



# Bats (Mammalia: Chiroptera) in urban–rural interfaces: community structure associated with pathogen screening in São Paulo—the largest metropolitan region in Brazil

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## Abstract

Little is known about the influence of the urban environments on bat species ‘ecology. The urbanization process potentially lead to critical ecological changes in bat communities’ intra and interspecific pathogenic transmissions dynamics. To date, the monitoring of pathogens in bats in Brazil has only been done with bats found dead or alive in households, from rabies surveillance systems. The present work aimed to investigate how urbanization influenced bat richness, relative abundance and pathogen occurrence. Most captured bats were Phyllostomidae, especially *Sturnira lilium*, *Artibeus lituratus*, *A. fimbriatus*, *Glossophaga soricina*, and *Platyrrhinus lineatus*, among others. From preserved-rural towards urban areas the lesser the bat richness, the higher the relative abundance of the captured bats. Noise level, luminosity and relative humidity correlated with bat abundance. The proportion of genders, sexually active bats and their size (weight, right forearm length, and body condition index) were stable throughout the investigation. Still, the proportion of pregnant females was higher in Spring and the number of juveniles in Summer, evidencing the seasonality of reproduction. Several Enterobacteria were isolated, evidencing a significant role of bats in the circulation of pathogens of medical and veterinary interest. These results are crucial in the pursuit of a harmonious coexistence between humans, bats and domestic animals in areas with different levels of anthropization.

**Keywords** Phyllostomidae · Richness · Abundance · Enterobacteria · Sao Paulo · Brazil

## Introduction

The heterogeneity of the environment in terms of human pressure, phytophysiology and economic exploitation influence the richness and composition of the bat community, either locally or regionally (Jung and Threlfall 2015). Anthropogenic changes can have positive or negative effects on bat species, leading some to population decline or local extinction and others to the benefit of new opportunities for shelter and food (Threlfall et al. 2011). Bats are among the vertebrate taxa best adapted to the urban environment (Nunes et al. 2017). Of the 182 species recorded in Brazil (Abreu et al. 2022), at least 84 (47.2%) occur in urban areas (Nunes et al. 2017). Among them, generalist species benefit most from artificial shelters, food items, such as fruit trees and insects attracted by urban lighting (Nunes et al. 2017; Straka et al. 2019), and even swimming pools as water sources (Nystrom and Bennet 2019). Some studies regarding

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the structure of bat populations in Brazilian urban areas were published (Esbérard et al. 2014; Nunes et al. 2017). In the Metropolitan Area of São Paulo (MASP), this type of survey is sparse in space and time, based on secondary data from bat rabies surveillance systems (Silva et al. 1996), convenience samples or animals deposited in collections (Garbino 2016), making it difficult to determine the sampling effort and its representativeness.

Bats have characteristics (flight, hibernation, life history and migration) that make them ideal hosts for pathogens, especially viruses (Banerjee et al. 2020; Irving et al. 2021). They are the second largest and one of the most diverse groups of mammals and the only ones capable of genuine flight (Neuweiler 2000). The high energy demand required for flight and their increased body temperature at night (during torpor) may select pathogens that can either survive in the host or be transmitted (Neuweiler 2000; Kunz and Fenton 2003). Most species are long-lived and gregarious, facilitating intra- and inter-specific transmission of pathogens. Moreover, vertical transmission allows pathogens to persist in colonies (Kunz and Fenton 2003) and alteration of migration routes through land use alteration allows the geographic spread of pathogens (Beltz 2018).

The study of urban bats and associated zoonoses is a topic of recent global interest. Bats (and other mammals) are susceptible to lyssaviruses such as rabies virus (RABV), filoviruses (Ebola and Marburg), paramyxoviruses (Hendra and Nipah), and coronavirus (CoV, such as SARS-CoV and MERS-CoV) (Calisher et al. 2006; Kessler et al. 2018; Beltz 2018). Furthermore, they are susceptible to bacterial infections, whether transmitted by arthropods (*Bartonella* sp., *Borrelia* sp. and *Rickettsia* sp.) or not (*Leptospira* sp., *Yersinia* sp., *Pasteurella* sp., *Mycoplasma* sp.), protists (*Babesia* sp., *Toxoplasma gondii*, *Eimeria* sp., *Cryptosporidium* sp., *Trypanosoma* sp. and *Leishmania* sp.) and fungi (*Histoplasma capsulatum*, *Blastomyces dermatidis*, *Pneumocystis* sp., *Coccidioides* sp., *Encephalitozoon* sp., *Candida* sp., *Malassezia* sp. and *Pseudogymnokscus destructans*, which causes the “white-nose syndrome”) (Kunz and Parsons 2009). Except for the “white-nose syndrome”, which causes population declines of bats in North America, the other diseases described here have zoonotic potential. In the MASP, one of the urban areas with the ample richness of bat species (Nunes et al. 2017), the description of pathogens in bats refers to *Candida* sp. (Botelho et al. 2012), *Rickettsia* spp. (D’Auria et al. 2010), *Toxoplasma gondii* (Cabral et al. 2013), *Leptospira* spp. (Bessa et al. 2010), and rabies virus (Favoretto et al. 2002; Sodr e et al. 2010).

In a gradient from a forested area towards the urban matrix, a reduction in richness and an increase in the abundance of some bat species is expected (Hourigan et al. 2006). It is expected that this increase in bat abundance can be accompanied by an increase in the frequency of pathogens.

At the interface between urban and rural areas, there is a knowledge gap regarding the selection of bat species by urban areas and the consequent transmission of RABV strains (De Lucca et al. 2013; Dias et al. 2019) and other pathogens between them. Thus, the present work aimed to investigate how the landscape components influence richness and relative abundance of bat species and pathogen occurrence on an urban–rural–natural gradient.

## Material and methods

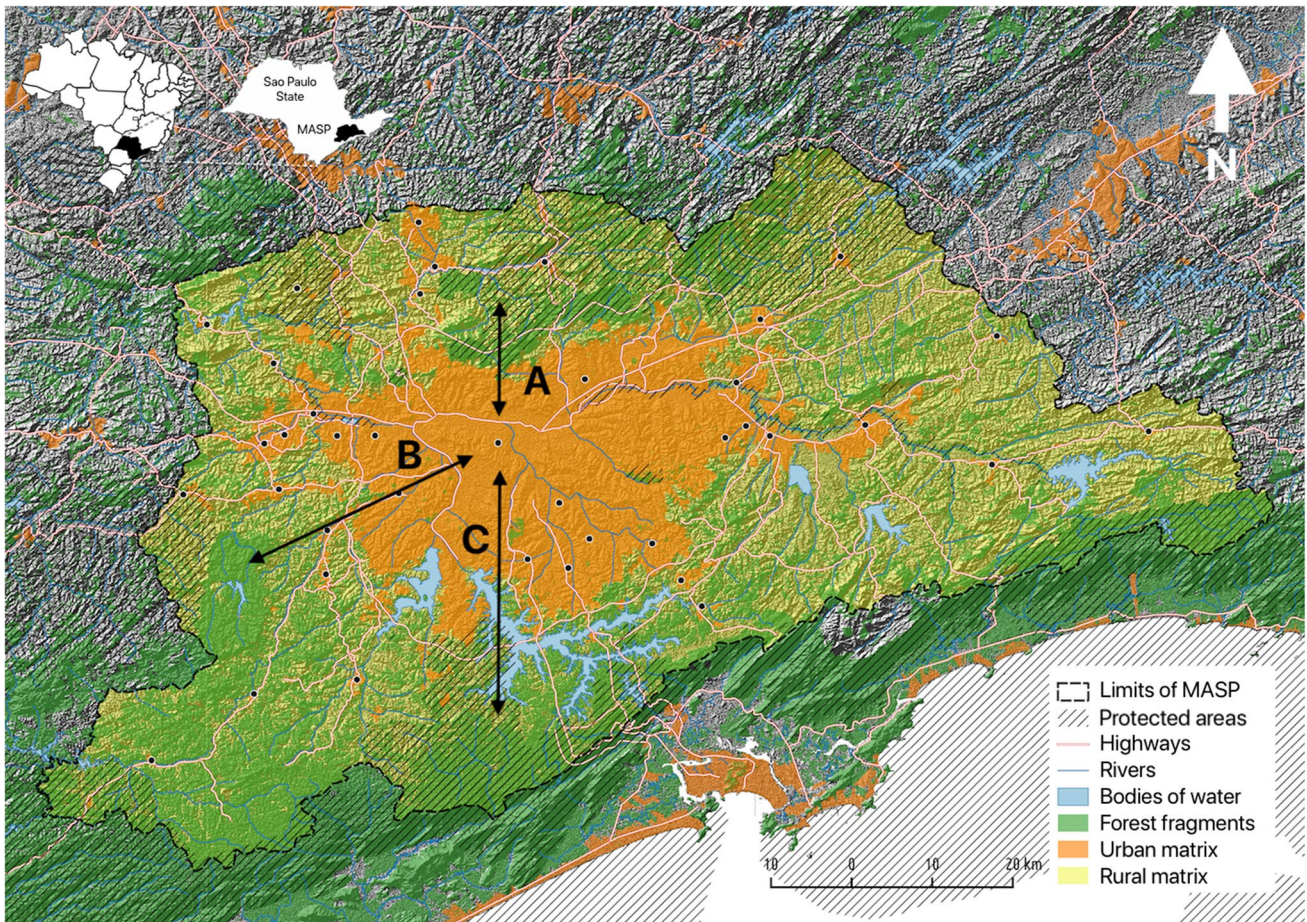
### Study area

The Metropolitan Area of São Paulo (MASP) comprises an area of 7,946.96 km<sup>2</sup> (about 3.2% of the total area of the State of São Paulo), home to 21,571,281 inhabitants (about 47.4% of the total population of the State). It comprises 39 municipalities that make up the largest, most important national wealth center, corresponding to about 18% of the Brazilian gross domestic product (GDP) and 54% of the State GDP (EMPLASA 2019). The MASP has important industrial, commercial and financial centers and an extensive rural matrix responsible for the supply of horticultural products.

The vegetation cover is essentially fragmented with some protected areas, mainly to the north, to the west, to the south, and even within the urban matrix. A detailed map of the study area was elaborated in QGIS software, using open source databases on protected areas, highways, rivers, forest fragments and urban–rural matrix (Fig. 1).

Considering that landscape components (fragmentation, composition, etc.) influence the richness and abundance of bat species in the gradient between the urban matrix and conserved areas (Hourigan et al. 2010; Threlfall et al. 2011), some regions of the MASP would be eligible for carrying out field surveys, as shown in Fig. 1. The parameters used to choose the axis B are described elsewhere (Mello 2022), but briefly, because it presented a distinct gradient between a large forest fragment, rural area and urban matrix. Moreover, the landscape gradient over the axis was stratified to allow the selection of the sampling areas, also according to Mello (2022). The other axis (A and C) were not chosen because either there was an abrupt change from the urban matrix to the forest fragment (absence of rural area in between) (axis A) or the forest fragment, although present, was found in the slope of the mountain range of Serra do Mar (axis C), ranging from around 800 to 0 m altitude.

