Bats (Mammalia, Chiroptera) from the Nísia Floresta National Forest, with new records for the state of Rio Grande do Norte, northeastern Brazil

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Abstract: The state of Rio Grande do Norte is considered a data gap for bat species records in Brazil. The state is also currently target of large economic projects with potential impacts on bats, especially wind farms and mining enterprises. In addition, Rio Grande do Norte has few conservation units in which there is no systematic study on bat fauna. The Nísia Floresta National Forest (NFNF), a federally protected area of 174 hectares, is located in the eastern coast of Rio Grande do Norte and corresponds to one of the last remnants of Atlantic Forest in the state, in its northernmost limits. A bat inventory was conducted in NFNF using mist nets set at ground level, from sunset to sunrise, from December 2011 to December 2012, totaling 25 sampling nights. We captured 1,379 bats belonging to four families and 16 species. *Artibeus planirostris* (Phyllostomidae) was the most frequently captured species (n = 685; 50%), followed by *Myotis lavali* (Vespertilionidae) (n = 248; 18%) and *Phyllostomus discolor* (Phyllostomidae) (n = 147; 11%). *Peropyrexy leucoptera, Phyllostomus discolor, Phyllostomus hastatus, Lophostoma brasiliense, Lasiusse blossevillii, Myotis lavali, and Promops nasatus* are new records for Rio Grande do Norte, increasing the current number of bat species from 25 to 32 in this state. Further inventories, especially using acoustic surveys with bat detectors, might add more species to the NFNF bat list.

Key-words: Atlantic Rainforest, chiropteran inventory, conservation units, Neotropical biodiversity, protected areas.

Morcegos (Mammalia, Chiroptera) da Floresta Nacional de Nísia Floresta, com novos registros para o estado do Rio Grande do Norte, nordeste do Brasil

Resumo: O estado do Rio Grande do Norte é considerado uma lacuna de informações sobre ocorrência de morcegos no Brasil. O estado também é atualmente alvo de grandes empreendimentos com potencial impacto sobre a quiropterofauna, especialmente no setor de energia eólica e mineração. Além disso, apresenta poucas unidades de conservação, e estes não possuem sua quiropterofauna estudada de maneira sistematizada. A Floresta Nacional de Nísia Floresta (FNNF), uma unidade de conservação federal de 174 hectares, localiza-se na costa leste do Rio Grande do Norte e corresponde a um dos últimos remanescentes de Mata Atlântica no estado e no limite norte do bioma. Foi realizado um inventário de morcegos na FNNF com a utilização de redes de neblina armadas no nível do solo, do por do sol ao amanhecer, de dezembro de 2011 a dezembro de 2012, totalizando 25 noites de amostragem. Nós capturamos 1379 morcegos pertencentes a quatro famílias e 16 espécies. *Artibeus planirostris* (Phyllostomidae) foi a espécie mais frequentemente capturada (n = 685; 50%), seguida por *Myotis lavali* (Vespertilionidae) (n = 248; 18%) e *Phyllostomus discolor* (Phyllostomidae) (n = 147; 11%). *Peropyrexy leucoptera, Phyllostomus discolor, Phyllostomus hastatus, Lophostoma brasiliense, Lasiusse blossevillii, Myotis lavali, e Promops nasatus* são novos registros para o Rio Grande do Norte, aumentando o número atual de espécies de morcegos no estado de 25 para 32. Inventários adicionais, especialmente utilizando amostragens acústicas com detectores de morcegos, tendem a acrescentar novas espécies à lista de morcegos da FNNF.

