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Zoonotic Bacteria and Microbiome Diversity in Brazilian Bats: Implications for Public Health

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ZOONOTIC BACTERIA AND MICROBIOME DIVERSITY IN BRAZILIAN BATS: IMPLICATIONS FOR PUBLIC HEALTH

Thesis manuscript presented to the Federal University of Lavras (Universidade Federal de Lavras), as part of the requirements of the Graduate Program in Veterinary Sciences, with a concentration in Animal Health and Public Health, for the attainment of the title of Doctor.

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AMANDA CARVALHO ROSADO FERREIRA

Bactérias zoonóticas e diversidade do microbioma em morcegos brasileiros: implicações para a saúde pública

Zoonotic Bacteria and Microbiome Diversity in Brazilian Bats: Implications for Public Health

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciências Veterinárias, área de concentração em Sanidade Animal e Saúde Coletiva, para a obtenção do título de Doutor.

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I dedicate this thesis to the bat research community, whose efforts have advanced our understanding of wildlife, zoonotic diseases, and the One Health framework.

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RESUMO

A proximidade entre morcegos, humanos e animais domésticos, decorrente do comportamento sinantrópico adquirido pelos morcegos, representa um risco potencial para o surgimento de novas doenças emergentes. No que diz respeito aos patógenos bacterianos, estudos que buscam detectar sua presença em morcegos têm aumentado recentemente. No entanto, ainda não há conclusões definitivas sobre o risco real que esses animais podem representar na transmissão desses patógenos para animais de criação ou para humanos. Diante disso, o objetivo desta tese é contribuir para a literatura fornecendo mais evidências sobre a detecção de patógenos bacterianos em morcegos, utilizando técnicas moleculares de reação em cadeia da polimerase (PCR) e sequenciamento. Esta tese é composta por três capítulos: (1) Avaliação molecular de patógenos bacterianos zoonóticos em uma população diversificada de morcegos do Brasil; (2) Detecção de patógenos bacterianos zoonóticos em morcegos de ecossistemas de vereda no Cerrado brasileiro; e (3) Análise metataxonômica de amostras retais de morcegos sinantrópicos de Montes Claros, Minas Gerais, Brasil. Até o momento, DNA de bactérias patogênicas de importância zoonótica, como *Brucella* spp., *Yersinia enterocolitica*, *Salmonella* spp., *Bartonella* spp., *Staphylococcus aureus*, *Coxiella burnetii* e *Listeria monocytogenes*, foi identificado entre os morcegos brasileiros estudados. Além disso, os resultados de sequenciamento identificaram gêneros importantes, como *Mycobacterium*, *Escherichia*, *Brucella*, *Clostridium*, *Klebsiella*, entre outros. Esses achados ampliam o conhecimento atual sobre patógenos bacterianos e a diversidade microbiana em morcegos no Brasil, reforçando a necessidade de vigilância contínua para melhor compreender seu papel potencial na ecologia de doenças zoonóticas e suas implicações para a saúde pública.

PALAVRAS-CHAVE: Chiroptera, zoonoses bacterianas e Saúde Única

ABSTRACT

The proximity among bats, humans and domestic animals, due to the synanthropic behavior acquired by bats, represents a potential risk for the emergence of new emerging diseases. In relation to bacterial pathogens, studies seeking to detect their presence in bats have been growing lately. However, they are still not categorical about the real risk that these animals may present in the transmission of these pathogens to livestock animals or to humans. In view of this, the aim of this thesis is to contribute to the literature by providing more evidence on the detection of bacterial pathogens in bats using molecular techniques of polymerase chain reaction and sequencing. This thesis is composed of three chapters: (1) Molecular evaluation of zoonotic bacterial pathogens in a diverse bat population from Brazil; (2) Detection of zoonotic bacterial pathogens in bats from vereda ecosystems in the Brazilian Cerrado; and (3) Metataxonomic analysis of rectal samples of synanthropic bats from Montes Claros, Minas Gerais, Brazil. So far, DNA from pathogenic bacteria of zoonotic importance such as *Brucella* spp., *Yersinia enterocolitica*, *Salmonella* spp., *Bartonella* spp., *Staphylococcus aureus*, *Coxiella burnetti* and *Listeria monocytogenes* have been identified among the Brazilian bats studied. In addition, sequencing results have identified important genus such as *Mycobacterium*, *Escherichia*, *Brucella*, *Clostridium*, *Klebsiella* and others. These findings expand current knowledge about bacterial pathogens and microbial diversity in bats in Brazil and reinforce the need for continued surveillance to better understand their potential role in the ecology of zoonotic diseases and their implications for public health.

