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Inventory

Updated list of non-volant small mammals from the Serra da Bocaina National Park, southeastern Brazil

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Abstract: In the core of the Atlantic Forest biome, the Serra da Bocaina National Park (SBNP) is located in the Atlantic Forest Southeast area of endemism for vertebrates. Filling gaps in knowledge about the spatial distribution and occurrence of species in national parks is of fundamental importance to know how many species are protected and to guide conservation initiatives. Here we updated the non-volant small mammal species list of the SBNP, providing new data on species list and abundance, with species identified mainly by karyotype and/or molecular analysis. Twelve sampling sessions with a capture-mark-recapture approach were carried out in four sites in the SBNP from 2013 to 2016, during the paving works of the state highway RJ-165 (Estrada Parque Paraty-Cunha), municipality of Paraty, state of Rio de Janeiro, Brazil. Non-volant small mammals (Rodentia and Didelphimorphia) were sampled using Sherman® and Tomahawk® live traps (18,987 trap-nights) and pitfall traps (4,591 trap-nights). Thirty-two species (11 marsupials and 21 rodents) were recorded from 1,185 captured specimens. Species richness ranged from 18 to 28 between sites. Ten and 11 species were exclusively captured in live traps and pitfall traps, respectively. The observed richness (32 species) represented 91.4% of the estimated species richness for the study area. Sites 2 and 4 were the most similar to each other regarding species composition, and site 3 was the most dissimilar. The species with highest relative abundance were *Euryoryzomys russatus* (14%) and *Delomys dorsalis* (14%), while six species had relative abundances lower than 1%. Fourteen and 17 species were identified by karyotype and molecular analysis, respectively. The present study added 22 species to the park's non-volant small mammals list, which now has 37 species with confirmed occurrence. This species richness found in the SBNP is one of the highest ever recorded for the group of non-volant small mammals in protected areas of the Atlantic Forest in Brazil, corroborating the Serra da Bocaina region as a biodiversity hotspot.
Keywords: *Atlantic Forest; cytb; cytogenetics; faunistic inventory; molecular identification; species richness.*

Lista atualizada dos pequenos mamíferos não-voadores do Parque Nacional da Serra da Bocaina, sudeste do Brasil

Resumo: No cerne do bioma Mata Atlântica, o Parque Nacional da Serra da Bocaina (PNSB) está localizado na área Sudeste de endemismo para vertebrados na Mata Atlântica. Preencher lacunas de conhecimento sobre a distribuição espacial e ocorrência das espécies em parques nacionais é de fundamental importância para saber quantas espécies estão protegidas e orientar iniciativas de conservação. Aqui atualizamos a lista de espécies de pequenos mamíferos não-voadores do PNSB, fornecendo novos dados sobre a lista de espécies e abundância, com espécies identificadas principalmente por análises cariotípicas e/ou molecular. Doze sessões de amostragem com uma abordagem de captura-marcação-recaptura foram realizadas em quatro áreas no PNSB de 2013 a 2016, durante as obras de pavimentação da rodovia estadual RJ-165 (Estrada Parque Paraty-Cunha), município de Paraty, estado do Rio de Janeiro, Brasil. Os pequenos mamíferos não-voadores (Rodentia e Didelphimorphia) foram amostrados usando armadilhas de captura viva Sherman® e Tomahawk® (18.987 armadilhas-noite) e armadilhas de queda (4.591 armadilhas-noite). Trinta e duas espécies (11 marsupiais e 21 roedores) foram registradas em 1.185 espécimes capturados. A riqueza de espécies variou de 18 a 28 entre as áreas de amostragem. Dez e 11 espécies foram capturadas exclusivamente em armadilhas de captura viva e armadilhas de queda, respectivamente. A riqueza observada (32 espécies) representou 91,4% da riqueza de espécies estimada para a área de estudo. As áreas 2 e 4 foram as mais semelhantes entre si quanto à composição de espécies, e a área 3 foi a mais dissimilar. As espécies com maior abundância relativa foram *Euryoryzomys russatus* (14%) e *Delomys dorsalis* (14%), enquanto seis espécies tiveram abundâncias relativas inferiores a 1%. Quatorze e 17 espécies foram identificadas pelo cariótipo e por análise molecular, respectivamente. O presente estudo acrescentou 22 espécies à lista de pequenos mamíferos não-voadores do parque, que passou a contar com 37 espécies com ocorrência confirmada. Essa riqueza de espécies encontrada no PNSB é uma das maiores já registradas para o grupo dos pequenos mamíferos não-voadores em áreas protegidas da Mata Atlântica no Brasil, corroborando a região da Serra da Bocaina como um *hotspot* de biodiversidade.

Palavras-chave: *Mata Atlântica; cytb; citogenética; inventário faunístico; identificação molecular; riqueza de espécies.*

Introduction

The Atlantic Forest exhibits some of the highest rates of species diversity and endemism on the globe (Myers et al. 2000), with 8% of the world's vertebrate species occurring in this biome, 3% of those being endemic (Figueiredo et al. 2021). The species richness of vertebrates, except for reptiles and bats, increases towards the core of the Atlantic Forest (i.e., the Serra do Mar region), a pattern often attributed to the great topographic variation in this region (Figueiredo et al. 2021). Topography is also the main factor explaining the spatial pattern of species richness of Atlantic Forest marsupials, while temperature seasonality is the most critical driver of endemic species richness (Delciellos et al. 2022). Species richness of South American rodents is correlated with latitude, but also with the existing altitudinal gradient on the continent (Maestri & Patterson 2016).

In the Serra do Mar region, the Serra da Bocaina National Park (SBNP) is located in one important center of endemism for small mammals in the Atlantic Forest (Dalapicolla et al. 2021). The Atlantic Forest Southeast area of endemism, as established by Dalapicolla et al. (2021), is the largest of the seven areas of endemism identified by these authors and the one with the largest number of protected areas that cover 55% of its extension. Despite this relative high level of protection, the southeastern Atlantic Forest, as well as the rest of the biome, is still severely threatened due to intense historical and current deforestation pressure (Rezende et al. 2018). Additionally, existing protected areas do not always overlap with diversity and endemism hotspots, as observed for marsupials (Delciellos et al. 2022) and small mammals (Dalapicolla et al. 2021). Thus, filling knowledge gaps on species occurrence and spatial distribution is of fundamental importance to obtain a more complete overview of how much of the Atlantic Forest species are protected and to guide conservation initiatives such as management and creation of protected areas in identified biodiversity hotspots.