Palavras-chave: Mata Atlântica, inventário de quirópteros, unidade de conservação, biodiversidade Neotropical, áreas protegidas.
Introduction

Brazil is a mega-biodiversity country (Mittermeier et al. 1997) with one of the highest species richness of bats in the world. Currently, nine families, comprising 178 species, are known in the Brazilian territory (Nogueira et al. 2014). Nevertheless, information on distribution and occurrence of bat species in Brazil is highly fragmented. There are no records of bat species for nearly 60% of the Brazilian area, and 8% of the country can be considered minimally surveyed in terms of bats (Bernard et al. 2011). One of the most significant data gaps about bat occurrence in Brazil corresponds to the state of Rio Grande do Norte (Bernard et al. 2011), located in the northeastern part of the country. Currently, Rio Grande do Norte is the state with the lowest number of localities surveyed for bats (= 12) and the lowest bat richness (= 24 species) in northeastern Brazil (Garcia et al. 2014). Concomitantly, this state has been attracting large economic projects with potential impact on bats, such as wind farms and mining development.

The east coast of Rio Grande do Norte corresponds to the northern limit of the Atlantic Forest biome (IBGE 2004), a hotspot for biodiversity conservation due to high rates of endemism and habitat loss (Myers et al. 2000). Five of the 10 bat species currently considered endemic to Brazil occur exclusively in the Atlantic Forest (Nogueira et al. 2014). The region originally covered an area of 150 million hectares, but currently, only 12% of the original vegetation remains, mostly distributed in small forest fragments below a size of 50 hectares (Ribeiro et al. 2009). Similarly, the current area of the Atlantic Forest corresponds to 12% of its original domain in the state of Rio Grande do Norte, with about 27,000 hectares of natural non-forest vegetation (including mangroves and salt marshes) and 16,000 hectares of forest remnants (Fundação SOS Mata Atlântica & INPE 2014). One of these remnants is the Nísia Floresta National Forest (Floresta Nacional de Nísia Floresta), a federally protected area of 174 hectares created in 2001 (MMA 2012).

Although the coastline of Rio Grande do Norte is considered an area of high biological importance and a priority area for biodiversity conservation of the Atlantic Forest (Conservation Internacional do Brasil et al. 2000), this region is poorly surveyed in terms of bats. The scarce information about bat occurrence along the coastal region of Rio Grande do Norte is dispersed in a few research papers and non-published dissertations and theses (reviewed by Garcia et al. 2014). Bats are diverse and abundant (Altringham 1996), indicators of habitat disturbance (Jones et al. 2009), and play important ecological roles as insect controllers (Boyles et al. 2011), pollinators (Fleming et al. 2009), and seed dispersers (Lobova et al. 2009) in the Neotropics. Considering the lack of information about the bat fauna in Rio Grande do Norte and the biological relevance of the remaining Atlantic Forest fragments in this state, the objective of this study was to carry out the first bat inventory at the Nísia Floresta National Forest, northeastern Brazil.

Material and Methods

1. Study Area

Our study was carried out at the Nísia Floresta National Forest (hereafter termed NFNF), a federal protected area located in the municipality of Nísia Floresta, state of Rio Grande do Norte, northeastern Brazil (latitude: 06°05′12.4″ S; longitude: 35°11′04.0″ W). The NFNF covers an area of 174.95 ha, with a maximum altitude of 100 m asl (MMA 2012). The climate in this region is classified as tropical savannah (Aw) (Peel et al. 2007) with an average temperature of 27°C (maximum: 30°C; minimum: 21°C) (IDEMA 2013). Normal annual rainfall is 1,522 mm, with a distinctive rainy season from March to August and a dry season from September to February (IDEMA 2013).

The study area is located in the Atlantic Forest biome (IBGE 2004), in a region originally covered by rainforest that has been historically exploited for sugarcane production. The NFNF area has been used as a forestry experiment station from the 1960s to the early 1980s and consists of both secondary native forest (= 60% of the total area) and experimental forestry sites (= 40% of the total area) (MMA 2012). Within the native forest, the predominant vegetation is seasonal semideciduous forest (“floresta estacional semidecidual”) (= 45% of the NFNF total area) in the west-central part of the NFNF, followed by coastal tableland (“tabuleiro litorâneo”) (= 15% of the NFNF total area) in the northern part of the NFNF (MMA 2012).