Along Axis B, six sampling areas were chosen, one for each stratum, according to Mello (2022) (Fig. 2): **Area 1** (representing Stratum 1—“environmental preservation”)—Morro Grande Reserve—area of about 10,000 ha of dense montane Atlantic rain forest and altitude of approximately



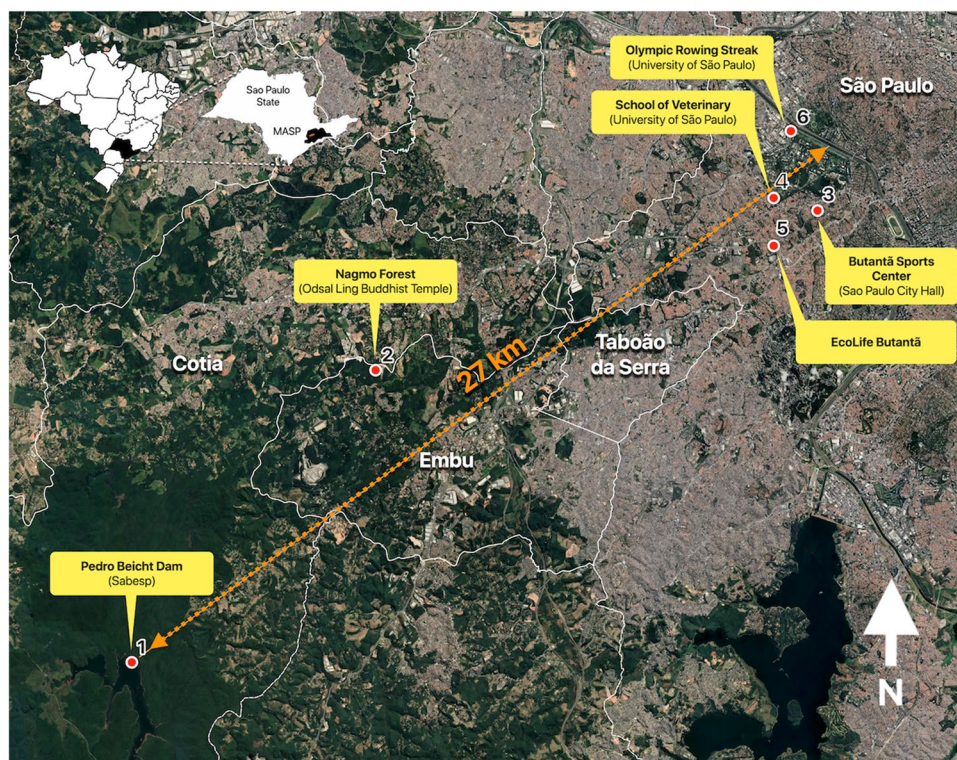
**Fig. 1** Map of the Metropolitan Area of Sao Paulo, Brazil, highlighting three eligible axes for bat survey. The dots represent the city centers and the arrows, the possible study axes

900 m. The bat capture area was located on the banks of the Pedro Beicht dam at the end of Morro Grande Trail (-23.71495° S; -46.96019° W); **Area 2** (representing Stratum 2—“rural”)—Nagmo Forest from the Odsal Ling Buddhist Temple—fragment of secondary Atlantic rain forest with the presence of exotic and ornamental plant species with about 100 ha and an average altitude of 800 m, surrounded by rural areas and condos. The place had been closed for almost 40 years and was recently acquired by the Buddhist Temple, which proceeded with the cleaning of the site and the opening of trails (-23.62412° S; -46.87513° W); **Area 3** (representing Stratum 3—“houses”)—Butantã Sports Educational Center from the City Hall of Sao Paulo—municipal club surrounded by houses, with the presence of several fruit trees, including papaya (*Carica papaya*), several banana trees (*Musa sp.*), an avocado (*Persea americana*) and a large fig tree (*Ficus sp.*), located near a gate ball field at an average altitude of 800 m (-23.57519° S; -46.72,369° W); **Area 4** (representing Stratum 4—“slums”)—School of Veterinary Medicine of the University of Sao Paulo (FMVZ-USP)—back of the faculty grounds, composed of a small fragment

of secondary Atlantic rain forest, with the presence of several *Piper sp.* and an average altitude of 800 m. In addition, the presence of a line of *Eucalyptus sp.* along the avenue in front (-23.57045° S; -46.73918° W); **Area 5** (representing Stratum 5—“verticalization”)—EcoLife Condominium—a vegetable garden and orchard area next to the condominium garage building, with 1,800 m<sup>2</sup>, on the banks of an important highway, with several fruit trees including avocado (*Persea americana*), banana (*Musa sp.*), mulberry trees (*Morus sp.*) at an average altitude of 800 m (-23.58556° S; -46.73969° W); **Area 6** (representing Stratum 6—“industrial warehouses”)—University of São Paulo Olympic Rowing Streak—western end of the rowing streak, with a predominance of exotic non-fruit trees and few bananas (*Musa sp.*) and guava trees (*Psidium guajava*) at an average altitude of 760 m (-23.55051° S; -46.73969° W).

FMVZ-USP was chosen, even though it does not belong to Stratum 4, it is close to the São Remo slum (about 500 m). The University of São Paulo Olympic Rowing Streak was chosen because it is close (100 m) to the industrial zone of Stratum 6. Contact had been made with the Museum

**Fig. 2** Sampling areas for bat survey in the Metropolitan Area of Sao Paulo, Brazil



of Technology of São Paulo, located inside Stratum 6, but it may not have been possible, as it was closed during the COVID-19 pandemic.

### Bat capture and bat population structure

A training was organized between October 19 and 21, 2020, along with the pilot-project in one of the sampling areas (Area 5, see below), addressing techniques for capturing and handling bats, using the same procedures of the field campaigns, according to Kunz and Parsons (2009). The participants could only participate in the training if they presented the result of serology for antibodies against vaccine RABV. The license for carrying out the pilot project, issued by the Ministry of the Brazilian Environment, is the same as for field collections, SISBio number 71136.

Four field campaigns were conducted, and each sampling point was visited four times, as close as possible to one capturing session per season of the year (spring, summer, autumn and winter). The negotiations for the authorization of the sampling areas chosen began in March 2021. However, with the resurgence of the COVID-19 pandemic, the permissions to carry out the field work were only given in June 2021, except for the Temple Buddhist Odsal Ling, who authorized the carrying out of activities from the second field campaign on. The campaigns were carried out in: 1<sup>st</sup> campaign from June 14 to 18, 2021; 2<sup>nd</sup> campaign from

October 2 to 7, 2021; 3<sup>rd</sup> campaign from January 29 to February 3, 2022; 4<sup>th</sup> campaign: April 25 to 30, 2022.

Sampling sessions included nocturnal captures of bats and were carried out in the sampling areas. One sampling area was selected per stratum, according to the inclusion criteria: safety, presence of bush or tree vegetation and preferably, with water collections (streams, rivers or lakes), as the presence of forest edges and water collections increases the capture success (Kunz and Parsons 2009). Climate conditions (rain and cloud cover), meteorological (temperature in °C and relative humidity in %) and environmental parameters (noise level in dB and luminosity in lux) were measured in every sampling session at 10 pm using a thermometer-hygrometer-lux meter-decibel meter device. These results (for each sampling session) were correlated with the total abundance of captured bats through Pearson (or Spearman) correlation coefficient, using the ‘cor()’ function of R.

Five to seven mist nets were used (ATX of three sizes: 3 × 2.5 m, 7 × 2.5 m, and 9 × 2.5 m) and mounted on 3 m high aluminum rods. Its arrangement was determined in site and could be in I, T, V, Y or Z arrangement, using one or more nets. Capture effort (net area \* hours opened) was calculated considering the number of repetitions (capture sessions) and mist nets, according to Straube and Bianconi (2002) (Tables 1, 2). Captures were made near abandoned buildings, tunnel exits, palm trees or fruit trees. The nets were opened at dusk and closed after 4–5 h, being checked every 20 min. Rainy days or days with very low temperatures

**Table 1** Number of bats captured, by species, field campaigns, and stratified by sampling area

Families/Species/ Camping Areas	Field Campaign 1						Field Campaign 2						Field Campaign						Field Campaign 4						Total
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	
Phyllostomidae	1	-	-	-	21	10	34	7	14	1	32	46	7	10	5	6	16	8	5	11	2	236			
<i>Anoura caudifera</i>	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1			
<i>Artibeus fimbriatus</i>	-	-	-	-	7	2	12	-	3	1	2	14	-	2	3	-	3	-	6	-	-	55			
<i>Artibeus lituratus</i>	-	-	-	-	6	1	11	1	3	3	14	6	1	1	2	1	5	6	2	-	-	60			
<i>Carollia perspicillata</i>	-	-	-	-	-	-	-	-	-	-	3	1	-	-	-	-	-	-	-	-	-	4			
<i>Desmodus rotundus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1			
<i>Glossophaga soricina</i>	-	-	-	-	-	4	-	-	1	-	-	-	1	6	-	1	1	3	-	-	-	17			
<i>Platyrrhinus lineatus</i>	-	-	-	-	6	-	2	-	5	-	-	1	-	1	1	-	1	-	-	-	-	17			
<i>Platyrrhinus recifinus</i>	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	3			
<i>Pygoderma bilabiatum</i>	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1			
<i>Sturnira lilium</i>	1	-	-	-	1	3	8	6	2	2	12	24	4	-	-	5	3	1	5	2	-	77			
Vespertilionidae	2	-	-	-	-	2	4	-	-	-	2	1	-	-	-	-	-	-	-	-	-	11			
<i>Eptesicus brasiliensis</i>	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1			
<i>Eptesicus diminutus</i>	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1			
<i>Myotis levis</i>	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1			
<i>Myotis nigricans</i>	2	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4			
<i>Myotis riparius</i>	-	-	-	-	-	1	2	-	7	14	1	34	47	7	10	5	6	16	8	5	11	247			
Total	3	-	-	-	21	12	38	7	14	1	34	47	7	10	5	6	16	8	5	11	2	247			

**Table 2** Sampling effort and meteorological parameters obtained during the field campaigns and stratified by sampling area

Parameter	Field Campaign 1						Field Campaign 2						Field Campaign 3						Field Campaign 4					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Sampling effort (h*area of mist nets)	756	-	864	738	554	792	792	792	900	792	636	792	864	600	0	594	738	855	792	900	810	594	738	855
Temperature (°C)*	13.0	-	16.3	17.0	14.6	16.9	14.0	17.1	20.3	16.6	18.4	19.0	21.5	23.8	-	23.2	21.0	24.0	19.4	22.6	20.3	24.2	25.0	20.1
Relative humidity (%)*	93.8	-	86.8	80.8	83.0	80.4	93.4	89.1	89.5	86.6	86.7	86.0	73.8	79.4	-	85.0	86.2	78.0	80.0	72.0	85.4	76.9	66.5	87.8
Noise level (dB)*	42.5	-	47.2	63.2	60.0	60.1	42.5	37.6	47.0	54.0	55.5	55.0	30.0	33.0	-	41.6	55.0	50.0	13.6	10.0	40.0	39.0	50.0	45.0
Luminosity (lux)*	0.04	-	0.18	0.46	1.50	0.44	0.01	0.08	0.11	0.75	1.86	2.70	0.02	0.12	-	0.60	0.41	0.20	0.02	0.60	0.18	0.20	0.50	0.08
Sky condition	C	-	O	O	O	O	D	D	D	D	D	O	O	D	R	R	D	R	O	O	O	O	O	D

\*Obtained at 10 pm. Sky condition: O = clear sky; C = cloudy; D = drizzle; R = rain

were avoided to reduce the probability of the bats dying from hypothermia. The captured bats were placed in cloth bags for later handling.