KEYWORDS: Chiroptera, Bacterial Zoonoses and One Health

IMPACT INDICATORS

This thesis aimed to expand knowledge regarding the diversity of zoonotic bacteria associated with Brazilian bats through the molecular investigation of bacterial pathogens, the characterization of the microbiome of synanthropic bats, and a synthesis of available scientific evidence on the subject. The results have a potential impact on the fields of health, the environment, and technology by providing novel information on the occurrence of zoonotic bacterial pathogens in bats across various Brazilian ecosystems, including vereda wetlands in the Cerrado and urban environments. These findings contribute to strengthening health surveillance within the One Health framework, supporting researchers, environmental agencies, and public officials in understanding the interactions between wildlife, the environment, and humans. Conducted under the Long-Term Ecological Research Program (PELD), the study also provides data to support future wildlife monitoring initiatives in conservation areas in Minas Gerais, thereby contributing to biodiversity conservation strategies and zoonosis surveillance. From a technological standpoint, the application and standardization of various molecular approaches for pathogen detection and microbiome analysis strengthen the methodologies used in wildlife research. The thesis also contributed to human resource development by involving undergraduate and graduate students in research activities, laboratory training, and data analysis, thereby fostering cooperation among the Federal University of Lavras (UFLA), the State University of Montes Claros (Unimontes), and partner institutions. Although the direct impacts are primarily scientific, the knowledge generated has the potential to inform public policies regarding epidemiological surveillance, wildlife conservation, and the prevention of zoonotic diseases. The work is aligned with the Sustainable Development Goals (SDGs) of the 2030 Agenda, particularly SDG 3 (Health and Well-being), SDG 4 (Quality Education), SDG 15 (Life on Land), and SDG 17 (Partnerships and Means of Implementation).

SUMMARY

GENERAL INTRODUCTION

The order Chiroptera (bats) is the second largest in terms of diversity with approximTELY 25% of living mammal species (SIMMONS, 2005). They are widely distributed throughout the world, with the exception of the (colder) polar regions. Throughout the evolutionary process, these mammals have acquired various characteristics that contribute to their wide distribution and adaptation to different habitats. The ability to fly allows bats to travel long distances, to occupy different habitats and thereby play important roles in ecosystem function. Nectarivores and frugivores, for example, are essential for pollinating various plants and dispersing seeds, acting as helpers in restoring destroyed habitats. Insectivores also play an important role in agriculture, helping to reduce agricultural pests.

On the other hand of being essential to the balance of ecosystems, these animals also bring with them a public health alert. Since the early 1990s, with the association of hematophagous bats with the rabies virus (CARINI, 1911), there has been a growing number of studies showing that these animals can harbor and sometimes even participate in the transmission cycle of numerous pathogens, such as viruses, bacteria and fungi (EMMONS, 1958; CALISHER et al., FERREIRA et al., 2024). Most of the published studies are related to viral pathogens, but recently there has also been an increase in studies on bacteria associated with bats, which may represent a zoonotic risk. Pathogenic bacterial species, such as *Salmonella*, *Yersinia*, *Leptospira* and others have been detected in bats, so far (FERREIRA et al., 2024).