Bat surveys took place at the experimental forestry area in the southeastern part of the NFNF. This area exhibits both regenerating seasonal semideciduous forest and introduced plants, including native and exotic species planted during forestry experiments. The dominant tree species are Tipirira guianensis (family Anacardiaceae), Byrsonima crassifolia (family Malpighiaceae), Mischocarpus sundaeus (family Sapindaceae), Cordia nodosa (family Boraginaceae), Plathymenia reticulata (family Leguminosae), Caesalpinia ferrea (family Leguminosae), and exotic species of Eucalyptus (family Myrtaceae) (MMA 2012). Tipirira guianensis (locally called “cuiúba”) is common throughout the entire area, while the density of the other species varies among different sites within the NFNF experimental forest zone (MMA 2012).

2. Sampling

Bat surveys were conducted using mist nets (12 × 3 m, mesh 19 mm, five shelves, Ecotone®) from December 2011 to December 2012. Sampling was performed during a total of 25 nights, one in December 2011 and two consecutive nights per month from January to December 2012. At each night, eight mist nets were set at ground level along forest trails and edges. The mist nets were set at different sites within the NFNF experimental forestry area in December 2011 and at the same spots from January to December 2012 (six along the first 300 m of the NFNF main trail and two at forest edges near the trailhead). Nets were opened at sunset and remained open for 11 h. The total sampling effort was 79,200 h.m² (3,168 h.m² per night), calculated according to Straube & Bianconi (2002) by multiplying the following factors: mist net area (m²), hours of exposure (h), number of nets, and number of sampling nights.

Captured bats were removed from nets, kept in individual cotton bags, and checked for sex and reproductive status (according to Racey 2009), age (according to Brunet-Rossini & Wilkinson 2009), forearm length (using a digital caliper), and body mass (with a spring scale). Bats were then immediately released at the capture site, except a number between 1–10 individuals per species which were killed by ether inhalation and collected as voucher specimens (permit SISBIO/ICMBio #30730-2), as well as individuals who accidentally died during capturing or handling processes. These specimens were fixed in formaldehyde (10% solution), preserved in ethanol (70% solution), and deposited in the Mammal Collection of the Federal University of Pernambuco (UFPE).

Voucher specimens were taxonomically identified according to the keys and descriptions of Araújo & Langguth (2010), Cloutier & Thomas (1992), Fonseca & Pinto (2004), Greenhall et al. (1983), Gregorini & Taddei (2002), Jones & Hood (1993), Lim et al. (2010), Moratelli et al. (2011), Reis et al. (2007), Simmons & Voss (1998), Velazco (2005), and Webster (1993). In specimens for which morphological identification was not completely conclusive, tissue samples (liver pieces) were used to molecular identification. DNA extraction was conducted using the Biopur Mini Spin Plus kit according to the manufacturer’s instructions. A conserved region of 360 bp of the cytochrome subunit b gene was amplified by PCR with the universal primers L14841 (5′-AAA AAG CTT CCA TCC AAC ATC TCA GCA TGA TGA AA-3′) and H15149 (5′-AAA CTG CAG CCC TCT AGA ATG ATA TTT GTC CTC A-3′).
(Kocher et al. 1989). Amplifications were performed in a final volume of 25 μl containing 12.5 μl of 2X Taq Master Mix (Vivantis Technologies), 0.5 μl of MgCl2 (50 mM), 1.0 μl of each primer (2.0 mM), and 10 ng of extracted DNA, according to Bobrowiec et al. (2015). The PCR products were purified and sequenced with the sequencing kit Big Dye® Terminator v3.1 (Life Technologies), according to the protocol provided, in both forward and reverse directions, in ABI 3500 automatic sequencer. To obtain a consensus sequence, we aligned and edited the sequences obtained in the program BioEdit v. 7.2.5 (Hall 1999). Then, we compared the sequences to homologous sequences of Cytb deposited in GenBank. In addition, we aligned the consensus sequences with sequences of the possible species, based on information on distribution and occurrence of bat species in northeastern Brazil.