Captured bats were identified using available identification keys (Bredt et al. 2002; Díaz et al. 2016; Reis et al. 2017). If needed for proper identification, the bats were euthanized (as long as they were not juvenile, pregnant or lactating females) using isoflurane, administered in an inhalation chamber. Euthanasia was performed in the field to prevent the individual from suffering wounds or prolonged stress. All gathered information was recorded in a specific form. All the information of the captured bats was recorded following a standard data structure.

The management of the bats consisted of: (1) installation of a numbered metallic ring on the left forearm in males and right forearm in females (in all bats captured in first campaign and unmarked bats in the subsequent campaigns); (2) verification of sex by observing the genitals; (3) measurement of the body mass (in g), using a 300 g precision scale; (4) measurement of the length of the right forearm and wing-span (mm), using an analogical caliper; (5) determination of reproductive status (active or inactive), through observation of testes in the scrotum of males and pregnancy or lactation in females; (6) determination of the approximate age (juvenile or mature), by verifying the consolidation or not of epiphyses and diaphyses of the metacarpophalangeal joints, through transluminescence, using a flashlight; (7) identification of wounds, such as perforations of the wing, amputation of digits or pinna, etc.; (8) counting (if present) the number of bat flies (Streblidae or Nycteribiidae) on the bat skin.

## Data analysis

Characteristics of the bats' life history, such as type of roosts, probable colony sizes, feeding habits, and the reproductive patterns (monoestric or polyestric), available in the literature (Neuweiler 2000; Almeida et al. 2002; Kunz and Fenton 2003; Pacheco et al. 2010; Meyers et al. 2017; Nunes

et al. 2017; Reis et al. 2017) were compiled and used in the analysis. The body condition index (BCI) was calculated for adults of each bat species, according to Azeredo et al. (2019), i.e., the calculation of the residuals ( $r_i = y_i - \hat{y}_i$ , where  $r$  = residual;  $y$  = actual weight (g);  $\hat{y}$  = weight estimated in function of the right forearm (mm)) of the linear regression model using the right forearm length (mm) as a function of the weight (g). This approach was used only for species whose captures were greater than or equal to three adults, except pregnant females.

For each life history parameter and biometric measurement (sex, estimated age, reproductive status, proportion of pregnant females, weight of adult individuals, forearm length, and infestation by bat flies), comparisons of categorical variables between different campaigns and sampling areas were made through chi-square ( $\chi^2$ ) using the 'chisq.test()' function of R. As for continuous variables, the comparisons between two mean values were made through the t-test using the 't.test ()' function or, for more than two groups, the one-way ANOVA using the 'lm' function of R. The post-hoc for the ANOVA was the Tukey test.

The similarity of the richness of captured bats between the sampling areas was determined by hierarchical clustering using the unweighted pair-group average (UPGMA) and choirs distance (which considers relative abundance) as distance matrix measure in the software Past 4.

## Collection of biological samples and laboratory procedures

The biological sample collection methods were performed according to Kunz and Parsons (2009). Saliva samples were duplicated from each captured bat for molecular diagnosis of rabies virus (RABV) using sterilized swabs. Rectal swabs were collected in duplicate for each animal (excluding those weighing < 25 g due to anatomical limitations) using sterile cotton swabs with an aluminum rod, because the bats should remain alive after the sample

collection. Fecal samples were collected when there was spontaneous defecation. Swabs or feces were used for molecular diagnosis of coronavirus (CoV) and culture and isolation of Enterobacteria. If possible, urine samples were also obtained when bats spontaneously urinated during handling. When possible, blood was collected by puncture of the pre-brachial or uropathagial veins and sample collection using capillary tubes or Pasteur pipettes, not exceeding 1% of the animal's weight. Local disinfection was performed with 70% alcohol before collection. All samples were kept in sterile microtubes (Eppendorf®) at 4 °C (maintained in a portable cooler connected in the car battery) until they were transported to FMVZ-USP. Euthanized bats and those that eventually died during handling were refrigerated at 4 °C and transported to FMVZ-USP for necropsy and organs harvested for diagnosis and isolation of RABV and *Leptospira* spp. All sample manipulation was performed in laminar flows level 2 biosafety. The necropsies were performed using individual sterile material. Cabin asepsis with was performed using 70% alcohol to prevent contamination.

The procedures were carried out following the ethical principles of animal experimentation, and were approved by the Ethics Committee on Animal Use at FMVZ-USP (protocol number 2332030320).

### Rabies virus detection

The viral RNA extraction from the captured bats' saliva samples was carried out according to the protocol used at the Laboratory of Viral Zoonoses at FMVZ-USP. Reverse transcription and amplification were performed with RABV504 sense and RABV304 antisense primers, according to Orciari et al. (2001), generating an amplicon of 210 bp. The samples were analyzed by electrophoresis in agarose gel (1.5%), using a Pasteur virus (PV) sample from RABV as a positive control and water treated with DEPC as a negative control.

The products were purified with a commercial kit and quantified in Nanodrop®. The sequencing reaction was performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems®). The nucleotide sequences were analyzed using FinchTV and Bioedit softwares for comparison with homologous sequences deposited in GenBank using the BLAST software. Alignment and the phylogenetic tree were obtained using the MEGA X software (Kumar et al. 2018).

### Coronavirus detection

The total RNA of the samples was extracted directly from the rectal swabs with Purelink RNA Mini Kit (Thermo Fisher Scientific™) and used for CoV research using

pan-coronavirus primers, capable of detecting all CoV genera directed to RNA-polymerase RNA-dependent described by Escutenaire et al. (2007) and adapted for conventional RT-PCR, with M-MLV (Thermo Fisher Scientific™) and Dream Taq Green PCR Mastermix (Thermo Fisher Scientific™). Swabs with vaccine samples of avian coronavirus and swabs without virus were used as positive and negative controls, respectively.

PCR bands of 179 bp, considered positive for coronavirus, underwent Sanger sequencing. After purification with Exosap It (Thermo Fisher Scientific™), the reaction was performed with BiGDye 3.1 (Thermo Fisher Scientific™), and the sequences were resolved with an ABI-3500 automatic sequencer (Thermo Fisher Scientific™).

The reads with a Phred score > 20 were used to assemble the respective contig sequences with CAP-Contig in Bioedit 7.0.5.3 software (Hall 1999), which were used to build a maximum likelihood tree with the Mega X software (Kumar et al. 2018). A replacement model estimated by BIC criteria and 1,000 bootstrap repetitions, together with sequences from the different homologous coronavirus genera and species, were used to determine the identity of each detected sequence. These analyzes were also performed at the Laboratory of Viral Zoonoses at FMVZ-USP.

### Leptospira spp. detection

The microscopic agglutination test (MAT) with a collection of 24 live antigens kept at the Laboratory of Bacterial Zoonoses at FMVZ-USP was used for the serological research of antibodies against *Leptospira* spp. Blood serum samples were diluted at 1:50 in sterile Sørensen saline (pH 7,4), and 50 µL of the sample dilutions were deposited in polystyrene microplates containing 96 wells. Then, 50 µL of the antigen was added to the wells, reaching a 1:100 dilution (screening). The microplates were incubated at 28°C for at least two hours for reading and interpretation. Each antigen was microscopically analyzed for its viability, purity and self-agglutination as a control for test validation. Reading was carried out using dark field microscopy to observe agglutinations, being considered reagents only for those samples that present at least 50% of agglutinated leptospiral in the 1:100 dilution.

Molecular analyses were performed after the DNA extraction of urine and kidney samples using the PureLink Genomic DNA Mini Kit (Invitrogen®) kit, according to the manufacturer's protocol. The extracted material was stored at -20°C until the moment of amplification. DNA amplification for *Leptospira* spp. was performed by PCR using the GoTaq™ Green Master Mix (Promega, Brasil®) and the Lep1 and Lep2 primers that amplify a region of 330 bp of the 16S rRNA gene (rrs) (Mérien et al. 1992). Positive samples in this first amplification were submitted to a

second PCR round for typing by applying pairs of primers that amplify a region of 549 bp of the SecY gene (Ahmed et al. 2009). Cultures of *L. interrogans* serovar Canicola and ultrapure water were used as positive and negative controls, respectively. Amplified products were analyzed by 1.5% (w/v) agarose gel electrophoresis with 0.5X TBE running buffer (0.045 M Tris–borate, 1 mM EDTA, pH 8.0). The gel was stained with SYBR Safe DNA gel stain (Invitrogen®) and subsequently photographed under ultraviolet light using a transilluminator.

### Culture and isolation of Enterobacteria

The fecal swabs were inoculated in a non-selective pre-enrichment medium (peptone water, DIFCO, BD) and incubated at 37 °C for 18–24 h. Then, 10 µl of each growth was streaked on MacConkey agar plates (Acumedia®). Also, another aliquot of 10 µl of each growth was streaked on XLT-4 agar plates, specific for *Salmonella* spp. (Waltman 2000). Colonies suggestive of *E. coli* or *Salmonella* spp. were separated and identified by Matrix Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry (MALDI-TOF MS). The identified isolates were stored in 1% Tryptic Soy Agar at room temperature and Brain Heart Infusion broth (DIFCO, BD®) at -20° C.

### Risk factors for microorganism infection

Besides the information gathered during the handling of the captured bats, other variables were used to perform a risk factor analysis. Feeding habits (general insectivore, aerial insectivore, frugivore, nectivore, polynivore and hematophagous) were compiled from Dias et al. (2019) and used to group the captured species using the unweighted pair-group average (UPGMA) and Raup-Crick as distance matrix measure (which considers absence-presence data) in the software Past 4.

Moreover, another set of new variables was created, also from a compilation from Dias et al. (2019), related to the possible roosts (abandoned house, bridge, building, cistern, fence, light pole, mine, roof lining, tunnel, banana tree, cave, foliage, forest, palm tree, rock crevice, tree hole, and tree top) for each captured species. These variables were also used in a discriminatory analysis to group the captured species through hierarchic clustering using the unweighted pair-group average (UPGMA) and Raup-Crick as distance matrix measure (which considers absence-presence data) in the software Past 4.

The risk analysis used the microorganisms with ten or more positive results (for viruses) or isolates (for bacteria) as dependent variables. In the first step, a univariate analysis was made comparing each independent variable with the dependent variable through chi-square ( $\chi^2$ ), using the

‘chisq.test ()’ command of R. For the qualitative variables, the comparison was made through the t-test using the ‘t.test ()’ command of R. The variables with  $p \leq 0.20$  were submitted to a multivariate analysis using stepwise logistic regression, using the ‘glm ()’ command of the *ISLR* package of R. Only the variables with  $p \leq 0.05$  were kept in the final model.