The SBNP was created 52 years ago by the Federal Decree 68,172 of March 4th, 1971 (IBAMA 2001), but a comprehensive survey of mammals had not been carried out until recently. From 2010 to 2016, we conducted a survey of non-volant small mammals in the southern portion of the park, in the state of Rio de Janeiro, as part of the program to evaluate environmental impacts of the paving works of the state highway RJ-165 (Estrada Parque Paraty-Cunha). Initially, from 2010 to 2011, three sampling sessions were carried out to inventory mammal species in the region, culminating in the publication of Delciellos et al. (2012). Here, we updated the SBNP non-volant small mammal species list with the data obtained from 2013 to 2016, providing new data on species occurrence and abundance.

Material and Methods

1. *Study area*

The study was conducted in four sites along the RJ-165 state highway (Estrada-Parque Paraty-Cunha), traversing the SBNP in the municipality of Paraty, Rio de Janeiro state, Brazil (Figure 1). The sites encompass an altitudinal range from 731 to 1,193 m a.s.l. (Delciellos et al. 2018). The climate of Paraty is type Af (Tropical Rainforest) following Köppen-Geiger classification, the mean annual temperature is 23.3 °C and the mean annual precipitation is 2,284 mm (https://pt.climate-data.org/). The super-humid season occurs from October to April and the humid season from May to September (https://pt.climate-data.org/). There is no water deficit in the region (https://pt.climate-data.org/). The vegetation is classified as ombrophilous dense montane forest (IBGE 2012). This study was part of the Mammal Monitoring Program

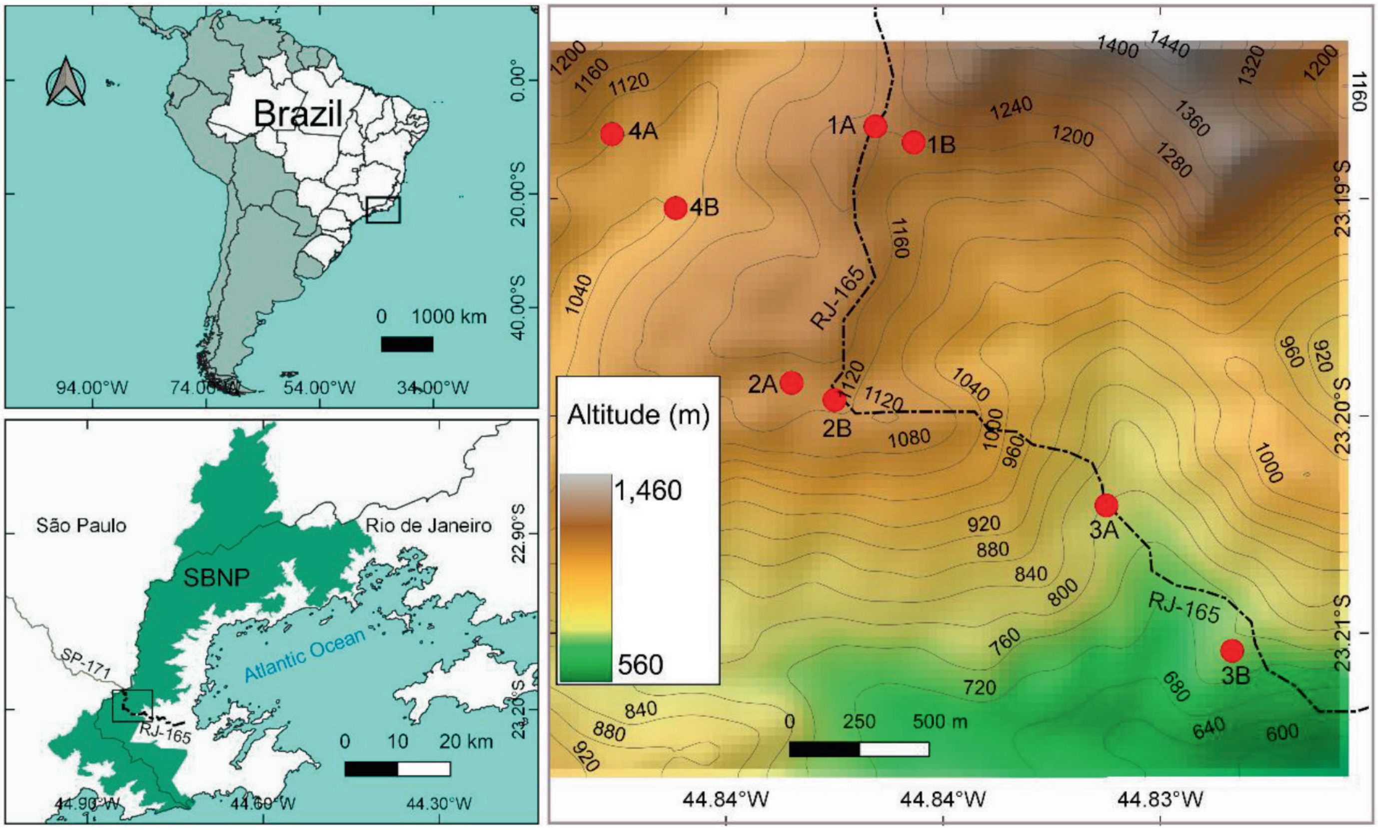

Figure 1. Study area in the Serra da Bocaina National Park (SBNP), municipality of Paraty, Rio de Janeiro state, Brazil. The Digital Elevation Model (MDE) database from the Instituto Brasileiro de Geografia e Estatística (IBGE 2023) was used to generate the contour lines and the topographic map. Topography is represented by shading. The study was carried out in four sites (1–4) distributed along the RJ-165 state highway, each site with two transects (A and B; red dots) for sampling non-volant small mammals.

of the roadworks project of the RJ-165 highway (IBAMA/MMA process no. 02001.003937/2008-18, authorizations no. 248/2013 and 610/2015).

2. *Sampling of non-volant small mammals*

Non-volant small mammals from the orders Didelphimorphia and Rodentia were sampled in twelve sampling sessions with a capture-mark-recapture approach carried out between June 2013 and December 2016 (Sampling sessions: 1 = June 2013; 2 = September 2013; 3 = December 2013; 4 = April 2014; 5 = June 2014; 6 = October 2014; 7 = January 2015; 8 = October 2015; 9 = December 2015; 10 = February 2016; 11 = July 2016; 12 = December 2016) using live traps (Tomahawk® and Sherman®) and pitfall traps. Two 290 m transects (A and B) with 30 trap stations were established at each sampling site. Trap stations consisted of either a Tomahawk trap (45 × 15 × 17 cm), placed on the ground and baited with a mixture of banana, bacon, grinded peanut and oat, or a Sherman trap (31 × 8 × 9 cm) set in the understory at a height of 1.5–2.0 m above the ground and baited with slices of banana. An additional 10 Sherman traps were set in the canopy (> 3.5 m above ground) along each transect during the first two sampling sessions, and then in the understory from the third session onwards, because the ropes that suspended the traps in the canopy were continually stolen. All traps were activated over five consecutive nights during each sampling session, and checked in the morning. The total sampling effort using live traps was 18,987 trap-nights.