Results

We captured a total of 1,379 bats of 16 species and four families (Table 1, Figure 1). The richest family was Phyllostomidae (12 species); the families Vespertilionidae, Emballonuridae, and Molossidae were represented by one to two species each. Artibeus planirostris (Phyllostomidae) was the most frequently captured species (50%), followed by Myotis lavali (Vespertilionidae) (18%) and Phyllostomus discolor (Phyllostomidae) (11%). The capture frequencies of the other species were less than 10%.

All captured species are vouchered by specimens in the Mammal Collection of the Federal University of Pernambuco (Appendix 1). Molecular taxonomic identification was carried out in three specimens (UFPE 3297, UFPE 3191, and UFPE 3198), in order to confirm the identification of the species from the genera Carollia and Myotis. The taxonomic identification of the specimens was confirmed as Carollia perspicillata and Myotis lavali (similarity was 100% in all cases; see gene sequences in the online Supplementary Material 1 and 2).

Discussion

Although our sampling was limited to one habitat type (experimental forestry area) within the NFNF, this is the first systematic inventory of bats in a protected area in the state of Rio Grande do Norte. Our results are regionally relevant and provide new information on bat composition in this state with a poorly known and described bat fauna. Approximately 44% of all captured species are new state records; we report for the first time the occurrence of Myotis lavali, Phyllostomus discolor, Phyllostomus hastatus, Lophostoma brasiliense, Lasiusus blossevillii, Peropteryx leucotera, and Promops nasutus in Rio Grande do Norte. In addition, we confirm the occurrence of seven species (Artibeus lituratus, Artibeus planirostris, Carollia perspicillata, Desmodus rotundus, Platyrhinus lineatus, Sturnira lilium, and Trachops cirrhosus) by providing voucher specimens, which were lacking in the previous records in grey and peer-reviewed literature (reviewed by Garcia et al. 2014). Only the previous records of Dermanura cinerea and Glossophaga soricina are vouchered by specimens in collections (Handley Jr. 1987, Webster 1993). The seven new species records increase the current number of bat species from 25 to 32 in Rio Grande do Norte (Barros 2014, Garcia et al. 2014).

Artibeus planirostris is a primarily frugivorous bat (Hollis 2005) and accounted for about 50% of total bat captures. This high frequency is probably associated with the availability of food resources in our study area, in which several fruit trees could be identified near the main forest trail. These bats were often captured carrying fruits of Ficus spp. (family Moraceae) and Cecropia spp. (family Urticaceae), which are largely consumed by Artibeus species in the Neotropics (Lobova et al. 2009). Artibeus planirostris is also often captured by mist-netting in the urban area of Natal city; this species accounted for 96% (N = 260) of bat captures in green areas within the campus of the Federal University of Rio Grande do Norte (UFRN) (M.A.S. Barros, unpublished data). This suggests that A. planirostris is an abundant bat species both in natural and anthropic areas in the Atlantic Forest biome in this state. Apart from the present

Table 1. Numbers of bat captures by mist-netting in forest trails and edges at the Nísia Floresta National Forest (Floresta Nacional de Nísia Floresta), state of Rio Grande do Norte, northeastern Brazil, from December 2011 to December 2012.

