrocentric Y chromosome. *Marmosops incanus* (Lund, 1840) male MZNB 241 showed 2n = 14 (Figure 6C), with a medium submetacentric X chromosome and a small Y chromosome.

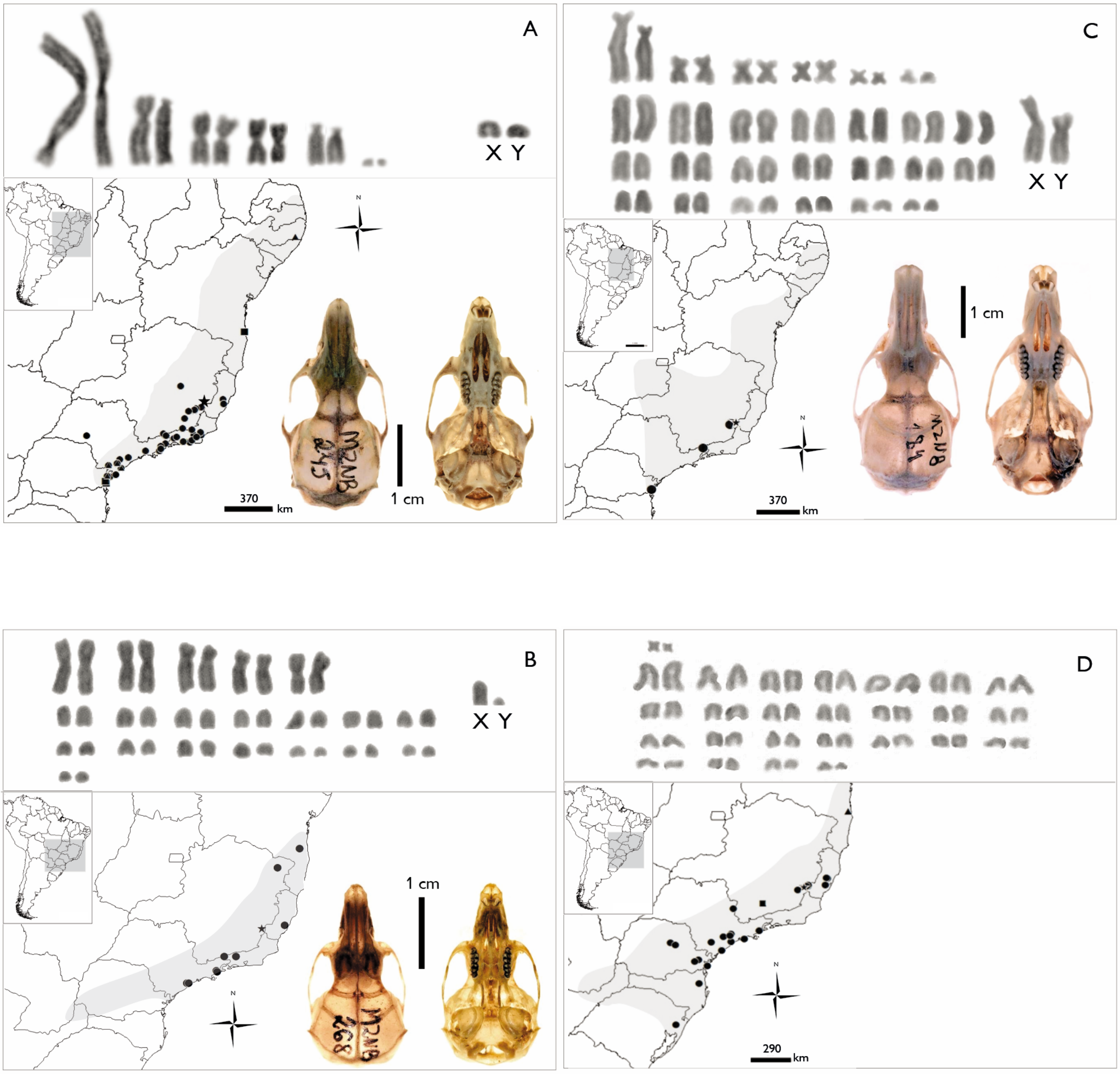

Figure 3. Giemsa staining karyotypes, maps of species distribution in gray (according to Patton *et al.*, 2015), with study area (black star) and localities of karyotyped specimens from previous studies (black circle; for localities and sources see Table 2), and ventral and dorsal skull views of: A) *Akodon cursor* male MZNB 164 with 2n = 14 and FNa = 21; B) *Blarinomys breviceps* male MZNB 268 with 2n = 42 and FNa = 50; C) *Oxymycterus dasytrichus* male MZNB 171 with 2n = 54 and FNa = 64; D) *Thaptomys nigrita* female MZNB 275 with 2n = 52 and FNa = 52 (with no indication of sexual pair, and with no figured skull). Black square = locality with different karyotype from the specimens of the study area.