Twenty pitfall traps (60 L plastic buckets) were set at each sampling site, totaling 80 buckets in the study area. In each site, buckets were buried in the ground, 10 m apart. A drift fence of plastic sheeting was set 0.5 m high, the base buried up to 0.1 m, and extended along the ground connecting the buckets to orientate the capture of wandering animals. Ideally, at each site these 20 buckets were installed in two transects, each with 10 buckets. Eventually, the 10 buckets sequence could not be done because of rough terrain, thus we deviate from the obstacle (e.g., rocks) and, in this case, the distance between buckets has become larger than 10 m. Pitfall traps were activated five consecutive nights during each sampling session. They were checked in the morning during the first seven sampling sessions, and then three times a day from the eighth session onwards to reduce mortality in the buckets by hypothermia due to the combination of low temperatures and high rainfall in the study area. Increasing the frequency of bucket checks was an imposition by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. Before this imposition, animal mortality in the buckets was high despite all possible measures to avoid eventual deaths, such as holes in the buckets, styrofoam plates to avoid drowning, and hydrophobic cotton pieces for the animals to warm up. The total sampling effort using pitfall traps was 4,591 trap-nights.

The mammal specimens captured were identified in the field to species or genus, sexed, weighed using a spring balance, measured (heady-body and tail lengths), and marked with a numbered ear-tag (at first capture). Unidentified specimens were collected, taxidermized, and deposited at the Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil (Table 1). Some specimens were karyotyped (Table 2) and/or had the mitochondrial DNA sequenced and analyzed (Table 3). The threatened status of species followed the classifications at regional (states of Rio de Janeiro and São Paulo; Bergallo et al. 2000, São Paulo 2018), national (MMA 2022) and global (IUCN 2022) levels (Table 1).

3. Karyotypic and molecular analysis

Karyotypes were prepared in the field and chromosomes in metaphases from 26 specimens were obtained by *in vitro* culture (culture of bone marrow grown in Dulbecco´s MEM with 10% fetal bovine serum and colchicine) plus ethidium bromide, following Geise (2014) with modifications – culture kept at 36.5 °C for 1h40 min. Conventional staining with Giemsa 5% was used to observe diploid number (2n), fundamental number of autosomes (FNa), and chromosome morphology. Microscopic analyzes were performed on the optic photomicroscope (Nikon Eclipse 50i), using an increase of 1,000 with an immersion objective of 100 plus 10 ocular lenses. Karyotypes were mounted, compared with those available in the literature. Chromosomes in metaphases were deposited at the Laboratório de Mastozoologia, Universidade do Estado do Rio de Janeiro (Geise & Aguieiras 2021). Chromosome morphology follows Levan et al. (1964).

Genomic DNA of 87 specimens belonging to 11 rodent and two marsupial genera was extracted from the liver and/or epithelial tissue using the salt protocol and proteinase K (Bruford et al. 1992). In the Polymerase Chain Reaction (PCR), the primers MVZ05 and MVZ16 (Smith & Patton 1993) were used to amplify the first 801 base pairs (bps) of the mitochondrial Cytochrome b (cytb) gene. Cytb is widely used in mammals as a species identification tool (Bradley & Baker 2001, Agrizzi et al. 2012). PCR was performed using 2.5 µl of 10× buffer, 1.0 µl of $MgCl_2$ at 50 mM, 0.5 µl of deoxynucleotide triphosphate mix (10 mM of each nucleotide), 0.3 µl of each primer at 10 µM, 3 units of Taq Platinum (Invitrogen Corporation, Carlsbad, California) and 1.0 µl of template DNA, totalizing 25 µl of final volume of PCR reaction. The PCR was carried out with an initial denaturation temperature of 94 °C for 5 min followed by 39 cycles (30 s of denaturation at 94 °C, 45 s at 48 °C for primer annealing and 45 s at 72 °C for extension of the molecule) and a final extension at 72 °C for 10 min. The PCR products were purified using ExoSAP enzymes (GE Healthcare Life Sciences). The sequencing reactions were run using BigDye Terminator 3.1 (Applied Biosystems, Inc.) and the same primers used for the PCR. The sequences were read in an ABI 3500 automated capillary sequencer (Applied Biosystems, Inc.) and aligned using Geneious Prime software (Biomatters Ltd, Auckland, New Zealand). After that the sequences were submitted to the BLAST tool incorporated in Geneious for the certification of the correct sequencing process.

For the molecular identification by phylogenetic reconstruction, additional sequences were obtained from GenBank (http://www.ncbi.nlm.nih.gov/), together with sequences of closely related taxa, included as outgroups (See Data Availability). The sequences obtained during

Table 1. Updated list of non-volant small mammals from the Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro state, Brazil. Abundance is the number of individuals per species during the study period, from June 2013 to December 2016. * = Previous records in the SBNP (Delciellos et al. 2012), not recorded from June 2013 to December 2016. Legend for type of record: CA = Capture; LT = Live trap; PIT = Pitfall trap; RO = Roadkill; VO = Visual observation. Legend for methods of species identification: K = Karyotype; M = Morphology; MA = Molecular analysis. Legend for status of conservation: DD = Data deficient; NT = Near threatened; VU = Vulnerable; EW = Extinct in the wild; IUCN = International Union for Conservation of Nature (IUCN 2022); RJ = state of Rio de Janeiro (Bergallo et al. 2000); SP = state of São Paulo (São Paulo 2018). Legend for voucher specimens: MN = Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil.

Species	Type of record	Method of species identification	Abundance	Status of conservation	Voucher specimens
Order Didelphimorphia					
Family Didelphidae					
Caluromys philander (Linnaeus, 1758)	CA(LT), RO	M	2		MN81492
**Chironectes minimus* (Zimmermann, 1780)	VO	M	–	NT (RJ)	Not collected
Didelphis aurita Wied Neuwied, 1826	CA(LT, PIT), RO, VO	M	72		MN83220, MN83787
Gracilinanus microtarsus (Wagner, 1842)	CA(LT, PIT)	M	63	NT (RJ)	MN81007, MN81045, MN83207, MN83210
Marmosa paraguayana Tate, 1931	CA(LT)	M	33		MN81032
Marmosops incanus (Lund, 1840)	CA(LT, PIT)	M	6		MN80996, MN81020, MN81044, MN81048, MN81049, MN81060