| Taxon                    | Number of females | Number of males | Total number | Frequency |
|--------------------------|-------------------|-----------------|--------------|-----------|
| **Family Emballonuridae**|                   |                 |              |           |
| Peropteryx leucotera     | –                 | 1               | 1            | 0.1%      |
| **Family Phyllostomidae**|                   |                 |              |           |
| Artibeus lituratus (Offer, 1818) | 7 | 4 | 11 | 0.8% |
| Artibeus planirostris (Spix, 1823) | 342 | 343 | 685 | 49.7% |
| Carollia perspicillata (Linnaeus, 1758) | 22 | 18 | 40 | 2.9% |
| Dermanura cinerea (Gervais, 1856) | 16 | 13 | 29 | 2.1% |
| Desmodus rotundus (É. Geoffroy, 1810) | – | 1 | 1 | 0.1% |
| Glossophaga soricina (Pallas, 1766) | 63 | 57 | 120 | 8.7% |
| Lophostoma brasiliense (Peters, 1866) | 3 | 5 | 8 | 0.6% |
| Phyllostomus discolor (Wagner, 1843) | 71 | 76 | 147 | 10.7% |
| Phyllostomus hastatus (Pallas, 1767) | 6 | 5 | 11 | 0.8% |
| Platyrhinus lineatus (É. Geoffroy, 1810) | 24 | 17 | 41 | 3.0% |
| Sturnira lilium (É. Geoffroy, 1810) | 8 | 26 | 34 | 2.5% |
| Trachops cirrhosus (Spix, 1823) | 1 | – | 1 | 0.1% |
| **Family Molossidae**    |                   |                 |              |           |
| Promops nasutus (Spix, 1823) | – | 1 | 1 | 0.1% |
| **Family Vespertilionidae** |                   |                 |              |           |
| Lasiusus blossevillii ([Lesson, 1826]) | – | 1 | 1 | 0.1% |
| Myotis lavali (Moratelli, Peracchi, Dias & Oliveira, 2011) | 5 | 243 | 248 | 18.0% |
| **Total**                | 1379              |                 | 100.0%      |           |

*According to Nogueira et al. (2014).
study, there are currently six additional records of *A. planirostris* in Rio Grande do Norte: in Ponta Negra, Natal (Varela-Freire 1997), and in five caves, one (Gruta da Carrapateira) in Felipe Guerra (Ferreira et al. 2010, Cordero-Schmidt et al. 2016), one (Caverna do Serrote Preto) in Lajes, one (Gruta Casa de Homens) in Caraúbas, and two (Caverna Lajedo Grande and Caverna da Pedrada) in Governador Dix-Sept Rosado (Cordero-Schmidt et al. 2016).

*Myotis lavali*, the second most frequently captured species (18%), is a recently described species (Moratelli et al. 2011) whose distribution limits and natural history are poorly known. Our record is a new marginal point of occurrence and extends the distribution of the species by about 470 km southeast (from Russo–CE) and about 210 km north (from São Lourenço da Mata–PE) (Moratelli & Wilson 2013). In northeastern Brazil, this species occurs mainly in xeric forests and shrublands within the Caatinga biome.
Kwiecinski, Trachops, Artibeus planirostris occurs both in
Peropteryx leucoptera, Promops nasutus
probably occurring in the states of Ceará, Paraíba, Pernambuco, Alagoas,
and Sergipe. The low number of records is possibly due to the foraging
behavior of the genus Promops, whose species hunt insects in the open
space high above the ground or canopy (Schnitzler & Kalko 2001), and are
therefore rarely captured by mist-netting at the ground level. The species
Peropyrtex leucopera is also poorly recorded in northeastern Brazil; it
was observed in Sapé in the state of Paraiba (Feijó & Langguth 2011), in
Itamaracá (Cruz et al. 2002) and Formoso (Guerra 1980) in the state of
Pernambuco, and in Capela in the state of Sergipe (Mikalauskas et al. 2014).
This species apparently has a disjunct geographic distribution, occurring
throughout northern South America and separately in the coastal region
of extreme northeastern Brazil (Mikalauskas et al. 2014). Our record extends
the distribution of Peropyrtex leucopera to 110 km north (from Sapé–PB)
in the northeastern region of Brazil, corresponding to the northern limit
of distribution of this species in the Atlantic Forest biome.