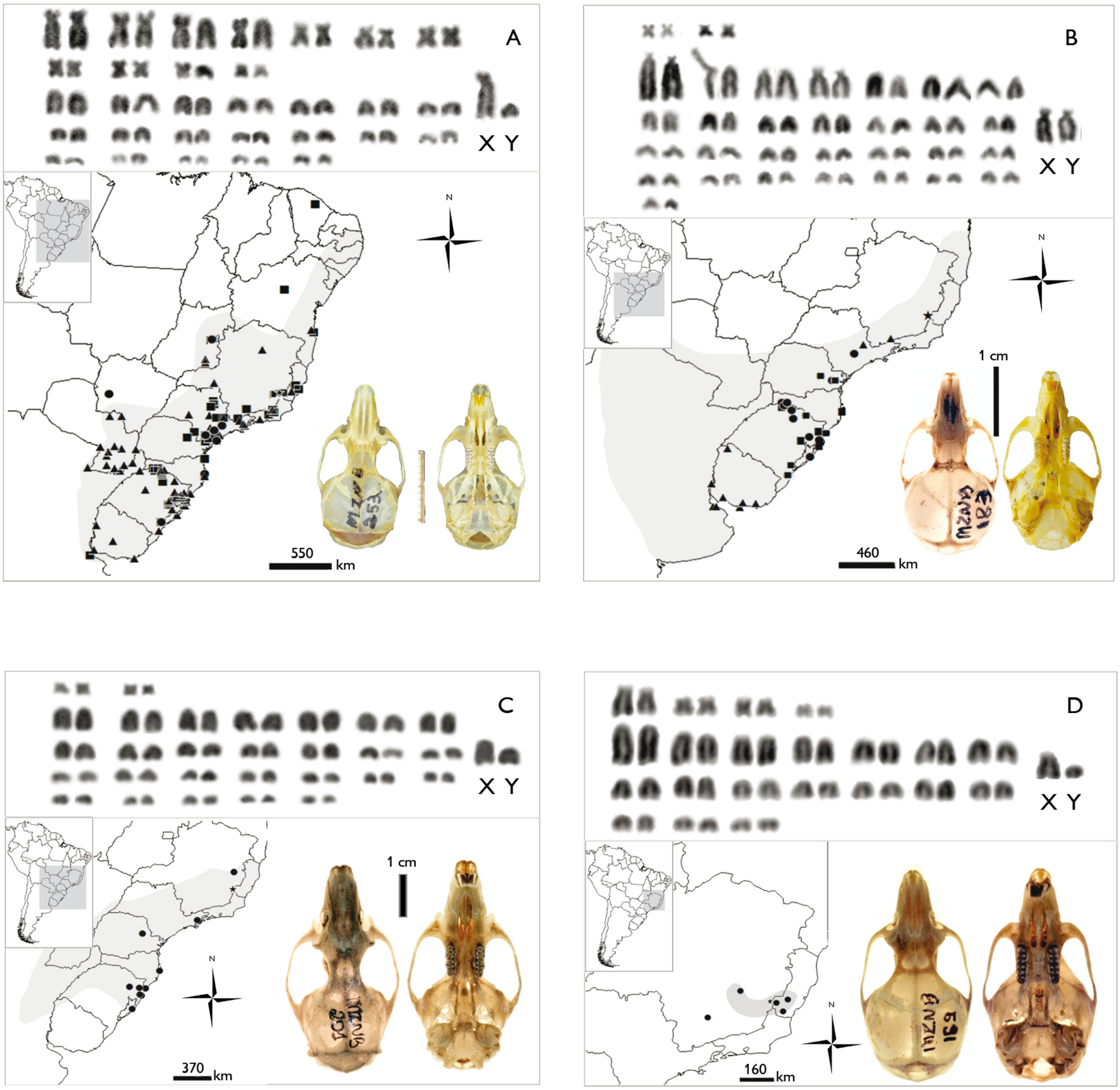

Figure 4. Giemsa staining karyotypes, maps of species distribution in gray (according to Patton *et al*., 2015), with the study area (black star) and localities of karyotyped specimens from previous studies (black circle; for localities and sources see Table 2), and ventral and dorsal skull views of: A) *Oligoryzomys nigripes* male MZNB 179 with 2n = 62 and FNa = 80 (skull MZNB 253); B) *Oligoryzomys flavescens*, MZNB 183, 2n = 64 and FNa = 66; C) *Sooretamys angouya* male MZNB 197 with 2n = 58 and FNa = 60 (skull MZNB 201); D) *Rhipidomys tribei* male MZNB 169 with 2n = 44 and FNa = 50. Black square = locality with different karyotype from the specimens of the study area. Black triangles= localities with the same and different karyotypes.