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Species	Type of record	Method of species identification	Abundance	Status of conservation	Voucher specimens
Marmosops paulensis (Tate, 1931)	CA(LT)	MA	1	NT (RJ), VU (SP)	MN83200
Marmosops spp.	CA(LT, PIT)	M	138		
Metachirus myosuros (Temminck, 1824)	CA(LT)	M	15		MN81834, MN83232
**Monodelphis americana* (Müller, 1776)	CA(PIT)	M	–		MN77794
Monodelphis iheringi (Thomas, 1888)	CA(PIT)	MA	9	VU (SP), DD (IUCN)	MN81006, MN81017, MN81046, MN81844
Monodelphis pinocchio Pavan, 2015	CA(PIT)	M, MA	2		MN83182
Monodelphis scalops (Thomas, 1888)	CA(PIT)	MA	7	NT (RJ)	MN81015, MN81040, MN81041, MN81481, MN81498, MN81840, MN83024
Monodelphis spp.	CA(PIT)	M	57		
Philander quica (Temminck, 1824)	CA(LT, PIT), RO	M	39		MN83784
Order Rodentia					
Family Sciuridae					
Guerlinguetus brasiliensis (Gmelin, 1788)	CA(LT)	M, MA	28		MN80992, MN81003, MN81009, MN81013, MN81026, MN81029, MN81057, MN81469, MN81471, MN81473, MN81486, MN83223, MN83783, MN83786
Family Cricetidae					
Abrawayaomys ruschii Cunha & Cruz, 1979	CA(PIT)	K, M, MA	5		MN81816, MN83192, MN83194
**Akodon cursor* (Winge, 1887)	CA(PIT)	K	–		MN77792
Akodon montensis Thomas, 1913	CA(PIT)	MA	2		Not collected
Akodon spp. or *Castoria angustidens*	CA(LT, PIT)	M	54		
Blarinomys breviceps (Winge, 1887)	CA(PIT)	K, M	6	EW (RJ)	MN77786, MN81027, MN81483
Brucepattersonius nebulosus E. F. Abreu-Júnior, J. F. Vilela, A. U. Christoff, V. H. Valiati & A. R. Percequillo, 2019	CA(PIT)	K, MA	2		MN83197
Brucepattersonius soricinus Hershkovitz, 1998	CA(LT, PIT)	M, MA	11	DD (IUCN)	MN81004, MN81809, MN81810, MN83198
Brucepattersonius spp.	CA(PIT)	M	4		
Castoria angustidens (Winge, 1887)	CA(LT)	K, M, MA	1		MN77791, MN83198

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Species	Type of record	Method of species identification	Abundance	Status of conservation	Voucher specimens
Delomys dorsalis (Hensel, 1872)	CA(LT, PIT)	K, M	166		MN77787, MN77788
Drymoreomys albimaculatus Percequillo, Weksler, and Costa, 2011	CA(PIT)	K, M, MA	4	NT (IUCN)	MN81462, MN83791
Euryoryzomys russatus (Wagner, 1848)	CA(LT, PIT)	K, M, MA	168		MN80997, MN81008, MN81050, MN81811, MN81818, MN83235
Juliomys ossitenuis Costa, Pavan, Leite & Fagundes, 2007	CA(LT, PIT)	K, MA	6		MMN81807, MN81847, MN81852
Juliomys pictipes (Osgood, 1933)	CA(PIT)	K, MA	16		MN77793, MN80998, MN81005, MN81841, MN81843, MN81863, MN83190, MN83217
Juliomys spp.	CA(LT, PIT)	M	3		
Nectomys squamipes Brants, 1827	CA(LT)	K, M	10		MN81059, MN81828, MN83789
**Oecomys catherinae* Thomas, 1909	CA(LT)	M	–		Not collected
**Oligoryzomys flavescens* (Water house, 1837)	CA(LT, PIT)	M	–		Not collected
Oligoryzomys nigripes (Olfers, 1818)	CA(PIT)	K	1		MN81014
Oligoryzomys spp.	CA(LT, PIT)	M	86		
Oxymycterus dasytrichus (Schinz, 1821)	CA(PIT)	M, MA	2		MN81850, MN83191
Oxymycterus spp.	CA(LT, PIT)	M	10		
Rhipidomys itoan B. M. A. Costa, Geise, Pereira & L. P. Costa, 2011	CA(LT, PIT)	K, M, MA	35		MN81016, MN83201, MN83221
Sooretamys angouya (G. Fischer, 1814)	CA(LT), RO	K, M, MA	23		MN80994, MN81025, MN81028, MN81061, MN81812, MN81826, MN81851, MN83193 MN83212, MN83218
Thaptomys nigrita (Lichtenstein, 1829)	CA(LT, PIT)	K, M	57	VU (RJ)	MN81812, MN81851, MN83193
Family Echimyidae					
Phyllomys nigrispinus (Wagner, 1842)	CA(LT, PIT)	K, MA	4		MN83181, MN83183, MN83187, MN83196
Phyllomys sulinus Leite, Christoff & Fagundes, 2008	CA(LT)	MA	1	DD (IUCN)	MN81062
Phyllomys spp.	CA(PIT)	M	2		
Trinomys dimidiatus (Günther, 1876)	CA(LT)	K, MA	34	VU (SP)	MN81018, MN81813

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Table 2. Updated list of karyotyped rodent specimens captured in the Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro state, Brazil. Legend: F = Female; M = Male.

Species	Voucher number	Sex	Diploid number	Fundamental number of autosomes	Reference
Abrawayaomys ruschii	MN83194	F	58	58	Present study
Akodon cursor	MN77792	F	14	20	Delciellos et al. (2012)
Blarinomys breviceps	MN77786	F	28	Not determined	Delciellos et al. (2012)
Blarinomys breviceps	MN81027	M	28	50	Present study
Brucepattersonius nebulosus	MN83197	F	52	52	Present study
Castoria angustidens	MN77791	F	46	46	Delciellos et al. (2012)
Delomys dorsalis	MN77787, MN77788	F	82	80	Delciellos et al. (2012)
Drymoreomys albimaculatus	MN81462	F	62	62	Present study (Delciellos et al. 2015)
Euryoryzomys russatus	MN80997, MN81827	F	80	88	Present study
Juliomys ossitenuis	MN81807, MN81847, MN81852	M/M/F	20	36	Present study (Delciellos et al. 2020)
Juliomys pictipes	MN81841, MN81843, MN83190	F	36	34	Present study (Delciellos et al. 2020)
Nectomys squamipes	MN81828	M	56	56	Present study
Oligoryzomys nigripes	MN81014	F	62	79	Present study
Phyllomys nigrispinus	MN83183, MN83187, MN83196	F	85/84/85	–	Present study (Delciellos et al. 2018)
Rhipidomys itoan	MN81016	M	44	50	Present study
Sooretamys angouya	MN83184, MN83185, MN83197	F/M/M	58	60	Present study
Thaptomys nigrita	MN81812, MN81851, MN83193	M	52	52	Present study
Trinomys dimidiatus	MN81018, MN81813	M	60	116/114	Present study