Among the bat species previously recorded in Rio Grande do Norte,
Desmodus rotundus and Trachops cirrhosus (cited as Trachops sp.) were
observed inside caves in the western region of the state in the Caatinga
biome (Ferreira et al. 2010). Therefore, we recorded these bat species
for the first time in an Atlantic Forest area in the state of Rio Grande do
Norte. The species Artibeus lituratus, Carollia perspicillata, Sturnira
lilium, Dermanura cinerea, Glossophaga soricina, and Phyltryrinus
lineatus were recorded in Atlantic Forest areas near NFNF (≈18–22 km).
Artibeus lituratus, Carollia perspicillata, and Sturnira lilium occur at
the Parkes Estadual do Jiqui in the municipality of Parnamirim, a
398 hectares protected area that exhibits forest remnants and hydric
resources (Farias 2009); Dermanura cinerea, Glossophaga soricina,
and Phyltryrinus lineatus were recorded in the Parkes Estadual do Jiqui
and also in the municipality of Natal (Handley Jr. 1987, Webster 1993,
Varela-Freire 1997, Farias 2009). Artibeus planirostris occurs both in
the Atlantic Forest and Caatinga biomes in the state of Rio Grande do
Norte (Ferreira et al. 2010, Varela-Freire 1997, Cordero-Schmidt et al.
2016, Present study).

Although the NFNF is a relatively small protected area in regeneration
surrounded by farms and croplands, it apparently is an important habitat
for bats. The bats from NFNF are morphologically and ecologically diverse,
including species that feed primarily on fruits (Artibeus planirostris,
Artibeus lituratus, Dermanura cinerea, Phyltryrinus lineatus, Sturnira
lilium, and Carollia perspicillata), pollen/nectar (Glossophaga soricina
and Phyllostormus discolor), arthropods and small vertebrates (Trachops
cirrhosus and Phyllostormus hastatus), insects (Peropyrtex leucopera,
Myotis lalvi, Lasius blossevilli, Promops nasutus, and Lophostoma
brasiliense), and blood (Desmodus rotundus). Thus, the bats recorded
in the present study provide ecosystem services such as seed dispersal,
pollination, and control of insect populations in the region of the NFNF.
Bats from the family Phyllostomidae corresponded to the largest number
of captures, which is in accordance with most inventories in the Neotropics
(e.g. Bernard et al. 2001, Esbéard et al. 2006, Novaes & Laurindo 2014).
This pattern is related both to the high abundance of phyllostomid bats in
Brazilian tropical areas and to the selectivity of the mist-netting method,
which tends to capture bats that forage near the ground and vegetation
(such as fruit-eating and nectar-feeding bats). Further inventories, especially
using acoustic surveys and sampling areas further north of the NFNF, might
add more species to the present bat list.

Supplementary material

The following online material is available for this article:

Supplementary Material 1: Consensus sequences of a specimen of
Carollia perspicillata (Chiroptera, Phyllostomidae), collected as voucher
(Mammal Collection of the Federal University of Pernambuco; collection
number UFPE 3297) in 2012 at the Nisia Floresta National Forest, Rio Grande do Norte, northeastern Brazil.

Supplementary Material 2: Alignment of consensus sequences of two specimens of Myotis lalavi (Chiroptera, Vespertilionidae), collected as vouchers (Mammal Collection of the Federal University of Pernambuco; collection numbers UFPE 3191 and 3198) in 2012 at the Nisia Floresta National Forest, Rio Grande do Norte, northeastern Brazil, and sequences from Myotis nigricans, Myotis levis and Myotis lalavi.

Appendix 1: List of bat specimens collected as vouchers at the Nisia Floresta National Forest (Floresta Nacional de Nisia Floresta), state of Rio Grande do Norte, northeastern Brazil, from December 2011 to December 2012. This material is deposited in the Mammal Collection of the Federal University of Pernambuco (Universidade Federal de Pernambuco).

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Author Contributions

Marília A. S. Barros: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Camila Martins Gomes Morais: Contribution to data collection; Contribution to manuscript preparation.

Bruna Marisa Braga Figueiredo: Contribution to data collection; Contribution to manuscript preparation.

Gustavo Andrade de Moura Júnior: Contribution to data collection; Contribution to manuscript preparation.

Fernanda Ito dos Santos: Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Enrico Bernard: Contribution to critical revision, adding intellectual content.

Conflicts of interest

The authors declare no conflict of interest.

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