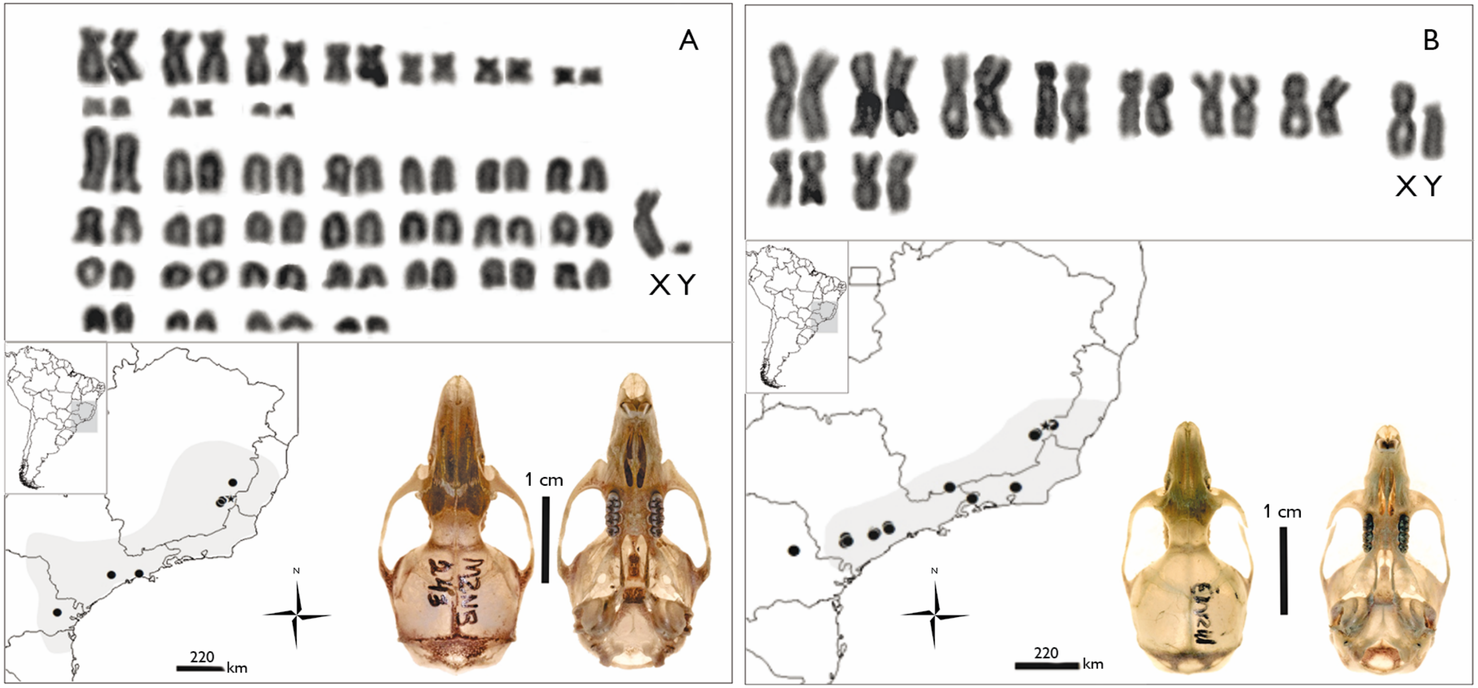

Figure 5. Giemsa staining coloration karyotypes, maps of species distribution in gray (according to Patton *et al.*, 2015 and this study), with the study area (black star), and localities of karyotyped specimens from previous studies (black circle; for localities and sources see Table 2), and skull ventral and dorsal views of: A) *Delomys sublineatus* male MZNB 216 with 2n = 72 and FNa = 90, and skull of female MZNB 243, B) *Juliomys ossitenuis* male MZNB 207 with 2n = 20 and FNa = 36.