Aspects as the ability to adapt to different habitats and to modulate the immune system have been raised as important reasons why these animals are associated with numerous pathogenic microorganisms (BANERJEE ET AL., 2020) . Indeed, despite of the increasing anthropogenic actions and environmental catastrophes, which lead to the constant fragmentation of forest habitats, can alter host-pathogen interactions and influence the circulation of infectious agents in wild animal populations (HADDAD et al., 2015; MCKINNEY, 2002). In this context, these animals have demonstrated the ability to establish themselves in a new habitat, urban centers, increasing contact between wild animals, domestic animals, and humans (RUSSO E ANCILLOTTO, 2015; NUNES et al., 2017). In these places they are able to benefit from urban conditions, such as abandoned construction areas, parks and urban vegetation, where they find shelter and food. This synanthropic behavior, an adaptation to living in close proximity to humans, raises public health concerns, since this closeness can increase the risk of disease transmission. In view of this, the aim of this thesis is to expand scientific knowledge about the presence of bacterial pathogens in bats, contributing new

evidence on this field, as well as gather important information from the current literature in this regard.

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ARTICLE 1

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Molecular evaluation of zoonotic bacterial pathogens in high diversity of bats from Brazil

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Abstract

The bats have stood out for their flexibility and adaptation to urban centers; moreover, are described as potential carriers of pathogens, which altogether raises One health concerns. Therefore, the presence of potentially pathogenic bacteria (*Salmonella* spp., *Leptospira* spp., *Bartonella* spp., *Yersinia enterocolitica*, *Staphylococcus aureus*, *Rickettsia* spp., *Pasteurella multocida*, *Coxiella burnetii*, and *Listeria monocytogenes*) in bat species in Brazil was investigated. A total of 283 bat liver samples belonging to 78 bat species from six Brazilian states were retrieved from the Mammal Collection of the Federal University of Lavras. DNA was extracted from liver samples and tested for the presence of the described pathogens using PCR and qPCR techniques. The results showed that 5.65% of the bats were positive for at least one pathogen, with the most commonly observed being *Salmonella* spp. and *Y. enterocolitica*. The positive samples were mainly from Minas Gerais state, with a higher prevalence in males and aerial insectivorous species. These results highlight the importance of monitoring these mammals as potential vectors/reservoirs of zoonotic pathogens and contribute to a broader understanding of the role of bats for public and animal health.

Key words: reservoirs, zoonoses, Chiroptera, One health, *Salmonella, Yersinia enterocolitica*.

1. Introduction

Over the years, there has been an increase in anthropogenic activities (e.g. mining and extraction of other natural resources) around the world resulting in the destruction and fragmentation of native habitats, and native vegetation suppression for agriculture and pastures, urbanization, pollution [1, 2]. These processes has caused various ecosystem effects, such as defaunation, that is the reduction of populations and the extinction of some animal native species [2, 3]. These changes force some wild animals to migrate to urban areas, where they find new opportunities for survival. Among synanthropic animals, some bats species (order Chiroptera) stand out significantly part of the species due their remarkable ability to adapt to urban centers [4]. A combination of factors can permit a bat species to adapt to urban environments, such as being generalist with high niche breadth (trophic guilds and roost ecology) or abiotic tolerance (noise, light, pollution, temperature, relative humidity) [4, 5]. Indeed, buildings, parks, and luminosity can offer several possibilities of shelter and food, especially to frugivorous and the aerial insectivore bat species (particularly the highly specialized free-tailed – Molossidae, and evening bats - Vespertilionidae) [5, 6]. Therefore, studies focusing on the biology of those synanthropic bat species, including their endoparasite diversity and potential health risks are very relevant to One Health [7, 8]. This is particularly important given the associations between bats and viruses such as rabies in Latin America, Ebola in Central Africa, and more recently the possibility of Sars-Covid in Asia [9]. In addition, it is important to study bat species that are potentially synanthropic or even that will never be, but they are of scientific interest. This is because it is known that endo- and ectoparasites negatively affect the fitness of many bat species, and our knowledge on uncommon bat species are scarce or even non-existent [10, 11].