## Results

### Bat captures and bat population structure

A total of 247 bats were captured (Table 1), of which 103 bats (41.7%) were captured during the third field campaign (summer), 72 (29.1%) during the second (spring), 48 (19.4%) during the fourth (autumn), and 24 (9.8%) during the first field campaign (winter). The highest abundance among bat families was Phyllostomidae ( $n = 236$ , 95.5%), followed by Vespertilionidae ( $n = 11$ , 4.5%). As for species, the highest relative abundance was that of *Sturnira lilium* ( $n = 77$ , 31.2%), followed by *Artibeus lituratus* ( $n = 60$ , 24.4%), *Artibeus fimbriatus* ( $n = 55$ , 22.4%), *Glossophaga soricina* and *Platyrrhinus lineatus* ( $n = 17$ , 7% each), *Carollia perspicillata*, *Myotis nigricans* and *Myotis riparius* ( $n = 4$ , 1.6% each), *Platyrrhinus recifinus* ( $n = 3$ , 1.2%), and *Anoura caudifera*, *Desmodus rotundus*, *Eptesicus brasiliensis*, *Eptesicus diminutus*, *Myotis levis* and *Pygoderma bilabiatum* ( $n = 1$ , 0.33% each). A total of four bats were euthanized for identification (one *M. levis*, one *M. nigricans*, one *M. riparius*, and one *E. brasiliensis*).

Four bats from Campaign 1 were recaptured in Campaign 2 (all from Area 5): one *A. lituratus*, one *A. fimbriatus*, and two *P. lineatus*, one of which was recaptured for the second time in Campaign 3. Moreover, one *A. fimbriatus* from Campaign 1 was recaptured in Campaign 3 in Area 5. Three bats captured in Campaign 2 were recaptured in Area 2: *A. lituratus*, *A. fimbriatus*, and *P. lineatus*. In Area 2 during Campaign 4, one *A. fimbriatus* from the Campaign 2 and two bats (one *A. fimbriatus* and one *S. lilium*) from Campaign 3 were recaptured. In Area 4 during Campaign 4, two *S. lilium* from Campaign 2 and one *S. lilium* from Campaign 3 were recaptured. In Area 5, two *A. fimbriatus* were recaptured, one from Campaign 1 and another, which had been captured in the Campaigns 1 and 2.

In Campaign 1, no wounds or scars were observed in the captured bats. In Campaign 2, six bats were regarded with wounds and scars: in Area 1, one *G. soricina* with a fracture on the left 2<sup>nd</sup> finger; in Area 2, one *A. lituratus* with scars in the back and one *A. fimbriatus* with scars in the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> fingers and the back; in Area 5, one *A. fimbriatus* with a fracture in the right 2<sup>nd</sup> finger and right-wing perforated between 2<sup>nd</sup> and 3<sup>rd</sup> fingers, one *A. lituratus* with a scar in the nose leaf, and one *A. fimbriatus* with multiple scars in both



wings. In Campaign 3, five bats were observed with wounds and scars: in Area 1, one *A. lituratus* with a scar on the head; in Area 2, one *A. fimbriatus* with scars on both wings; in Area 4, one *S. lilium* with scars in the right wing and one *S. lilium* with a granuloma in the right hind limb; in Area 6, one *A. lituratus* with a scar in the nose leaf. In Campaign 4, two bats were observed with wounds and scars: in Area 1, one *S. lilium* with a missing left thumb; in Area 4, one *S. lilium* with a granuloma in the right hind limb (recapture from Campaign 3).

The climate conditions and meteorological and environmental parameters measured in each sampling session are shown in Table 2.

Capture effort was not correlated to the abundance of captured bats if we consider all sampling efforts of the four field campaigns, although it showed a significant inverse correlation during the first campaign ( $r^2=0.85$ ,  $p=0.026$ ). Temperature ( $^{\circ}\text{C}$ ), relative humidity (%) and noise level (dB) were not correlated with the abundance if we consider the four field campaigns. Luminosity (lux) was not correlated to the abundance of captured bats if we consider all sampling efforts of the four field campaigns. Still, it showed a significant direct correlation during the first ( $r^2=0.82$ ,  $p=0.03$ ) and fourth ( $r^2=0.80$ ,  $p=0.015$ ) campaigns and a slight inverse correlation during the third campaign ( $r^2=0.48$ ,  $p=0.19$ ).

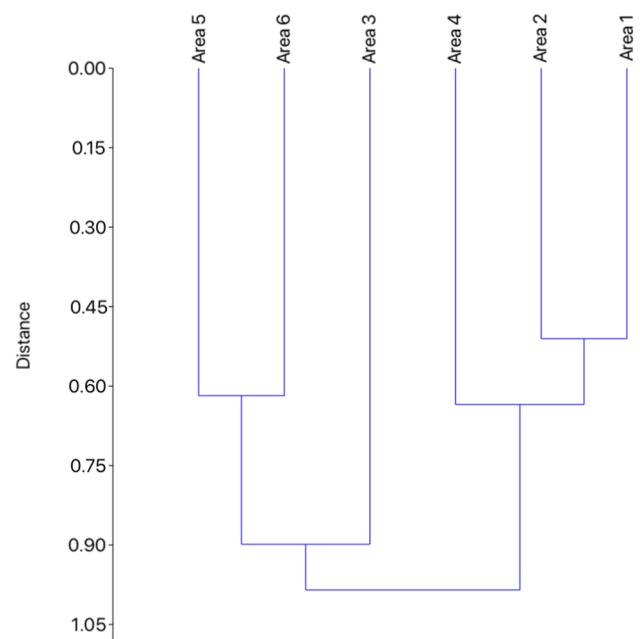
A dendrogram of the hierarchical clustering of the richness of captured bats in each sampling area is shown in Fig. 3.

Areas 1 and 2 are the ones where the richness is most similar (~51%), grouping with Area 4, which is ~35% similar to those cited. In another cluster, Areas 5 and 6 are ~37% similar, grouping with Area 3, which is only ~10% similar to those cited.

The number of captured bats by gender and by field campaign, is shown in Table 3. No difference between the genders has been observed between the field campaigns ( $\chi^2=5.55$ ,  $df=3$ ,  $p=0.14$ ) and sampling areas ( $\chi^2=2.39$ ,  $df=5$ ,  $p=0.79$ ).

The number of captured bats by age group (juvenile or mature) and by field campaign is shown in Table 4. A difference among the age groups between campaigns was observed ( $\chi^2=18.66$ ,  $df=3$ ,  $p=0.00032$ ), with precisely a higher proportion of juvenile bats in Campaign 3 (32.5%) than in Campaigns 2 (11.1%) ( $\chi^2=10.59$ ,  $df=1$ ,  $p=0.0011$ ) and 4 (6.7%,  $\chi^2=11.44$ ,  $df=1$ ,  $p=0.00072$ ). No difference has been observed between the sampling areas ( $\chi^2=8.45$ ,  $df=5$ ,  $p=0.13$ ).

The number of sexually active individuals by field campaign is shown in Table 5. No difference between the field campaigns (Yates  $\chi^2=5.16$ ,  $df=3$ ,  $p=0.16$ ) and sampling areas (Yates  $\chi^2=4.61$ ,  $df=5$ ,  $p=0.46$ ) was observed.



**Fig. 3** Hierarchical grouping dendrogram of bat species richness by sampling area. Cophenetic correlation coefficient (CCC)=0,70

The proportion of pregnant females (among mature females) by field campaign is shown in Table 6. A difference in the proportion of pregnant females between campaigns was observed ( $\chi^2=17.086$ ,  $df=3$ ,  $p=0.00068$ ), with precisely a higher proportion of pregnant females in Campaign 2 (85.7%) than in Campaigns 3 (34.8%) ( $\chi^2=11.78$ ,  $df=1$ ,  $p=0.00060$ ) and 4 (0%) (Yates  $\chi^2=14.62$ ,  $df=1$ ,  $p=0.00013$ ). No difference has been observed between the sampling areas ( $\chi^2=4.36$ ,  $df=5$ ,  $p=0.50$ ).

The mean weight of the adult individuals by field campaign is shown in Table 7. When considering females and males together, the mean weight of the individuals captured in Field Campaign 1 = 46.48 g, Campaign 2 = 46.74 g, Campaign 3 = 45.19 g, and Campaign 4 = 44.02 g. No difference in mean (females + males) weight was observed between campaigns ( $f=0.10$ ;  $p=0.96$ ), nor among females ( $f=0.16$ ;  $p=0.92$ ), nor males ( $f=0.34$ ;  $p=0.79$ ). Moreover, no significant difference was observed in weight among females and males between the field campaigns.

The mean weight of the adult individuals (females and males together) in Area 1 = 42.21 g, Area 2 = 48.06 g, Area 3 = 58.87 g, Area 4 = 33.20 g, Area 5 = 43.67 g, and Area 6 = 65.23 g. A difference among the mean (females + males) weight was observed between the sampling areas ( $f=2.42$ ;  $p=0.037$ ), precisely a marginal difference between Areas 4 and 6 ( $Q=4.035$ ;  $p=0.053$ ). No difference in mean weight among females ( $f=1.79$ ;  $p=0.12$ ) and males ( $f=1.39$ ;  $p=0.23$ ) were observed between the sampling areas.

**Table 3** Sex of captured bats, by species, field campaign, and stratified by sampling area

Families/ Species/ Sampling Area	Field Campaign 1						Field Campaign 2						Field Campaign 3						Field Campaign 4						Total
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	
Phyllostomidae	0/1	-	-	-	11/10	4/6	15/19	4/3	4/10	0/1	17/15	15/31	3/4	6/4	2/3	4/2	10/6	4/4	5/0	5/6	1/1	110/126			
<i>Anoura caudifera</i>	-	-	-	-	-	-	0/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/0			
<i>Artibeus fimbriatus</i>	-	-	-	-	4/3	1/1	4/8	-	1/2	0/1	0/2	4/10	-	1/1	2/1	2/1	-	-	4/2	-	-	23/32			
<i>Artibeus lituratus</i>	-	-	-	-	2/4	0/1	7/4	0/1	1/2	7/7	2/4	-	1/0	0/1	0/2	1/0	4/1	3/3	1/1	-	-	29/31			
<i>Carollia perspicillata</i>	-	-	-	-	-	-	-	-	-	1/2	0/1	-	-	-	-	-	-	-	-	-	-	1/3			
<i>Desmodus rotundus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/0	-	-	-	-	-	1/0			
<i>Glossophaga soricina</i>	-	-	-	-	-	0/4	-	0/1	-	-	-	-	0/1	5/1	-	0/1	1/0	1/2	-	-	-	7/10			
<i>Platyrrhinus lineatus</i>	-	-	-	-	3/3	-	0/2	2/3	-	-	0/1	-	0/1	0/1	-	1/0	-	-	-	-	-	6/11			
<i>Platyrrhinus recifinus</i>	-	-	-	-	1/0	-	-	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-	2/1			
<i>Pygoderma bilabiatum</i>	-	-	-	-	-	-	-	-	-	1/0	-	-	-	-	-	-	-	-	-	-	-	1/0			
<i>Sturnira lilium</i>	0/1	-	-	-	1/0	3/0	4/4	4/2	0/2	8/4	9/15	-	2/2	-	-	3/2	1/2	0/1	5/0	0/2	40/37				
Vespertilionidae	0/2	-	-	-	-	1/1	2/2	-	-	1/1	1?	-	-	-	-	-	-	-	-	-	4/6+1?				
<i>Eptesicus brasiliensis</i>	-	-	-	-	-	0/1	-	-	-	1/0	-	-	-	-	-	-	-	-	-	-	-	1/0			
<i>Eptesicus diminutus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/1			
<i>Myotis levis</i>	-	-	-	-	-	-	-	-	-	0/1	-	-	-	-	-	-	-	-	-	-	-	0/1			
<i>Myotis nigricans</i>	0/2	-	-	-	-	-	1/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/3			
<i>Myotis riparius</i>	-	-	-	-	11/10	5/7	17/21	4/3	4/10	0/1	18/16	15/31+1?	3/4	6/4	2/3	4/2	10/6	4/4	5/0	5/6	1/1	114/132+1?			
Total	0/3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			