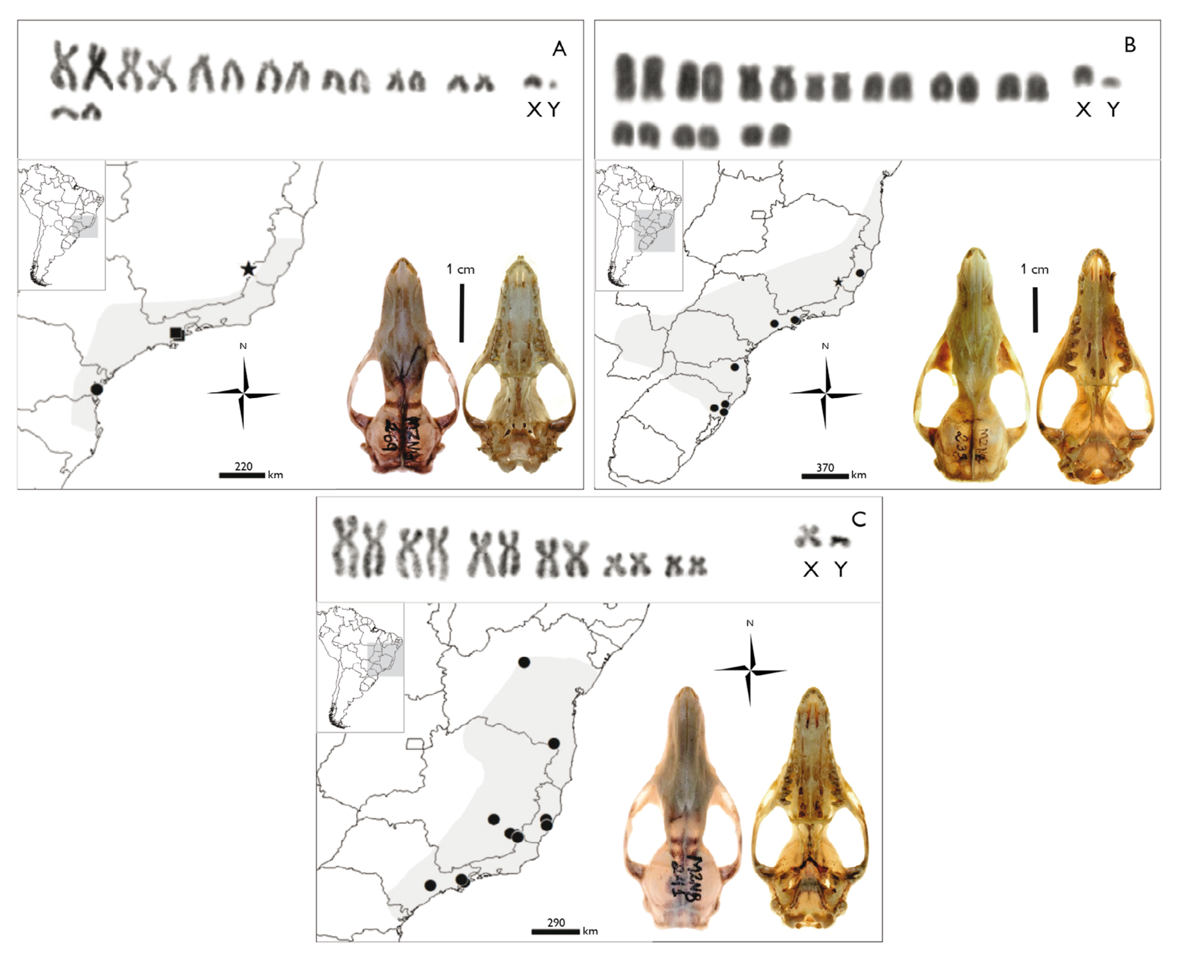

Figure 6. Giemsa staining karyotypes, maps of species distribution (modified from Pine & Handley Jr., 2008 [2007]) in gray, with the study area (black star), and localities of karyotyped specimens from previous studies (black circle; for localities and sources see Table 2), and ventral and skulls dorsal views of: A) *Monodelphis scalops* male MZNB 180 with 2n = 18 and FNa = 20, and skull of male MZNB 269, B) *Philander quica* male MNZB 165 (MBF145) with 2n = 22 and FNa = 20, and skull of male MZNB 239, C) *Marmosops incanus* male MZNB 241 with 2n = 14 and FNa = 24.

DISCUSSION

The karyotype herein reported for *Abrawayaomys ruschii* with 2n = 58, is similar to the single one previously reported for a specimen from Rio de Janeiro state (Pereira, L. *et al*., 2008). However, the autosomal fundamental number and the sexual chromosomes cannot be identified by Pereira, L. *et al.* (2008) due to the poor quality of the preparation. Herein the acrocentric X and Y sexual chromosomes, and the FNa of 60 is described for the first time. After G-banding, all chromosome pairs of this species could be identified (Figure 2). *Abrawayaomys ruschii* is a poor known species and is considered as *incertae saedis* (Ventura *et al*., 2013), whereas a recent study suggested its close relationship with the tribe Akodontini (Gonçalves *et al*., 2020). Some authors suggested the presence of a third and undescribed species of Minas Gerais *Abrawayaomys* (Pardiñas *et al*., 2009), however our data showed that in the localities sampled in Minas Gerais