A systemic review published a list of 84 species of urban bats recorded in Brazil [5], which represents almost half of the Brazilian species already described [45.16% (84/186)] [12], showing that the synanthropic behavior and omnipresence of these animals in large urban centers is notorious in the country. As a result, the close contact of these species with domestic animals and humans is imminent, which brings into focus a new concern about the transmission of zoonotic pathogens. It is important to note that not only urban bats raise One health concerns, but also the process of deforestation and consequent bat-human-domestic animal contact can support the zoonotic transmission chains [10].

Recent studies have confirmed the identification of important bacterial pathogens in bats in various locations around the world, including Brazil [13, 14]. A scoping review revealed more than 100 potentially pathogenic genera found in these animals, [13]. These encompassing bacterial genera that have been well described in the literature as being responsible for gastrointestinal syndromes, such as *Salmonella* spp., *Yersinia enterocolitica*, and *Listeria* spp.; those involved in fever illness, as *Coxiella burnetii*, *Rickettsia* spp., and *Bartonella* spp.; important opportunistic pathogens, as *Pasteurella multocida* and *Staphylococcus aureus*; as well as bacteria related to other clinical signs in humans, such as *Leptospira* spp. [13, 14].

Despite the amount of knowledge accumulated until date, reviews on the subject agree that there are still gaps in the understanding on the frequency and distribution of the bacterial pathogens identified by bat species worldwide. Studies aimed at filling these gaps can also contribute to risk assessment and to guide public and animal health surveillance systems. Thus, the aim of this study was to investigate the presence of potentially pathogenic bacteria in liver samples from bat species from Brazil collected in both urban and wild environments.

2. Materials and methods

2.1 Sampling

A total of 287 bat specimens were selected from the Coleção de Mamíferos da Universidade Federal de Lavras - CMUFLA (Mammal Collection of the Universidade Federal de Lavras). CMUFLA is part of the Centro de Biodiversidade e Patrimônio Genético - CEBIOMinas (Center of Biodiversity and Genetic Heritage) and has more than 4,000 bat specimens in its collection. The samples used in this study were selected based on two distinct criteria: 1) species represented by individuals collected in cities, rural areas, and 2) small forest fragments near the urban centers, and it is known or potentially can have a synanthropic behavior, and individuals representing rare species with scarce biological data known collected in wild environments. The 287 specimens came from 37 different regions, represented by municipalities from six Brazilian states, the majority from Minas Gerais [85.36% (245/287)], followed by Pará [7.32% (21/287)], Bahia and São Paulo [2.79% (8/287)], and Goiás and Tocantins [0.70% (2/287)] (Figure 1). One individual [0.35% (1/287)] had no data on geographical origin. The sample included animals collected from 2007 to 2020 [2007 (4); 2008 (11); 2009 (25); 2010 (2); 2011 (37); 2012 (41); 2013 (57); 2014 (3); 2015 (1); 2016 (14); 2017 (11); 2018 (14); 2019 (4); 2020 (14)], being 44.95% (129/287) sampled at dry season, whereas 37.98% (109/287) were sampled at the rainy season. Forty-nine specimens [17.07% (49/287)] did not have this information in the CMUFLA database. Seven families were represented, the majority of species being to Phyllostomidae [47.74% (137/287)], followed by Molossidae [27.53% (79/287)], Vespertilionidae [13.24% (38/287)], Emballonuridae [6.97% (20/287)], Mormoopidae [2.44% (7/287)], Natalidae [1.39% (4/287)], and Furipteridae [0.70% (2/287)]. The sampling consisted of 45 genus and 78 species of bats (Supplementary Material Table S1). Regarding feeding habits, aerial insectivorous were the most represented [52.26% (150/287)], followed by frugivorous [24.04% (69/287)], gleaner insectivorous/arthropod-feeding [9.06% (26/287)], nectarivorous [8.71% (25/287)], hematophagous [3.48% (10/287)], omnivorous [1.39% (4/287)], and carnivorous [1.05% (3/287)]. The majority were male [54.35% (156/287)], 41.81% (120/287) were female and 11 bats had no sex identification. All animals were classified as adult based on morphological criteria observing the completely ossified epiphysis of the fingers. Reproductive condition was recorded in