Table 3. List of specimens from which cytochrome b sequence data was identified with BLAST analysis and used for phylogenetic analyses. Legend: B ID% = Blast Percent Identity; BOCA = Field number from collected specimens in the Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro state, Brazil; BQC = Blast Query Cover; MN = Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Species	Specimens number	BQC% / B ID%	Outgroups	Model
Abrawayaomys ruschii	BOCA666/MN83194	99 / 99	*Delomys*	GTR + I+ G
Akodon montensis	BOCA1509	94 / 99	*Blarinomys*	HKY + I + G
	BOCA1513	94 / 99		
Brucepattersonius soricinus	BOCA1143	96 / 99	*Oxymycterus*	GTR + I + G
	BOCA1163	100 / 99		
	BOCA1155	99 / 99		
	BOCA1494	97 / 99		
Brucepattersonius nebulosus	BOCA667/MN83197	98 / 99	*Oxymycterus*	GTR + I + G
	BOCA795	100 / 100		
Castoria angustidens	BOCA221	92 / 97	*Blarinomys*	HKY + I + G
Drymoreomys albimaculatus	BOCA336/MN81462	100 / 100	*Cerradomys*	GTR + I + G

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Species	Specimens number	BQC% / B ID%	Outgroups	Model
Euryoryzomys russatus	BOCA1332/MN83235	100 / 99	*Cerradomys*	GTR + I + G
	BOCA1333	100 / 100		
	BOCA265	50 / 86		
	BOCA852	94 / 96		
	BOCA889	100 / 97		
Juliomys ossitenuis	BOCA357/MN81807	98 / 99	*Oxymycterus*	GTR + I + G
	BOCA365/MN81852	98 / 99		
	BOCA792/MN81847	98 / 98		
	BOCA 814	99 / 93		
	BOCA1324	98 / 99		
	BOCA1353	98 / 98		
Juliomys pictipes	BOCA18/MN80998	100 / 98	*Oxymycterus*	GTR + I + G
	BOCA47/MN81005	100 / 98		
	BOCA201	100 / 99		
	BOCA759/MN81841	100 / 98		
	BOCA766/MN81843	100 / 98		
	BOCA798	100 / 98		
	BOCA818	100 / 98		
	BOCA820	100 / 98		
	BOCA904	100 / 98		
	BOCA995	100 / 98		
	BOCA1026	100 / 95		
	BOCA1154/MN83217	100 / 98		
	BOCA1157	100 / 97		
	BOCA1231	100 / 97		
	BOCA1233	100 / 97		
	BOCA1355	98 / 98		
Marmosops paulensis	BOCA826/MN83200	100 / 99	*Didelphis*	GTR + I + G
Monodelphis iheringi	BOCA49/MN81006	100 / 99	*Didelphis*	GTR + I + G
	BOCA92/MN81017	100 / 99		
	BOCA241/MN81046	100 / 99		
	BOCA769/MN81844	100 / 99		
	BOCA1401	96 / 98		
	BOCA1403	99 / 99		
	BOCA1479	90 / 99		
	BOCA1504	99 / 99		
	BOCA1520	99 / 98		
Monodelphis pinocchio	BOCA328/MN83182	99 / 99	*Didelphis*	GTR + I + G
Monodelphis scalops	BOCA86/MN81015	100 / 99	*Didelphis*	GTR + I + G
	BOCA234/N81040	100 / 99		
	BOCA235/MN81041	100 / 99		
	BOCA404/MN81481	100 / 99		
	BOCA587/MN81498	100 / 99		
	BOCA756/MN81840	100 / 99		
	BOCA1034	100 / 99		
Oxymycterus dasytrichus	BOCA1510	100 / 99	*Delomys*	GTR + I + G
	BOCA1500	100 / 96		
Rhipidomys itoan	BOCA832	100 / 98	*Oxymycterus*	GTR + I + G
	BOCA1171/MN83221	100 / 99		

Continue...

...Continuation

Species	Specimens number	BQC% / B ID%	Outgroups	Model
Sooretamys angouya	BOCA356/MN83184	100 / 99	*Cerradomys*	GTR + I + G
	BOCA362/MN83185	100 / 99		
	BOCA677/MN83197	96 / 99		
	BOCA841	89 / 97		
Trinomys dimidiatus	BOCA59	99 / 99.87	*Clyomys*	HKY + I + G
	BOCA94/MN81018	99 / 99.87		
	BOCA168	99 / 99.87		
	BOCA225	99 / 99.87		
	BOCA283	99 / 99.87		
	BOCA383	99 / 99.87		
	BOCA465	99 / 99.75		
	BOCA528	99 / 99.87		
	BOCA551	99 / 99.75		
	BOCA576	99 / 99.87		
	BOCA597/MN81813	99 / 99.87		
	BOCA692	99 / 99.87		
	BOCA702	99 / 99.87		
	BOCA868	99 / 99.87		
	BOCA924	99 / 99.87		
	BOCA1061	99 / 99.75		
	BOCA1069	99 / 99.87		
	BOCA1134	99 / 99.87		
	BOCA1177	99 / 99.87		
	BOCA1190	99 / 99.75		
	BOCA1211	99 / 99.87		
	BOCA1220	99 / 99.87		
	BOCA1446	99 / 99.87		
	BOCA1472B	99 / 99.87		

this study were deposited in GenBank (See Data Availability). Bayesian Inference was run in MrBayes v3.2 (Ronquist et al. 2012) over 10^6 generations, with one tree being sampled every 10^3 generations, resulting in a total of 10^3 trees. We discarded the first 10% of the samples as burn-in and obtained a consensus from the remaining trees. Only the nodes with a Posterior Probability (PP) higher than 95% were considered robust. The jModelTest 2.1.7 program (Darriba et al. 2012) was used to establish the best evolutionary model for the data, using the Bayesian Information Criterion (BIC).

4. Statistical analysis

Sample sufficiency was evaluated by the calculation of the expected number of species (Sest) and species richness estimated using Chao 2, an incidence-based non-parametric estimator (Colwell & Coddington 1994), using EstimateS 9.1 software (Colwell 2013). The similarity among sites in presence/absence data for non-volant small mammal species was assessed using the Sorensen index (Bray-Curtis, single link method) in a cluster analysis (Mingoti 2007). The "% Information remaining" (i.e., a rescaling of Wishart's objective function; Bakker 2023) was used for dendrogram graphical representation, in the software PCOrd 4.14 (McCune and Meford 1999).