the species present is *Abrawayaomys ruschii*. The geographical distance (378 km) between the two areas where the karyologic studies have been published, one in the state of Rio de Janeiro and another in Minas Gerais state, suggest a conserved chromosome complement for *A. ruschii*.

Blarinomys breviceps' new karyotype herein described, with 2n = 42 and FNa = 50, differs from all others described for this species. Eight distinct karyomorphs have already been described for this monospecific genus with a variation in diploid numbers (2n) due to arrangements involving autosomes and the presence of b chromosomes (B): 2n = 52, 2n = 50 + 2B, 2n = 44 + 1B, 2n = 39 + 4Bs, 2n = 36 + 1, 2n = 34, 2n = 29 + 2Bs, 2n = 28 (Geise *et al*., 2008; Ventura *et al*., 2012). The map in Figure 3 shows localities with cytogenetic studies. All these karyotypes, including the one herein described, share the same fundamental autosomal number of 50 and the XY sexual system, with a variation in diploid numbers due to up 4 supernumerary chromosomes and Robertsonian arrangement (Ventura *et al*., 2012). *Blarinomys* is considered a fossorial rodent due to its adaptations to fossorial living (Teta & Pardiñas, 2015). Fossorial rodents often have a high level of intra and inter-specific polymorphism (Ipucha *et al*., 2008). The presence of two evolutive lineages of *Blarinomys breviceps* in the Atlantic Forest suggested by phylogenetic analysis (Ventura *et al*., 2012), indicate that further studies are necessary for understanding whether the karyologic variation of this taxon is polymorphism.