Obs: females/males

**Table 4** Age groups of captured bats, by specie, field campaign, and stratified by sampling area

Families/ Species/ Sampling Areas	Field Campaign 1						Field Campaign 2						Field Campaign 3						Field Campaign 4						Total
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	
Phyllostomidae	0/1	-	-	-	3/16+2?	1/9	3/30+1?	1/6	2/12	0/1	8/24	17/29	0/7	0/10	0/5	0/6	2/14	0/8	1/4	0/11	0/2	38/195+3?			
<i>Anoura caudifera</i>	-	-	-	-	-	0/1	0/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/1			
<i>Artibeus fimbriatus</i>	-	-	-	-	2/4+1?	0/2	1/11	1/2	0/1	1/1	1/13	0/2	0/3	0/3	0/3	0/6	6/48+1?								
<i>Artibeus lituratus</i>	-	-	-	-	0/5+1?	0/1	0/11	0/1	0/3	0/14	3/3	0/1	0/1	0/2	0/1	0/6	4/55+1?								
<i>Carollia perspicillata</i>	-	-	-	-	-	-	1/2	0/1	-	-	-	-	-	-	-	-	1/3								
<i>Desmodus rotundus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/0								
<i>Glossophaga soricina</i>	-	-	-	-	-	1/3	0/2	0/1	0/1	0/1	0/1	0/1	0/6	0/1	0/1	0/3	1/16								
<i>Platyrrhinus lineatus</i>	-	-	-	-	1/5	0/1	0/5	0/5	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	1/16								
<i>Platyrrhinus recifinus</i>	-	-	-	-	0/1	-	-	-	-	-	-	-	-	-	-	-	0/3								
<i>Pygoderma bilabiatum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/0								
<i>Sturnira lilium</i>	0/1	-	-	-	0/1	0/3	2/5+1?	1/5	1/1	5/7	13/11	0/4	0/5	0/3	0/1	1/4	0/2	23/53+1?							
Vespertilionidae	0/2	-	-	-	-	0/2	0/3+1?	-	-	0/2	1?	-	-	-	-	-	0/9+2?								
<i>Eptesicus brasiliensis</i>	-	-	-	-	-	0/1	-	-	-	0/1	-	-	-	-	-	-	0/1								
<i>Eptesicus diminutus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/1								
<i>Myotis levis</i>	-	-	-	-	-	-	-	-	-	0/1	-	-	-	-	-	-	0/1								
<i>Myotis nigricans</i>	0/2	-	-	-	-	0/1	0/1+1?	-	-	-	-	-	-	-	-	-	0/3+1?								
<i>Myotis riparius</i>	-	-	-	-	-	0/1	0/2	-	-	1?	-	-	-	-	-	-	0/3+1?								
Total	0/3	-	-	-	3/16+2?	1/11	3/33+2?	1/6	2/12	0/1	8/26	17/29+1?	0/7	0/10	0/5	0/6	2/14	0/8	1/4	0/11	0/2	38/204+5?			

Obs.: juvenile/adults

**Table 5** Reproductive status of adult bats, by species, field campaign, and stratified by sampling area

Families/ Species/ Sampling Areas	Field Campaign 1						Field Campaign 2						Field Campaign 3						Field Campaign 4						Total
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	
Phyllostomidae	-/0	-	-	-	1/2	2/1	12/2	2/0	4/5	-/0	7/0	6/2	1/0	6/1	2/2	1/0	1/1	3/2	3/-	0/2	0/0	51/20			
<i>Anoura caudifera</i>	-	-	-	-	-	-	-/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-/0		
<i>Artibeus fimbriatus</i>	-	-	-	0/1	1/0	1 <sup>p</sup> /1	3 <sup>p</sup> /1	-	1/0	-/0	-/0	3/1	-	1/1	2/0	1 <sup>p</sup> /1	-	-	0/0	0/0	0/0	12/6			
<i>Artibeus lituratus</i>	-	-	-	1/0	1 <sup>p</sup> /2	-/0	5 <sup>p</sup> /0	-	1 <sup>p</sup> /2	-	5 <sup>3.1,2p</sup> /0	1 <sup>p</sup> /1	0/-	-/0	-/2	0/0	3 <sup>1,2p</sup> /1	-	-	0/0	0/0	16/6			
<i>Carollia perspicillata</i>	-	-	-	-	-	-	0/0	-/0	-	-	-	-	-	-	-	-	-	-	-	-	-	0/0			
<i>Desmodus rotundus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/0			
<i>Glossophaga soricina</i>	-	-	-	-	0/0	-/0	-/0	-	-/0	-	-	-	-	5 <sup>2.1,2p</sup> /0	-	-	-/1	0/1	5/2	-	-	-			
<i>Platyrrhinus lineatus</i>	-	-	-	0/0	-/0	-/0	-/1	2 <sup>1,p</sup> /2	-	-	-	-	-	-	-	-	-	-	2/3	-	-	-			
<i>Platyrrhinus recifinus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/0	0/0	-	-			
<i>Pygoderma bilabiatum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/0			
<i>Sturnira lilium</i>	-/0	-	-	-	-	1 <sup>p</sup> /-	4 <sup>p</sup> /0	2 <sup>1,p</sup> /0	-/1	2 <sup>p</sup> /0	2 <sup>p</sup> /0	2 <sup>p</sup> /0	1 <sup>p</sup> /0	-	1 <sup>p</sup> /0	0/0	0/0	3 <sup>p</sup> /-	0/1	16/3	-	-			
Vespertilionidae	-/2	-	-	-	-	0/0	1/0	-	-	1 <sup>p</sup> /-	-	-	-	-	-	-	-	-	2/2	-	-	-			
<i>Eptesicus brasiliensis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/-	-	-	-			
<i>Eptesicus diminutus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>Myotis levis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>Myotis nigricans</i>	-/2	-	-	-	-	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/2	-	-	-			
<i>Myotis riparius</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/0	-	-	-			
Total	-/2	-	-	1/2	4/5	2/1	13/2	2/0	4/5	-/0	8/0	6/2	1/0	6/1	2/2	1/0	1/1	3/2	3/-	0/2	0/0	53/22			

Obs: mature females (lactating, <sup>p</sup>pregnant; <sup>p</sup>post-lactating/mature males (scrotum testicles)

**Table 6** Proportion of pregnant females (among adult females) by species, field campaign, and stratified by sampling area

Famílias/ Espécies/ Áreas	Campanha 1						Campanha 2						Campanha 3						Campanha 4						Total
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	
Phyllostomidae	-	-	-	-	0/1	0/1	2/2	12/12	1/2	2/4	-	4/7	1/6	1/1	2/6	0/2	0/1	0/3	0/3	0/3	0/3	0	0	25/51	
<i>Anoura caudifera</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Artibeus fimbriatus</i>	-	-	-	-	0	0	1/1	3/3	0/1	0/1	-	-	0/3	0/1	0/1	0/2	0/1	0/2	0	0	0	0	0	4/12	
<i>Artibeus lituratus</i>	-	-	-	-	0/1	0/1	-	5/5	-	1/1	-	2/5	1/1	0	-	-	0	0/3	0/3	0/3	0/3	0	0	9/16	
<i>Carollia perspicillata</i>	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	0	
<i>Desmodus rotundus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	0	
<i>Glossophaga soricina</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/5	-	-	-	0	0	-	0	-	2/5	
<i>Platyrrhinus lineatus</i>	-	-	-	-	0	0	-	-	1/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/2	
<i>Platyrrhinus recifinus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
<i>Pygoderma bilabiatum</i>	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	0	
<i>Sturnira lilium</i>	-	-	-	-	-	-	1/1	4/4	1/2	-	-	2/2	0/2	1/1	0/1	0/1	0	0/3	0/3	0/3	0/3	0	0	9/16	
Vespertilionidae	-	-	-	-	-	-	0	1/1	-	-	-	0/1	-	-	-	-	-	-	-	-	-	-	-	1/2	
<i>Eptesicus brasiliensis</i>	-	-	-	-	-	-	-	-	-	-	-	0/1	-	-	-	-	-	-	-	-	-	-	-	0/1	
<i>Eptesicus diminutus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Myotis levis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Myotis nigricans</i>	-	-	-	-	-	-	0	0	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	0	
<i>Myotis riparius</i>	-	-	-	-	0/1	0/1	2/2	13/13	1/2	2/4	-	4/8	1/6	1/1	2/6	0/2	0/1	0/1	0/3	0/3	0/3	0	1/1		
Total	-	-	-	-	0/1	0/1	2/2	13/13	1/2	2/4	-	4/8	1/6	1/1	2/6	0/2	0/1	0/1	0/3	0/3	0/3	0	26/53		

Obs: pregnant females/mature females

**Table 7** Mean weight (g) of adult bats, by species, field campaign, and stratified by sampling area

Families/ Species/ Sampling Areas	Field Campaign 1						Field Campaign 2						Field Campaign 3						Field Campaign 4						Total												
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6													
Phyllostomidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Anoura caudifera</i>	-	-	-	-	-	-	-9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-9		
<i>Artibeus fimbriatus</i>	62.5/56	75/61	64.5/57.1	69/56	-65.9	61.5/-	61.7/57.4	61.5/57	60.5/57	57/57	64/65	66.5/63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
<i>Artibeus lituratus</i>	66/77.5	-172.5	88.9/75	-170.6	91/76	85.6/73.4	105/64	72/-	-168	65/-	73/77	77.3/69.3	75/65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
<i>Carollia perspicillata</i>	-	-	-	-	-	-	16/17	17/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
<i>Desmodus rotundus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35/-		
<i>Glossophaga soricina</i>	-	-	-	-	-	-	-12	-13	-	-	-	-	-8	11.6/10	9/-	-9	-9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11/8.5			
<i>Platyrrhinus lineatus</i>	27.7/28	-125.5	31.5/26.3	-128	-122	28/-	23/19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>Platyrrhinus recifinus</i>	-127	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14/-			
<i>Pygoderma bilabiatum</i>	-	-	-	-	-	-	14/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>Sturnira lilium</i>	-122	24/-	24.7/23.5	43/42.7	-26	22.7/22	21.2/23.8	24/23	19/27	22/22.5	19/27	-122	19.8/-	-123	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Vespertilionidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>Eptesicus brasiliensis</i>	-	-	-	-	-	-	24/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24/-			
<i>Eptesicus diminutus</i>	-	-	-	-	-	-	-17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-17			
<i>Myotis lewis</i>	-	-	-	-	-	-	6/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-6			
<i>Myotis nigricans</i>	-17	6.5/-	7/6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-6			
<i>Myotis riparius</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6/7			
Total	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			

Obs: females/males

Moreover, no significant difference in weight between females and males was observed between the sampling areas.