Results

From 2013 to 2016, 32 species of non-volant small mammals (11 marsupials and 21 rodents) were recorded from 1,185 captured specimens (Table 1). Species richness ranged from 18 to 28 between sites (Site 1 = 26; Site 2 = 28; Site 3 = 18; Site 4 = 25). Ten species were exclusively captured with live traps (Sherman and Tomahawk) and 11 exclusively with pitfall traps (Table 1). The observed richness (32 species) was lower than the species richness estimated using Chao 2 (Mean ± SD = 35.06 ± 3.82) and represented 91.4% of the estimated species richness for the study area (Figure 2).

The genera with the highest relative abundance were *Euryoryzomys* (14%), *Delomys* (14%), and *Marmosops* (12%) (Figure 3). The genera with lower relative abundance (< 1%) were *Abrawayaomys*, *Blarinomys*, *Caluromys*, *Drymoreomys*, *Nectomys*, and *Phyllomys* (Figure 3). Four species are Near threatened, four are Vulnerable, and one is Extinct in the wild at regional level; and three are Data deficient and one is Near threatened at global level (Table 1). No species is threatened at national level (Table 1). In the cluster analysis comparing all sampling sites, sites 2 and 4 were the most similar to each other regarding species composition, and site 3 was the most dissimilar (Figure 4).

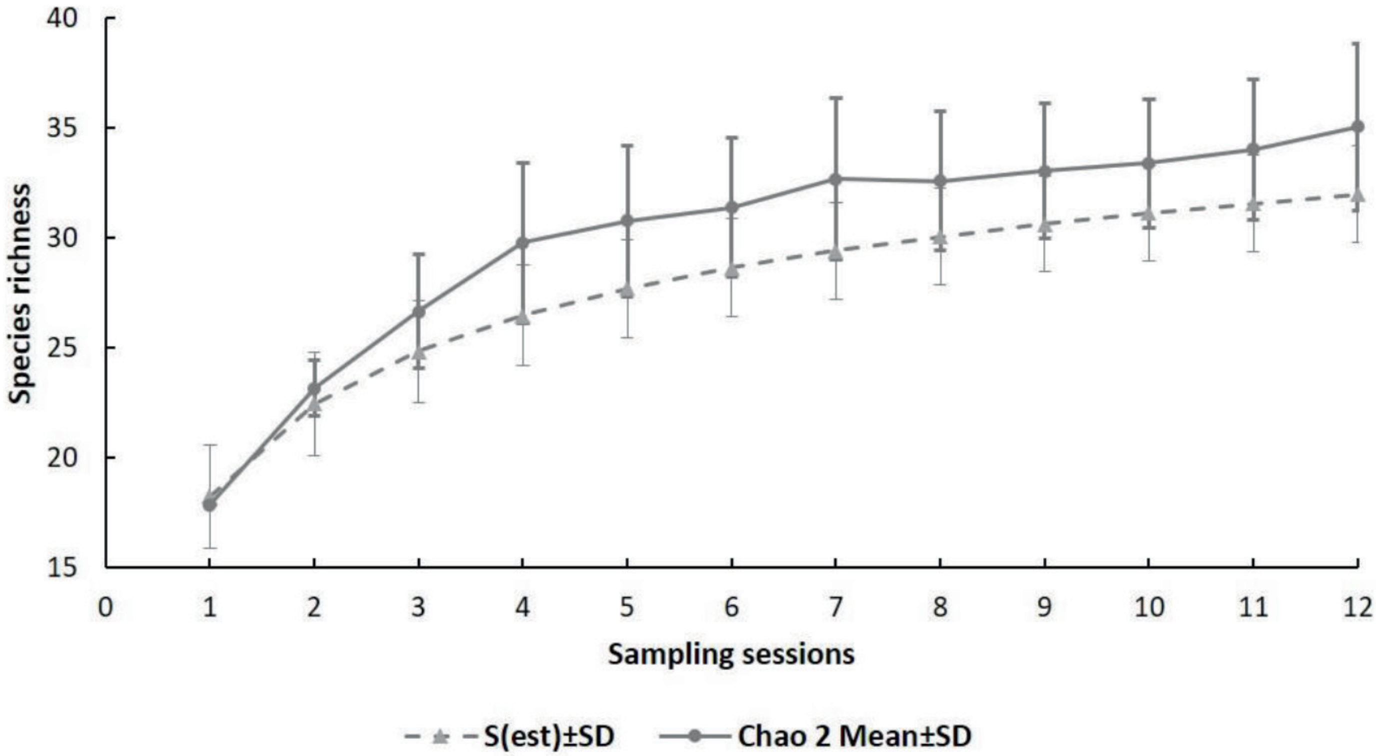

Figure 2. Expected number of species (Sest) and species richness estimated using Chao 2 (Chao 2 Mean) for non-volant small mammals (Didelphimorphia and Rodentia) recorded during twelve sampling sessions in the Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro state, Brazil. Bars are the standard deviation.