The tribe Akodontini included several genera that occur in Brazil (Patton *et al*., 2015), and we analyzed karyotypes of specimens belonging to four genera of this tribe, *Akodon*, *Blarinomys*, *Oxymycterus*, and *Thaptomys*. *Akodon cursor* karyotypes herein found were within the variation described for this species (Table 2). Polymorphism due to pericentric inversion and centric fusion/fission in the largest chromosome of the complement is well documented, with karyotypes ranging from 2n = 14 to 16 and FNa = 18 to 26 (Yonenaga, 1975; Yonenaga-Yassuda *et al*., 1983; Maia & Langguth, 1981; Kasahara & Yonenaga-Yassuda, 1984; Fagundes *et al*., 1998; Geise *et al*., 1998; Sbalqueiro & Nascimento, 1996; Ventura *et al*., 2004; Pereira, L. & Geise, 2007; Moreira *et al*., 2009). The 2n = 16 is fixed in the extreme north of the species distribution (Maia & Langguth, 1981) with a single record in the extreme south of distribution in Guaraqueçaba, Paraná state, where all three diploid numbers were found (Sbalqueiro & Nascimento, 1996). Despite this polymorphism, the karyotype of *Akodon cursor* is easily identified, and specimens were karyotyped throughout its distribution (Figure 3A).

Oxymycterus dasytrichus karyotype with 2n = 54 and FNa = 62 herein reported is similar to those already described for this species in Minas Gerais and Paraná states (Moreira *et al*., 2009; Mochi, 2014; Figure 3C). Despite variations in external and cranial morphology, all species of this genus share the same 2n and FNa (Mattevi *et al*., 1982; Sbalqueiro *et al*., 1982; Hershkovitz, 1998; Bonvicino, 2011), and this constancy leads to few karyologic studies carried out with *Oxymycterus* species.

Thaptomys nigrita karyotype showed 2n = 52 and FNa = 52 (Figure 3D), similar to those reported to specimens from Rio Grande do Sul, Paraná, São Paulo, Rio de Janeiro, Espírito Santo, and Minas Gerais states (Yonenaga *et al*., 1975; Paresque *et al*., 2004; Ventura *et al*., 2004, 2010; Moreira *et al*., 2009; Delciellos *et al*., 2012). Two other karyotypes were attributed to *T. nigrita*, one with 2n = 48-51 and FNa = 52 described for specimens from Luminárias in Minas Gerais state (Colombi, 2013), and another with 2n = 50 and FNa = 48 for specimens from Una, Bahia state (Ventura *et al*., 2004).

The tribe Oryzomyini included several genera that occur in Brazil, and we analyzed karyotypes of specimens belonging to the two genera of this tribe, *Oligoryzomys* and *Sooretamys* (Patton *et al*., 2015). The 2n = 62 is constant in *O. nigripes*, with variations in fundamental autosomal numbers from 78 to 80-82 due to pericentric inversion affecting up to four autosome pairs (pairs 2, 3, 4, 8) throughout its distribution, in Brazil (Yonenaga *et al*., 1976; Bueno *et al*., 1987; Zanchin, 1988; Almeida & Yonenaga-Yassuda, 1991;

Bonvicino & Weksler, 1998; Andrades-Miranda *et al*., 2000; Bonvicino *et al*., 2001a), Paraguay (Myers & Carleton, 1981), and Argentina (Espinosa & Reig, 1991). Despite variations in fundamental autosomal numbers due to pericentric inversions (Almeida & Yonenaga-Yassuda, 1991; Andrades-Miranda *et al*., 2000; Figure 4A), and polymorphism in the morphology of X chromosome (Paresque *et al*., 2007), *O. nigripes* can easily be identified by its chromosome complement. The karyotype data in the literature increased *O. nigripes*' geographic distribution west up to Morro do Chapéu in Bahia state, Pacoti in Ceará state, and Bodoquena in Mato Grosso do Sul state (Paresque *et al*., 2007; Figure 4A), showing the importance of karyologic data in the identification and delimitation of species distribution boundaries.

The *Sooretamys angouya* karyotype with 2n = 58 and FN = 60 is similar to the one found in other localities throughout the Atlantic Forest (Andrades-Miranda *et al*., 2000; Silva & Yonenaga-Yassuda, 2004; Di-Nizo *et al*., 2014). Polymorphism in diploid numbers (57-58) due to a Robertsonian rearrangement involving autosomes has already been reported in specimens identified as *Oryzomys ratticeps* (Kasahara & Yonenaga-Yassuda, 1984), as well as the presence of up to two supernumerary chromosomes, resulting in 2n = 60 (Silva & Yonenaga-Yassuda, 2004). The chromosome complement of *S. angouya* was known only in part of its distribution (Figure 4B), with no karyotype description available for its distribution in the west.