The mean right forearm length of the adult individuals by field campaign is shown in Table 8. When considering females and males together, the mean forearm length of the individuals captured in Field Campaign 1 = 57.89 g, in Campaign 2 = 52.65 g, in Campaign 3 = 56.04 g, and in Campaign 4 = 56.04 g. No difference in mean (females + males) forearm length was observed between campaigns ( $f=0.99$ ;  $p=0.40$ ), nor among females ( $f=2.28$ ;  $p=0.084$ ), nor males ( $f=0.97$ ;  $p=0.41$ ). Moreover, a difference in the mean forearm length among females (61.85 mm) and males (50.22 mm) has been observed only in Campaign 4 ( $t=2.61$ ;  $df=43$ ;  $p=0.012$ ).

The mean forearm length of the adult individuals (females and males together) in Sampling Area 1 = 55.43 mm, Area 2 = 57.51 mm, Area 3 = 48 mm, Area 4 = 46.33 mm, Area 5 = 53.98 mm, and Area 6 = 68.11 mm. A difference among the mean (females + males) forearm length was observed between the sampling areas ( $f=3.14$ ;  $p=0.0093$ ), precisely a marginal difference between Areas 2 and 4 ( $Q=3.92$ ;  $p=0.067$ ) and between Areas 4 and 6 ( $Q=4.76$ ;  $p=0.011$ ). Marginal differences in mean forearm length between females ( $f=2.18$ ;  $p=0.064$ ) and males ( $f=2.18$ ;  $p=0.061$ ) were observed in the sampling areas. Moreover, a marginal difference in the mean forearm length among females (60.77 mm) and males (51.32 mm) has been observed only in Area 1 ( $t=2$ ;  $df=44$ ;  $p=0.051$ ).

The mean condition mass index (BCI) of the adult individuals by field campaign is shown in Table 9. When considering females and males together, the mean BCI of the individuals captured in Field Campaign 1 = 0.04, Campaign 2 = 0.59, Campaign 3 = -1.01, and Campaign 4 = 0.85. No difference in mean (females + males) BCI was observed between campaigns ( $f=0.37$ ;  $p=0.78$ ), nor among females ( $f=0.82$ ;  $p=0.48$ ), nor males ( $f=0.077$ ;  $p=0.97$ ). Moreover, no significant difference in weight between females and males was observed between the field campaigns.

The mean BCI of the adult individuals (females and males together) in Sampling Area 1 = 0.52, Area 2 = -0.32, Area 3 = 0.12, Area 4 = -1.66, Area 5 = 0.52, and Area 6 = -0.48. No difference in mean (females + males) BCI was observed between sampling areas ( $f=0.42$ ;  $p=0.83$ ), nor among females ( $f=0.23$ ;  $p=0.95$ ), nor males ( $f=0.23$ ;  $p=0.95$ ). Moreover, no significant difference in weight between females and males has been observed between the sampling areas.

As for the ectoparasites, although no formal identification procedure has been applied, only bat flies were observed (Streblidae or Nycteribiidae). The proportion of bats infested by flies is shown in Table 10. The ratio of bats parasitized by flies in Campaign 4 (33.33%) was higher than in Campaign 3

(17.48%) ( $\chi^2=4.075$ ,  $df=1$ ,  $p=0.043$ ). The mean number of flies per captured bat in Campaign 4 (5.43) was higher than in Campaign 3 (2.97) ( $t=2.83$ ;  $df=50$ ;  $p=0.0067$ ). Moreover, a difference in the proportion of infested individuals between the sampling areas was observed (Yates  $\chi^2=29.18$ ,  $df=5$ ,  $p=0.000021$ ): Area 6 > Area 3 > Area 5 > Area 1 > Area 2 > Area 4. No difference in the mean number of bat flies was observed between the sampling areas ( $f=0.27$ ;  $p=0.93$ ).

### Risk factors for microorganism infection

Besides the variables gathered in the field work (field campaign, sampling area, species, family, weight, right forearm length, body condition index (BCI), age, sex, reproductive status, and presence of wounds, such as fractures or scars), two new variables were created: feeding habits and possible roosts. The hierarchical grouping of feeding habits resulted in six groups: (1) *Desmodus rotundus*; (2) *Myotis nigricans* + *Myotis levis* + *Eptesicus brasiliensis* + *Eptesicus diminutus* + *Artibeus lituratus*; (3) *Sturnira lilium* + *Platyrrhinus lineatus* + *Carollia perspicillata*; (4) *Artibeus fimbriatus* + *Pygoderma bilabiatum* + *Platyrrhinus recifinus*; (5) *Myotis riparius*; (6) *Anoura caudifera* + *Glossophaga soricina* (Fig. 4).

The hierarchical grouping of possible roosts resulted in seven groups: (1) *Myotis levis* + *Artibeus fimbriatus*; (2) *Artibeus lituratus*; (3) *Platyrrhinus lineatus* + *Myotis nigricans* + *Eptesicus brasiliensis*; (4) *Glossophaga soricina*; (5) *Myotis riparius* + *Anoura caudifera* + *Eptesicus diminutus*; (6) *Sturnira lilium* + *Pygoderma bilabiatum* + *Platyrrhinus recifinus*; (7) *Desmodus rotundus* + *Carollia perspicillata* (Fig. 5).

As for the microorganisms, no positive results for any viruses (RABV and CoV), *Salmonella* spp. and *Leptospira* spp. were observed. As for other bacteria, 252 isolates from 174 bats were obtained: *Acinetobacter* spp. ( $n=2$ ); *Acinetobacter baumannii* ( $n=1$ ); *Acinetobacter baylyi* ( $n=1$ ); *Aeromonas caviae* ( $n=1$ ); *Aeromonas hydrophila* ( $n=8$ ); *Aeromonas veronii* ( $n=1$ ); *Cedecea lapagei* ( $n=1$ ); *Cedecea neteri* ( $n=2$ ); *Citrobacter amalonaticus* ( $n=2$ ); *Citrobacter farmeri* ( $n=1$ ); *Citrobacter freundii* ( $n=21$ ); *Citrobacter gillenii* ( $n=1$ ); *Citrobacter koseri* ( $n=1$ ); *Citrobacter youngae* ( $n=1$ ); *Cronobacter sakazakii* ( $n=1$ ); *Enterobacter aerogenes* ( $n=1$ ); *Enterobacter asburgiae* ( $n=5$ ); *Enterobacter bugandensis* ( $n=1$ ); *Enterobacter cancerogenus* ( $n=5$ ); *Enterobacter cloacae* ( $n=29$ ); *Enterobacter hormaechei* ( $n=10$ ); *Enterobacter ludwigii* ( $n=1$ ); *Enterobacter xiangfangensis* ( $n=5$ ); *Escherichia coli* ( $n=71$ ); *Hafnia alvei* ( $n=1$ ); *Klebsiella* spp. ( $n=1$ ); *Klebsiella aerogenes* ( $n=2$ ); *Klebsiella oxytoca* ( $n=8$ ); *Klebsiella pneumoniae* ( $n=6$ ); *Klebsiella variicola* ( $n=2$ ); *Kluyvera ascorbata* ( $n=5$ ); *Kluyvera*

**Table 8** Mean right forearm length (mm) of adult bats by species, field campaign, and stratified by sampling area

Families/ Species/ Sampling Areas	Field Campaign 1						Field Campaign 2						Field Campaign 3						Field Campaign 4						Total						
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6							
Phyllostomidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Anoura caudifera</i>																															
<i>A. fimbriatus</i>																															
<i>A. lituratus</i>																															
<i>C. perspicillata</i>																															
<i>D. rotundus</i>																															
<i>G. soricina</i>																															
<i>P. lineatus</i>																															
<i>P. rhinus</i>																															
<i>P. recifinus</i>																															
<i>P. bilabiatum</i>																															
<i>S. litum</i>																															
<i>V. nidae</i>																															
<i>E. brasiliensis</i>																															
<i>E. diminutus</i>																															
<i>M. lewis</i>																															
<i>M. nigricans</i>																															
<i>M. riparius</i>																															
Total																															

Obs: females/males



*cryocrescens* (n=2); *Kosakonia cowanii* (n=8); *Leclercia adenocarboxylata* (n=1); *Morganella morgani* (n=4); *Pantoea* spp. (n=2); *Pantoea agglomerans* (n=1); *Pluri-bacter gergoviae* (n=2); *Proteus hauseri* (n=1); *Providencia alcalifaciens* (n=2); *Providencia rettgeri* (n=1); *Pseudeschherichia* spp. (n=1); *Pseudomonas aeruginosa* (n=4); *Serratia marcescens* (n=23); *Serratia nematodiphila* (n=2) (Table 11a and b). Of that, in 63 bats (23.25%) more than one bacterial isolate was obtained, and the maximum number of isolates in a single bat was five.

The logistic regression model considering the *C. freundii* isolates as dependent variable showed Sampling Area 2 (compared to Sampling Area 1) as a protection factor ( $p=0.0041$ ) (OR=0.30; 95%CI=0.091–0.91) and presence of wounds as a risk factor ( $p=0.075$ ) (OR=6.74; 95%CI=0.72–59.90). The regression model for *E. cloacae* showed buildings as roost as protection factor ( $p=0.039$ ) (OR=0.37; 95%CI=0.13–0.90). Finally, the regression model for *E. coli* showed Sampling Area 4 (compared to Sampling Area 1) as a risk factor ( $p=0.001$ ) (OR=10.33; 95%CI=2.79–50.88).

## Discussion

This study used a sampling design based on the rural–urban landscape’s stratification, considering its changes in a linear gradient. The small number of sampling areas per stratum and the small number of repetitions in each sampling areas were due to the restrictions imposed by the COVID-19 pandemic and the difficulty in finding safe sites with the possibility of field work. Moreover, the small window of the new moon (which is the time of choice to carry out bat captures, due to the low moon luminosity) was not big enough to perform more repetitions in the same stratum during a given field campaign. Conversely, the same patterns were observed, such as the stability of bat richness and relative abundance per sampling area.

The predominance of the family Phyllostomidae over Vespertilionidae was due to the use of capture methods based on ground mist nets, as observed in bat monitoring based on the same methodology (Bernard 2001). Moreover, the high relative abundance of *Sturnira lilium*, *Artibeus lituratus*, *A. fimbriatus*, *Glossophaga soricina*, and *Platyrrhinus lineatus* is in line with other studies performed in the same biome (Brito et al. 2010; Chaves et al. 2012; Garbino 2016) but different from passive surveillance studies (Silva et al. 1996; Dias et al. 2019). Schulze et al. (2000) point out that the great abundance of some frugivorous phyllostomids, such as *C. perspicillata* and *S. lilium*, are indicators of forest disturbances. In passive surveillance systems, bats are mainly obtained dead and rarely alive from households or public places, making the relative abundance of

Vespertilionidae and Molossidae higher. To fill this gap, bioacoustic studies should be implemented to understand bat richness and abundance.