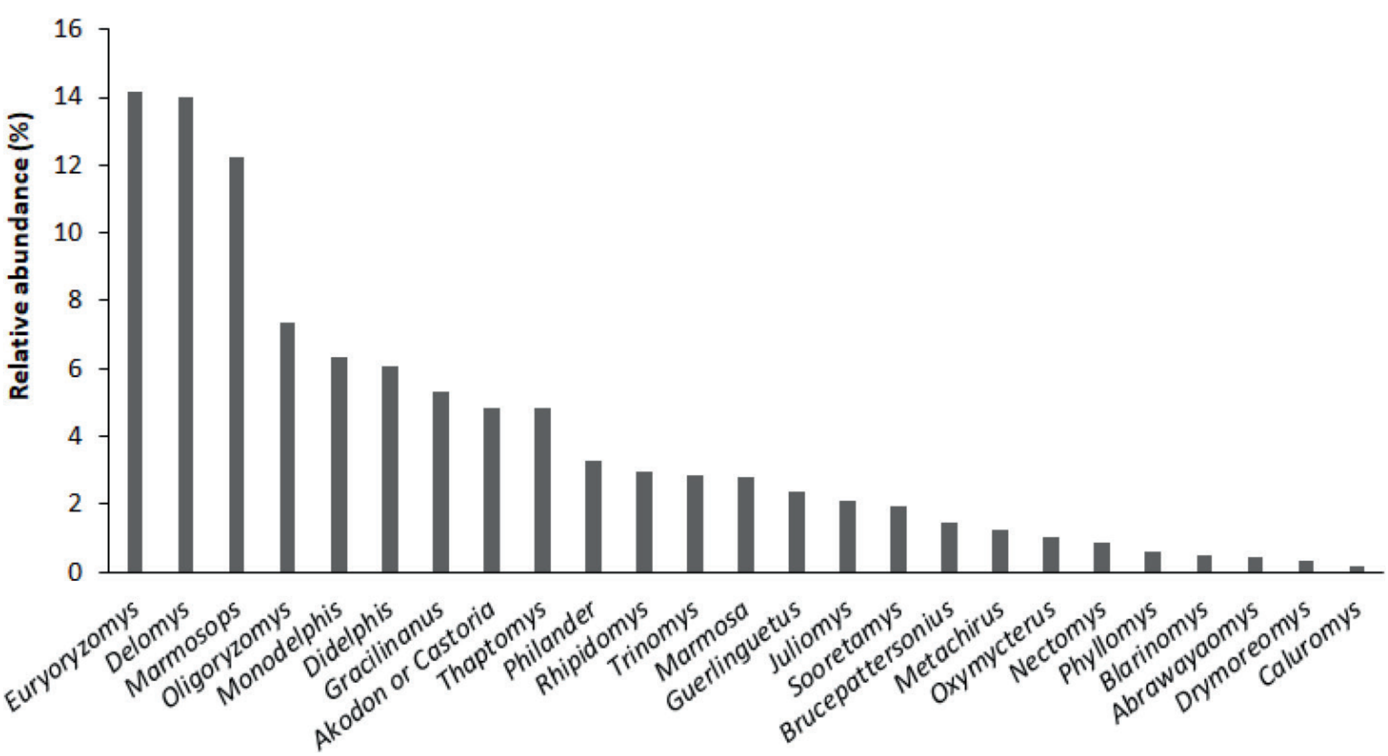

Figure 3. Relative abundance (%) for non-volant small mammals' genera (Didelphimorphia and Rodentia) recorded during twelve sampling sessions in the Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro state, Brazil. See Table 1 for the list of recorded species by genus.

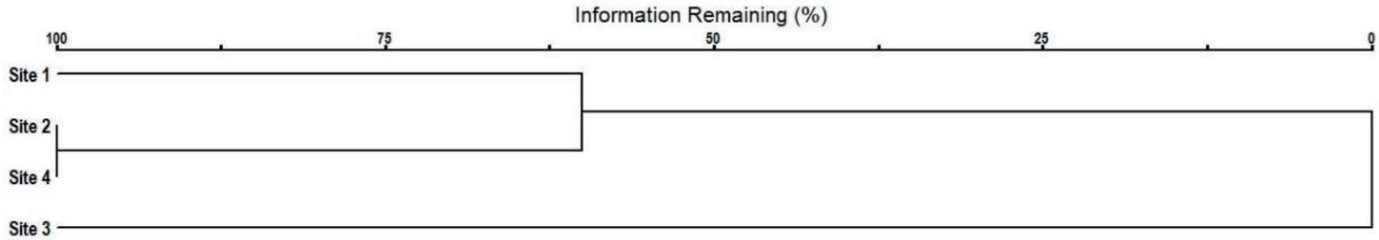

Figure 4. Cluster analysis of sampling sites (Sites 1 to 4) based on species composition of non-volant small mammals (Didelphimorphia and Rodentia) recorded during twelve sampling sessions in the Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro state, Brazil. The % information remaining is a rescaling of Wishart's objective function (Bakker 2023).

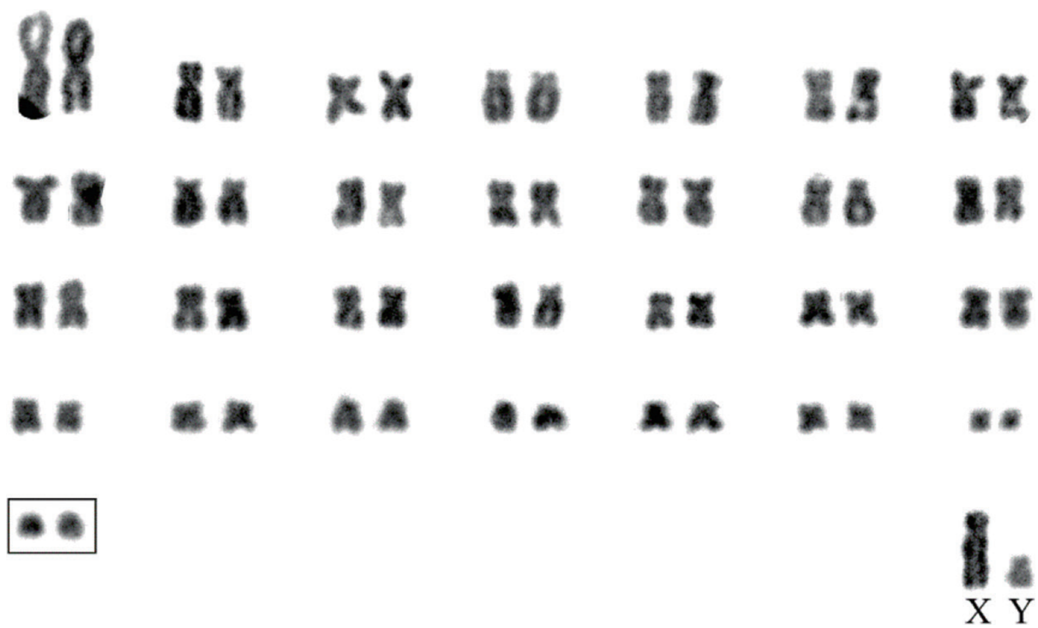

Figure 5. Karyotype (stained with conventional Giemsa) of a male of *Trinomys dimidiatus* (MN 81813) from the Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro state, Brazil. Chromosome complement with diploid number (2n) = 60 and Fundamental number of autosomes (FNa) = 114. The pair of acrocentric chromosomes is highlighted in the box.

Twenty-six specimens belonging to 14 rodent species were karyotyped (Table 2; See Data Availability). Undescribed chromosomal variation was found in *Trinomys dimidiatus* (MN 81813), that presented a distinct fundamental number of 114, composed by 28 pairs of biarmed and one pair of acrocentric chromosomes (Figure 5). The sexual pair is composed by X large submetacentric, and the Y is a small metacentric chromosome. The karyotypes of the other 13 species do not differ from the literature (Table 2).

Seventeen species were identified by molecular analysis, belonging to three families (Cricetidae, Echimiydae and Didelphidae) and 13 genera (Table 3; See Data Availability). Three genera were recorded with more than one species occurring in sympatry (*Brucepattersonius soricinus* and *B. nebulosus*; *Juliomys ossitenius* and *J. pictipes*; *Monodelphis iheringi*, *M. pinocchio* and *M. scalops*). The most common evolutionary model was General Time-Reversible (GTR + I + G) followed by Hasegawa-Kishino-Yano (HKY + I + G) (Table 3). The cytb gene was efficient in recovering the monophyly of the species and all species are formally described in the literature. Also, the blast tool of NCBI platform showed a great potential for the first screening of the analyzed specimens (Table 3).