the field and when the bats were reproductively active, they were classified into scrotum males, and pregnant, lactating, and post-lactating females; all animals listed as young or sub-adult were classified as young. Adults were more frequent [96.52% (277/287)] than young animals [3.48% (10/287)]. A full description of the animals can be found in Supplementary Material Table S1. All tissue samples were preserved in absolute alcohol and stored in microtubes kept in a freezer at -20°C until DNA extraction.

2.2 DNA extraction

The DNA of the samples was extracted using the "Genomic DNA Purification Kit" (Wizard®, Promega, USA), following the manufacturer's standards. To verify the quality and concentration of the extracted DNA, samples were analyzed in a spectrophotometer (Nanodrop®, Thermo Fisher Scientific, USA). Low-quality samples that could not be re-extracted were excluded from the analysis. The remaining samples were stored in a freezer at -20°C.

2.3 Molecular detection of pathogens

Conventional PCR assays were used to screen the samples for *Salmonella* spp. (*ompC*), *Leptospira* spp. (*16S sRNA* and *lipL32*), *Bartonella* spp. (*gltA*), *Y. enterocolitica* (*ail*), *S. aureus* (*nuc*), *Rickettsia* spp. (*gltA*), *P. multocida* (*kmt1*), and *C. burnetii* (*IS1111*) DNA. A real-time quantitative (q)PCR was used to search for the presence of *L. monocytogenes* DNA (*hlyA*). The sequence of primers used, positive controls, size of the amplified product, and references, are specified in Supplementary Material Table S2. Ultrapure sterile water was used as a negative control. The conventional PCR products were visualized by electrophoresis in 1.5% agarose gel stained with (0.5 mg / mL) using Tris-borate-EDTA (TBE) running buffer (89 mM Tris Base, 89 mM boric acid and 2 mM EDTA pH 8.0). The molecular weight marker of 100 bp DNA Ladder was used in all assays. The images were developed and recorded in a transilluminator device (L-PixChemiPhotoDigitizer - Loccus Biotecnologia, Brazil) for subsequent analyses.

2.4 Statistical analysis

A logistic multivariate model was the strategy used to investigate the relationship of the independent variables (family, genus, specie, feeding habit, collection season, sex, age, city and state) and the outcome (PCR results for selected bacterial pathogens), for determining the factors potentially associated with their occurrence [15]. The independent variables sex, family, feeding habit, age, and genus were selected for analysis based on their biological importance among the other independent variables in the data. Despite its importance, the variable related to season of sampling was not selected for analysis due to the percentage of more than 17% of animals that did not have this information. Subsequently, a Fisher exact test was performed to investigate the relationship between the dependents variables (PCR results) and the selected independents variables with a permissive p-value ≥ 0.20 for selection of the variables to be initially included in the logistic multivariate model [15]. The fitting of the models was assessed by considering the likelihood ratio of Fisher test result and its p-value, comparing the adjust of the complete model to its null model, being considered significant when p-value was < 0.05 [15]. The models were evaluated by testing the predictive ability of the model (sensibility and specificity) and goodness-of-fit with Pearson X^2 test, when pertinent [15].