Different bat richness was observed at both ends of the rural–urban axis: bat richness was lower in urban areas (Areas 3 and 6) and highest in preserved-rural areas (Areas 1 and 2). At the same time, a higher number of captured bats were observed in the preserved-rural regions, even though higher relative abundances were observed in urban areas, i.e., starting from preserved-rural towards urban areas the lesser the bat richness, the higher the relative abundance of captured bats. Hourigan et al. (2010) found a similar pattern, in which a greater number of species was found in intermediate urbanization levels, leading them to the conclusion that these environments harbor species from preserved and non-preserved areas, increasing their richness. Despite the apparent differences in the landscape, highlighted by Mello (2022), some variables may have influenced these results, such as luminosity. Luminosity has been associated with a high abundance of aerial insects and insectivore bats (especially Vespertilionidae) (Straka et al. 2019).

The low number of recaptures observed in the present work raises the discussion about whether the captured bats were sedentary or often foraging in the same areas. To adequately address this question, bat movement studies in the rural–urban gradient should be implemented, not only because of the interest of bat ecological but also the circulation of pathogens.

In Area 6, all of the captured bats were *Artibeus*, the heaviest bats captured in the present work, and in Area 4, most of the captured bats were *Sturnira lilium*, one of the lightest species in the current work. Even so, different-sized bats did not influence the comparisons, since these differences were not extreme, and only marginally observed between some sampling areas.

The higher proportion of pregnant females (in Spring) preceded the higher proportion of juvenile bats (in Summer). Since reproduction is energetically costly, food availability determines the reproduction timing (Crichton and Krutzsch 2000). Most bat species reproduce seasonally, as lactation and weaning coincide with peak food availability. In tropical areas where food is abundant year-round and temperature variations are not extreme, the rainfall seasonality (rainy season during summer in MASP) confers a greater or lesser degree of seasonality, which in the case of frugivorous and insectivorous bats plays a significant role, making most of the Phyllostomidae polyestrous and Vespertilionidae monoestrous (Crichton and Krutzsch 2000).

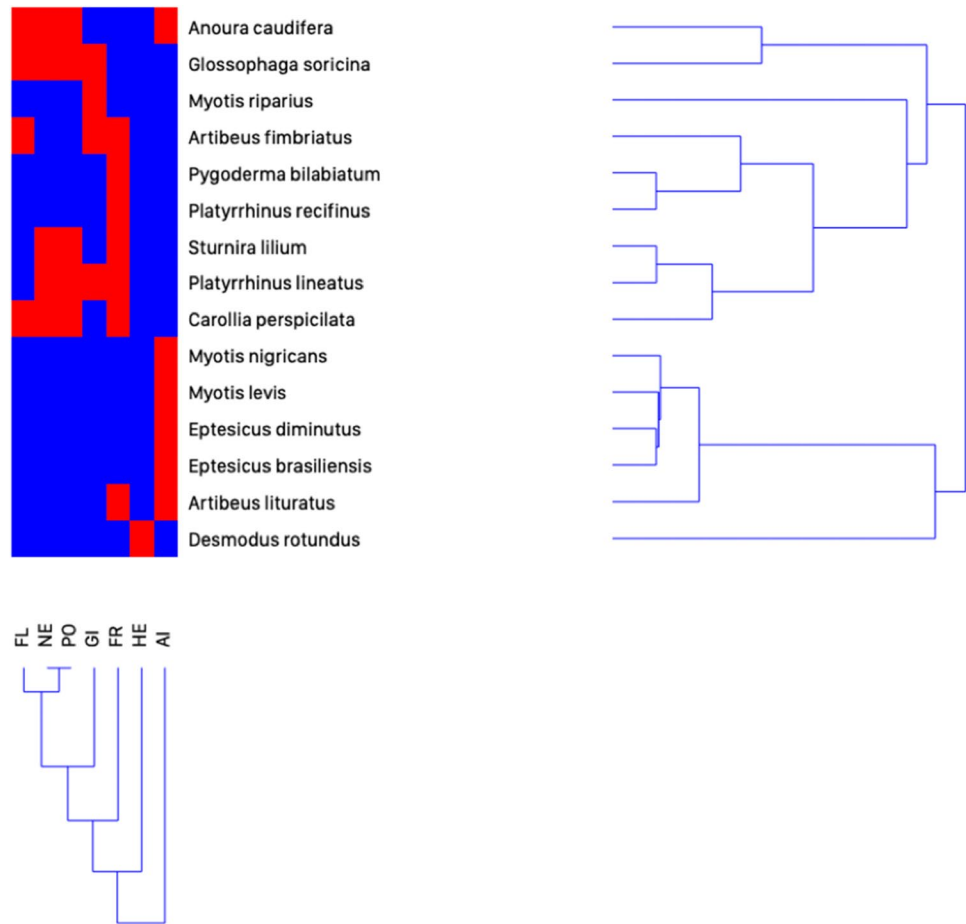
Bat flies of the families Streblidae and Nycteribiidae have already been described in the study area (Bertola et al. 2005). The environment can cause variation in fly abundance, different types of bat roosting can influence the degree of parasitism of these flies (Bordes et al. 2008; Pilosof et al. 2012). Even though

**Table 9** Mean body condition index of adult bats (excepting pregnant females) by species, field campaign, and stratified by sampling area

Families/Species/ Sampling Areas	Field Campaign 1						Field Campaign 2						Field Campaign 3						Field Campaign 4					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Phyllostomidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Anoura caudifera</i>																								
<i>Artibeus fimbriatus</i>					-0.59	-0.78	-2.29	-0.78	-2.29		0.83	12.21	8.06	-1.86	-3.78	-1.86	-3.78	-1.86	-3.78	-1.86	2.65	2.40	4.90	
<i>Artibeus lituratus</i>					-0.10	2.08	3.69	-6.01	0.47				1.30	-12.75	-4.90	-4.29	-3.29	0.30	-1.14					
<i>Carollia perspicillata</i>													0.33	-0.65										
<i>Desmodus rotundus</i>																								
<i>Glossophaga soricina</i>						1.19			2.59															
<i>Platyrrhinus lineatus</i>					0.52		-1.46		1.01				0.86											
<i>Platyrrhinus recifinus</i>					0.67																			
<i>Pygoderma bilabiatum</i>																								
<i>Sturnira lilium</i>	0.33				0.32	1.45	1.13	-1.15	4.20				-1.17	0.02	0.36	-0.63	2.15	0.02	-2.05	0.54				
Vespertilionidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Eptesicus brasiliensis</i>																								
<i>Eptesicus diminutus</i>																								
<i>Myotis levis</i>																								
<i>Myotis nigricans</i>	0.26						-0.26																	
<i>Myotis riparius</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



**Fig. 4** Hierarchical grouping dendrogram of the feeding habits of captured bats. Abbreviations: FL = flower eater; NE = nectivore; PO = polynivore; GI = general insectivore; FR = frugivore; HE = haematophagous; AI = aerial insectivore



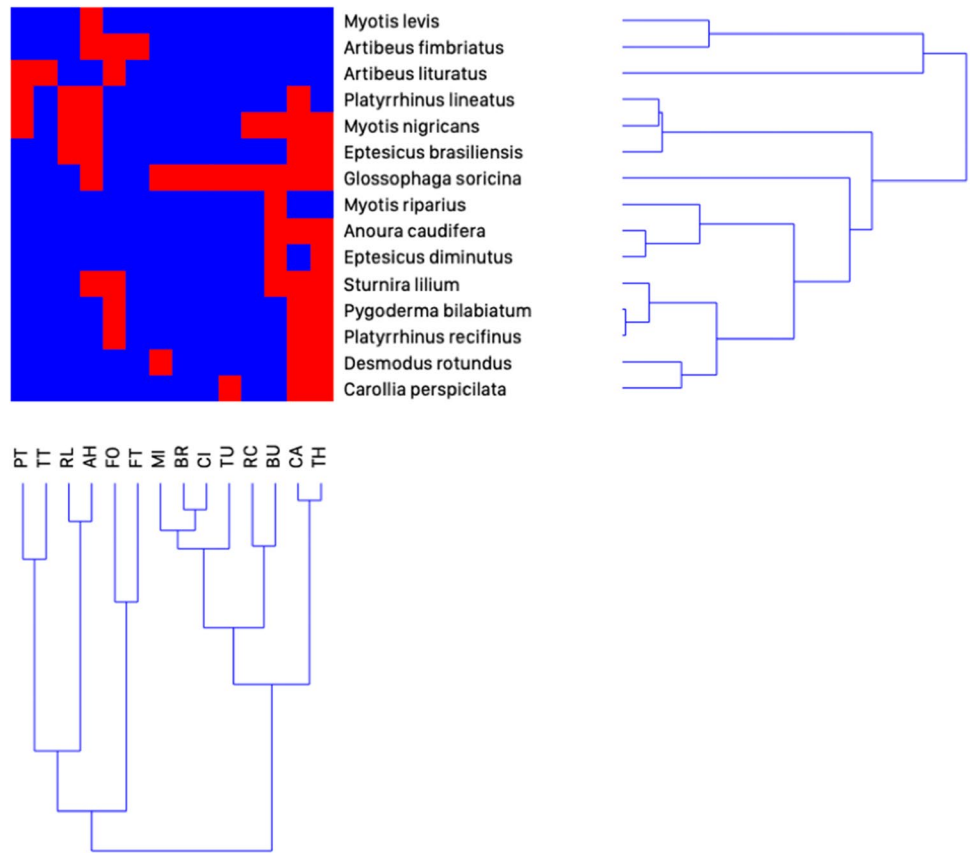
no formal identification of these ectoparasites has been made, and no observations of these parasites have been made in the first two field campaigns, some comments are noteworthy. The proportion of bats parasitized by flies (Streblidae or Nycteribidae) and the mean number of flies per captured bats in Campaign 4 (Autumn) was higher than in Campaign 3 (Summer). Bertola et al. (2005) observed a preference of the flies for bats' juvenile stage, which could partly explain these results, but bat species that co-inhabit roosts should also be taken into account. These authors also described the flies' preference for pregnant female bats. Still, unfortunately this could not be corroborated with the present work since no bat flies were collected during the Field Campaigns 1 and 2. The proportion of infested bats was higher in the urban areas than in the rural-preserved areas, except Sampling Area 4 (slums), which was the lowest (Area 6 > Area 3 > Area 5 > Area 1 > Area 2 > Area 4). Pilosof et al. (2012) found that the abundance of flies is affected by climatic and anthropogenic disturbances. Since bat flies are exposed to the environment during their life cycle, parasite abundance can be directly affected by local climatic conditions. For example, the development of bat flies is favored in warmer temperatures. Understanding the bat flies' transmission can help elucidate the

epidemiology of transmissible pathogens, as mentioned by Bertola et al. (2005), and further studies must be carried out.