Discussion

Thirty-two species were recorded in the present study, adding 22 species to the park's non-volant small mammals list. Most of these new records probably are due to the large sample effort carried out during a long period of time in the study area; species identification using a variety of methods, such as karyotypic and/or molecular analyses (e.g., *Juliomys* (Delciellos et al. 2020), *Phyllomys* (Delciellos et al. 2018)); or to species recently described, such as *B. nebulosus* (Abreu-Júnior & Percequillo 2019), *D. albimaculatus* (Percequillo et al. 2011), and *M. pinocchio* (Pavan 2015). Adding five species (*Akodon cursor*, *Chironectes minimus*, *Monodelphis americana*, *Oecomys catherinae*, and *Oligoryzomys flavescens*) exclusively recorded in a previous study (Delciellos et al. 2012), we obtain a list with 37 species of non-volant small mammals with confirmed occurrence in the SBNP (Table 1).

The species richness found in the SBNP (37 species) is one of the highest ever recorded for the group of non-volant small mammals in protected areas of the Atlantic Forest in Brazil, corroborating the region as a biodiversity hotspot (Dalapicolla et al. 2021, Delciellos et al. 2022). Similar species richness (37 species) was found for the Serra dos Órgãos National Park (Cronemberger et al. 2019), but in this park several areas were sampled and a higher sampling effort was carried out, including the longest small mammal monitoring study in Brazil (Gentile et al. 2023). The Bananal Ecological Station (BES) is located about 60 km from the study area in the SBNP, both protected areas being part of the same large remnant of Atlantic Forest in the Serra do Mar (Abreu-Júnior & Percequillo 2019). Thirty-two species were recorded in the BES, including rare endemic rodent species, such as *Phaenomys ferrugineus*, *Phyllomys kerri* and *Rhagomys rufescens* (Abreu-Júnior & Percequillo 2019), which were not registered within the SBNP. In other protected areas, species richness was frequently lower than that found in the SBNP, as in the Tinguá Biological Reserve (21 species; Travassos et al. 2018), Desengano State Park (21 species; Modesto et al. 2008), Morro Grande Forest Reserve (23 species; Pardini & Umetsu 2006), and Foz do Iguaçu National Park (24 species; Brocardo et al. 2019), but it is important to highlight that differences among methods used and sampling effort were not taken into account in this comparison among studies.

The use of pitfall traps in the Atlantic Forest is challenging, because of both the rough terrain with many rocks that make it difficult to install large buckets and the difficulty of keeping the animals alive once trapped in the buckets. The last situation is usually associated with a combination of low temperatures, high rainfall, and predators (Barros et al. 2015). However, the use of pitfall traps is highly recommended, as in the present study 13 out of 37 species were recorded exclusively using this method. Furthermore, in addition to capturing the most common species in the non-volant small mammals community, this sampling method was also helpful in capturing arboreal (e.g., *J. pictipes* and *J. ossitenuis*, Delciellos et al. 2020; *Phyllomys nigrispinus* and *P. sulinus*, Delciellos et al. 2018), rare (e.g., *D. albimaculatus*, Delciellos et al. 2015; *M. pinocchio*), and threatened species (e.g., *B. breviceps*, Delciellos et al. 2012).

Species richness and composition differed among the four sampling sites in the SBNP. Site 3 had the lowest species richness and it was the most dissimilar regarding species composition. The biotic and abiotic factors that cause this variation among sites were not evaluated in the present study. One of the possible explanations for the pattern we found is the large altitudinal gradient that exists in the SBNP. A large altitudinal gradient can be associated with a great variability in topography (Eisenlohr et al. 2013), which in turn can promote habitat heterogeneity (Rodrigues et al. 2020) and species diversity (Rodrigues et al. 2019). Topography (i.e., surface roughness) can also promote a higher species richness by providing a higher area availability and favoring speciation by restricting dispersal of individuals (Janzen 1967, Johnson et al. 2003, Delciellos et al. 2022). In the Atlantic Forest, a positive relationship between topography and species richness was found for tetrapods (Figueiredo et al. 2021) and marsupials (Delciellos et al. 2022).

Our study carried out at the SBNP revealed one of the highest diversities of non-volant small mammals ever recorded in the Atlantic Forest. If we add to the SBNP species list the species recorded exclusively in the BES, we obtain a surprisingly even higher species richness (42 species) for Serra da Bocaina region. However, despite being a center of endemism and a diversity hotspot for non-volant small mammals (Dalapicolla et al. 2021, Delciellos et al. 2022), the area of the SBNP located in the municipality of Paraty has been suffering with at least two main anthropic pressures in the last decade that are clearly identifiable. The first is an irregular and diffuse anthropic expansion in the park's surroundings in the municipality of Paraty, quantified by loss of forest cover and an increase in built-up areas or pasturelands (Welerson et al. 2021). The second is the paving of the RJ-165 road that crosses the SBNP, which provided increased traffic and vehicle speed, as well as easier access for humans and domestic animals to the park, and an increase in the number of wild animals being run over (Rodrigues 2020, Aguieiras 2021). The impact of these factors specifically on non-volant small mammals remains to be evaluated in future studies.

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Author Contributions

Ana Cláudia Delciellos: Substantial contribution in the concept and design of the study. Contribution to data collection, data analysis and interpretation, manuscript preparation, and critical revision, adding intellectual content.

Marcia Aguieiras: Contribution to data collection, data analysis and interpretation, manuscript preparation, and critical revision, adding intellectual content.

Roger Rodrigues Guimarães: Contribution to data collection, data analysis and interpretation, manuscript preparation, and critical revision, adding intellectual content.

Ana Carolina Loss: Contribution to data collection, data analysis and interpretation, manuscript preparation, and critical revision, adding intellectual content.

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Bruno Henrique de Castro Evaldt: Contribution to data collection, and data analysis and interpretation.

Marcelo de Assis Passos Oliveira: Contribution to data collection, data analysis and interpretation, and manuscript preparation.

Lena Geise: Contribution to data collection, data analysis and interpretation, manuscript preparation, and critical revision, adding intellectual content.

Oscar Rocha-Barbosa: Substantial contribution in the concept and design of the study.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Ethics

Specimens were handled following protocols approved by the American Society of Mammalogists (Sikes & Animal Care and Use Committee of the American Society of Mammalogists 2016); and captured and collected following the permissions of the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA/MMA process 02001.003937/2008-18, authorizations 248/2013 and 610/2015).

Data Availability

Delciellos, Ana Cláudia, 2023, "Replication Data for: Non-volant small mammals of the Serra da Bocaina National Park, southeastern Brazil: an updated species list with new data on karyotype description and phylogeny", https://doi.org/10.48331/scielodata.NPBXGK, SciELO Data, DRAFT VERSION, UNF:6:vFcDoxWDHKr71+QStMU7jQ== [fileUNF]

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