We karyotyped specimens belonging to one genus of the tribe Thomasomyini, *Rhipidomys* (Patton *et al*., 2015). The karyotype of *Rhipidomys tribei*, 2n = 44 and FNa = 50 (Figure 4D) has already been reported for specimens from Espírito Santo and Minas Gerais states (Zanchin *et al*., 1992a; Costa, B. *et al*., 2011; Carvalho, A., 2017). Other *Rhipidomys* species, such as *Rhipidomys cariri* from Pernambuco and Ceará states (Thomazini, 2009; Carvalho, A., 2017), *Rhipidomys Itoan* from São Paulo and Rio de Janeiro states (Geise, 1995; Di-Nizo *et al*., 2014; Carvalho, A., 2017), *R. gardneri* from Acre state (Patton *et al*., 2000) and *R. macconnelli* from Bolivar in Venezuela (Aguilera *et al*., 1994) share the same 2n = 44 and FNa = 50. However, these karyotypes differ from each other in their autosome complement morphology and in the presence of constitutive heterochromatin.

Recently the *Juliomys* genus was considered to belong to the tribe Wiedomyini tribe together with *Wiedomys*, *Phaenomys* and *Wilfredomys* (Gonçalves *et al*., 2020). The *Juliomys ossitenuis* karyotype herein reported is similar to the one found in specimens from Minas Gerais, Rio de Janeiro and Paraná states (Aguieiras *et al*., 2013; Costa, L. *et al*., 2007; Grazzini *et al*., 2015). This species showed a conservative karyotype throughout its distribution and a distribution greater than the one reported in the last compilation for the genus (Patton *et al*., 2015). We karyotype two genera of Sigmodontinae *incertae sedis*, *Abrawayaomys* (above discussed) and *Delomys*. *Delomys sublineatus* karyotype herein reported is similar to the one found throughout its distribution (Zanchin *et al*., 1992b; Gonçalves & Oliveira, 2014).

The Didelphidae species are characterized by a conservative karyotype with only three diploids numbers, 14, 18 and 22 (Reig *et al*., 1977; Carvalho, B. *et al*., 2002). This conserved chromosome complement led to few studies with this group (Figure 6). Data herein presented corroborated previous reports about *Monodelphis scalops* with 2n = 18 and FNa = 30 (Di-Nizo *et al*., 2014), *Marmosops incanus* with 2n = 14 and FNa = 24 (Carvalho, B. *et al*., 2002; Faria, 2008) and *P. quica* with 2n = 22 and FNa = 20 (Pereira, N. *et al*., 2008; Di-Nizo *et al*., 2014).

The different karyotype herein found for *Blarinomys* can be related to the topography of the area. The Serra da Mantiqueira contains the highest inselberg-type landforms in South America, and has a high generic and species-level diversity, probably due to the old age of the Atlantic forest and its flora and fauna (Por, 1992; Porembski & Barthlott, 2000).The southeastern region of Brazil, where the *Zona da Mata* and the Atlantic Forest are located, is formed by a topographical complex of mountains, which directly influence this biome's landscapes (Costa, L. *et al*., 2000) with altitudinal variations of 0 to 2,900 m. The studied area is postulated as a refugium in the Southeast Brazilian

Atlantic Forest (Porto *et al*., 2012), and the data herein reported, with a high number of little known and, or, endemic species, such as *Abrawayaomys ruschii* (Pardiñas *et al*., 2015), *Blarinomys breviceps* (Teta & Pardiñas, 2015), and *Phyllomys lundi* (Leite & Loss, 2015), is consistent with this hypothesis.

This study confirms the *Zona da Mata Mineira* as a hotspot region in the Atlantic Forest and showed the importance of karyotype analysis in identifying known and still unknown small mammals, in addition to likely contributing to biogeographic and taxonomic studies of these rodents.

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