A relatively high number of wounds and scars were observed in the captured bats. Bats could be injured during fights in roosts, i.e., some wounds could be specific to certain diseases. Instead, these results indicated no difference between specific and occasional wounds. No viruses (RABV and CoV) were detected in the sample, but RABV was previously isolated in the MASP, as described elsewhere (Favoretto et al. 2002; Sodr e et al. 2010), but the prevalence may be small and a large sample would be necessary to detect it, as described by Dias et al. (2019). Studies of bat CoV in Brazil are scarce (Brand o et al 2008; Lima et al. 2013; Asano et al. 2016), made up of small samples and low prevalences. More studies are necessary to describe the circulation of CoV in Brazil, especially after the COVID-19 pandemics.

No *Salmonella* spp. and *Leptospira* spp. were isolated in the sample, although these bacteria have already been described in urban and wild bats (Bessa et al. 2010; Iovine et al. 2015). The *Leptospira* results follow the literature review, showing that South America presents the lowest positive rate in MAT and molecular tests. Positive results ranged from 0% to 0.5% (0.1% ± 0.2%) and 0% to 100% (26.9% ± 23%), using MAT and

**Fig. 5** Hierarchical grouping dendrogram of captured bat roost types



molecular diagnostic, respectively worldwide (Esteves et al. 2022). *Salmonella* spp. belongs to the family Enterobacteriaceae and has been detected in bats worldwide at lower rates (Reyes et al. 2011; Islam et al. 2013), including in an insectivorous female bat of *Lasiurus blossevillii* in Brazil (Ferreira et al. 2021). Our results indicate that the studied bats may not be carriers of this pathogenic bacteria. Conversely, a high number of bacteria isolates was found in the sample, in particular *Citrobacter freundii* (n=21), *Enterobacter cloacae* (n=29), *E. hormaechei* (n=10), *Escherichia coli* (n=71), and *Serratia marcescens* (n=23). No differences in the proportion of bacteria isolates relative to the number of captured bats were observed between the field campaigns and sampling areas, except *E. hormaechei*, with a higher ratio of isolates per captured bats in Campaigns 3 and 4 than in Campaigns 1 and 2 and a higher proportion of isolates per captured bats in Campaign 3 than in Campaign 1.

The logistic regression models showed interesting insights. Sampling Area 2 (compared to Sampling Area 1) was a protection factor for the infection by *C. freundii*, and the presence of wounds was a risk factor. Area 2 had the highest number of captured bats, which may explain the high number of wounded bats. This result evidenced the opportunistic role of *C. freundii* and its association with skin wounds in bats, as demonstrated for other species (Kus and Burrows 2007).

For *E. cloacae*, using buildings as roost was a protection factor. *A. fimbriatus* and *S. lillium* were the species with the lowest prevalence of *E. cloacae*, 14.5 and 7.8%, respectively, if compared to *A. lituratus* (20%) and *C. perspicillata* (75%). The first two may roost in buildings, but this fact does not directly explain this protection factor, which should be further investigated. It is worth remembering that the types of roosts used by bats were generically assigned, according to Dias et al. (2019), but not necessarily observed in the field.

Sampling Area 4 (compared to Sampling Area 1) was a risk factor for the infection by *E. coli*. The abundant presence of water (due to the presence of a spring) and livestock in the FMVZ-USP, in addition to the precarious conditions of basic sanitation in the nearby slum, may explain this result. No significant logistic regression models were obtained for *E. hormaechei* and *S. marcescens*.

## Conclusions

Describing of the richness and abundance of bats (and other native species) in a gradient between areas of environmental preservation, rural and urban landscapes is essential for good planning of land use, which contributes

**Table 11** Number of bacteria isolates, by bat species, field campaign, and stratified by sampling area

Families/Species/ Sampling Areas	A spp	A bau	A bay	A cav	A hyd	A ver	C lap	C net	C ama	C far	C fre	C gil
Phyllostomidae	2	1	1	1	5	1	1	2		1	20	1
<i>Anoura caudifera</i>			1									
<i>Artibeus fimbriatus</i>	1	1		1	2						5	
<i>Artibeus lituratus</i>	1				1		1			1	3	
<i>Carollia perspicillata</i>												
<i>Desmodus rotundus</i>												
<i>Glossophaga soricina</i>								2				
<i>Platyrrhinus lineatus</i>					1							
<i>Platyrrhinus recifinus</i>												
<i>Pygoderma bilabiatum</i>												
<i>Sturnira lilium</i>					1	1					12	1
Vespertilionidae					3				2		1	
<i>Eptesicus brasiliensis</i>										1		
<i>Eptesicus diminutus</i>										1		
<i>Myotis levis</i>					1				1			
<i>Myotis nigricans</i>					2						1	
<i>Myotis riparius</i>												
Total	2	1	1	1	8	1	1	2	2	1	21	1
Families/Species/ Sampling Areas	C kos	C you	C sak	E aer	E asb	E bug	E can	E clo	E hor	E lud	E xia	E col
Phyllostomidae	1	1	1	1	4	1	5	29	10	1	4	70
<i>Anoura caudifera</i>												1
<i>Artibeus fimbriatus</i>	1	1	1		1		1	8	2			25
<i>Artibeus lituratus</i>					2	1		12	3	1	1	13
<i>Carollia perspicillata</i>							2	3				
<i>Desmodus rotundus</i>												
<i>Glossophaga soricina</i>									1			
<i>Platyrrhinus lineatus</i>												
<i>Platyrrhinus recifinus</i>												
<i>Pygoderma bilabiatum</i>												
<i>Sturnira lilium</i>				1	1		2	6	4		3	31
Vespertilionidae					1						1	1
<i>Eptesicus brasiliensis</i>												
<i>Eptesicus diminutus</i>												
<i>Myotis levis</i>												
<i>Myotis nigricans</i>					1							1
<i>Myotis riparius</i>											1	
Total	1	1	1	1	5	1	5	29	10	1	5	71
Families/Species/ Sampling Areas	H alv	K spp	K aer	K oxy	K pne	K var	K asc	K cry	K cow	L ade	M mor	Pa spp
Phyllostomidae		1	1	7	6	2	5	2	7	1	4	2
<i>Anoura caudifera</i>												
<i>Artibeus fimbriatus</i>						1	2	1	1	1	2	
<i>Artibeus lituratus</i>			1	3	1		2	1	2		1	1
<i>Carollia perspicillata</i>				1								
<i>Desmodus rotundus</i>												
<i>Glossophaga soricina</i>		1				1						
<i>Platyrrhinus lineatus</i>												
<i>Platyrrhinus recifinus</i>												
<i>Pygoderma bilabiatum</i>												

**Table 11** (continued)

Families/Species/ Sampling Areas	H alv	K spp	K aer	K oxy	K pne	K var	K asc	K cry	K cow	L ade	M mor	Pa spp
<i>Sturnira lilium</i>				3	5		1		4		1	1
Vespertilionidae	1		1	1					1			
<i>Eptesicus brasiliensis</i>									1			
<i>Eptesicus diminutus</i>	1											
<i>Myotis levis</i>												
<i>Myotis nigricans</i>			1	1								
<i>Myotis riparius</i>												
Total	1	1	2	8	6	2	5	2	8	1	4	2
Families/Species/ Sampling Areas	P agg	P ger	P hau	P alc	P ret	Ps spp	P aer	S mar	S nam	Total		
Phyllostomidae	1	2	1	1	1	1	4	23	2	238		
<i>Anoura caudifera</i>										2		
<i>Artibeus fimbriatus</i>							2	10	2	72		
<i>Artibeus lituratus</i>	1	2	1					6		62		
<i>Carollia perspicillata</i>								1		7		
<i>Desmodus rotundus</i>										0		
<i>Glossophaga soricina</i>					1			2		8		
<i>Platyrrhinus lineatus</i>							1			2		
<i>Platyrrhinus recifinus</i>								1		1		
<i>Pygoderma bilabiatum</i>										0		
<i>Sturnira lilium</i>				1		1	1	3		84		
Vespertilionidae				1						14		
<i>Eptesicus brasiliensis</i>										1		
<i>Eptesicus diminutus</i>										2		
<i>Myotis levis</i>										2		
<i>Myotis nigricans</i>										7		
<i>Myotis riparius</i>				1						2		
Total	1	2	1	2	1	1	4	23	2	252		

*A spp* Acinetobacter spp., *A bau* A. baumannii, *A bay* A. baylyi, *A cav* Aeromonas caviae, *A hyd* A. hydrophila, *A ver* A. veronii, *C lap* Cedecea lapagei, *C net* Cedecea neteri, *C ama* Citrobacter amalonaticus, *C far* C. farmeri, *C fre* C. freundii, *C gil* C. gilleni, *C kos* C. koseri, *C you* C. youngae, *C saz* Cronobacter sakazakii, *E aer* Enterobacter aerogenes, *E asb* E. asburgiae, *E bug* E. bugandensis, *E can* E. cancerogenus, *E clo* E. cloacae, *E hor* E. hormaechei, *E lud* E. ludwigii, *E xia* E. xiangfangensis, *E col* Escherichia coli, *H alv* Hafnia alvei, *K spp* Klebsiella spp. *K aer* K. aerogenes, *K oxy* K. oxytoca, *K pne* K. pneumoniae, *K var* K. variicola, *K asc* Kluyvera ascorbata, *K cry* K. cryocrescens, *K cow* Kosakonia cowanii, *L ade* Leclercia adenocarboxylata, *M mor* Morganella morgani, *Pa spp* Pantoea spp. *P agg* P. agglomerans, *P ger* Pluribacter gergoviae, *P hau* Proteus hauseri, *P alc* Providencia alcalifaciens, *P ret* P. rettgeri, *Ps spp* Pseudesccherichia spp., *P aer* Pseudomonas aeruginosa, *S mar* Serratia marcescens, *S nam* S. nematodiphila.

to the construction of a harmonious coexistence between humans, domestic animals and bats. In some situations, bats should receive food subsidies or even a strategic allocation of artificial or natural shelters, for example to promote the recovery of degraded areas or pest control. However, in other situations, their presence should be mitigated, especially if the risk of pathogens spillover is possible. For this, strategies such as urban cleaning effort, planting trees and plants that are not attractive and constructing buildings that prevent their use by bats could be used.

Moreover, the description of the circulating pathogens in bat populations allows the elaboration of contingency plans to mitigate the risk of spillover for humans and

animals. The opposite is also valid. Implementing these actions would require an intense inter-institutional relationship and could be justified not only for by bats, but for other species.

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**Data availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Competing interests** The authors declare no competing interests.

**Animal ethics** The procedures were carried out following the ethical principles of animal experimentation, and were approved by the Ethics Committee on Animal Use at School of Veterinary Medicine of the University of São Paulo (protocol number 2332030320). The animal capture license was obtained at the Brazilian Ministry of the Environment and Climate Change (SISBio 71136).

**Conflict of interest** All authors have seen and approved the final version of the submitted manuscript. The manuscript is original, hasn't received prior publication or isn't under consideration for publication elsewhere. There aren't competing interests and there's no financial/personal interest or belief that could affect the objectivity.

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