3. Results

Due to the low concentration of DNA extracted, four samples were excluded from analysis, all from Minas Gerais (Supplementary Material Table S3). Therefore, the presence of DNA of potentially pathogenic bacteria was investigated in the liver samples of 283 specimens. The frequency of detection animals positive for at least one of the searched pathogens was 5.65% (16/283). Among the nine pathogens investigated, the DNA of five of them (55.56%) was detected: *Salmonella* spp. [2.47% (7/283)], *Y. enterocolitica* [1.77% (5/283)], *S. aureus* [1.06% (3/283)], *C. burnetii* and *L. monocytogenes* [0.35% (1/283)] each. No positive specimens were identified for *Leptospira* spp., *Rickettsia* spp., *Bartonella* spp., and *P. multocida*. One positive adult male of *Anoura geoffroyi*, from

159 the municipality of Minduri, Minas Gerais, showed co-infection with *Salmonella* spp. and *S. aureus*.
160 In addition, two animals were positive in the PCR for the genus *Leptospira* (16S rRNA gene) but
161 negative in the amplification of the *lipL32* gene, which encodes a protein present in pathogenic
162 species, indicating the presence of saprophytic species (Appendix 1). These animals were not counted
163 as positive, since they were not considered infected by potentially zoonotic bacterial pathogens.

164 Positive samples were detected in 80% (4/6) of the political States. No DNA pathogen was
165 detected among the bats surveyed in states of Goiás and Tocantins. Most of the positive animals were
166 from the state of Minas Gerais [81.25% (13/16)], belonging to the municipalities of Mariléia (5),
167 Minduri (3), Caeté (1), Carrancas (1), Lavras (1), Muzambinho (1), and Unaí (1). For the other states
168 only one individual was detected as positive in each of them, in São Paulo, sample from Piquete, in
169 Bahia, sample from Santa Maria da Vitória, and in Pará, material from Parauapebas. The
170 Supplementary Material Figure S1 shows the location of the specimens by detected pathogen.
171 Considering the total number of individuals surveyed by state, the frequency of positives in São Paulo
172 and Bahia was 12.5% (1/8), followed by 6.61% (13/242) in Minas Gerais, and 4.76% (1/21) in Pará.
173 In relation to the season in which the positive animals were sampled, a similar number can be
174 observed for dry (7) and rainy (6) seasons, three animals had no information about the collection date.
175 Given the proportion of positive animals in relation to the studied population, the percentages
176 observed in the dry [5.42% (6/109)] and rainy [5.50% (6/109)] seasons were similar.

177 Of the seven families represented in this study, positive animals were detected in five
178 (71.43%). Among them, the family with the highest frequency of positives was Phyllostomidae
179 [37.5% (6/16)], followed by Molossidae [31.25% (5/16)], Vespertilionidae and Embaluronidae with
180 12.5% (2/16) each, and by Mormoopidae [6.25% (1/16)] (Figure 2A). Considering the frequency of
181 positives among the total number of individuals represented in each family, the Mormoopidae family
182 has the highest percentage of positives [14.26%, (1/7)], followed by the Emballonuridae [10.00%,
183 (2/20)], Molossidae [6.49%, (5/77)], Vespertilionidae [5.41%, (2/37)], and Phyllostomidae [4.41%,

(6/136)] (Figure 2 B). Regarding to bat genus, the 16 positive individuals belong to 23.91% (11/46) of the genera represented in this study.

Considering the feeding habit, most of the positive bats [62.5%, (10/16)] were aerial insectivorous followed by nectarivorous [12.5%, (2/16)]. The frugivorous, hematophagous, carnivorous, and gleaner insectivorous bats had positives samples with one representative each [6.25%, (1/16)] (Figure 2 C). In relation to the total population surveyed, the frequency of positives among carnivorous was 50% (1/2), among hematophagous 10% (1/10), nectarivorous 8.33% (2/24), insectivorous 6.80% (10/147), gleaner insectivorous 3.85% (1/26), and frugivorous 1.45% (1/69). None of the four omnivorous specimens exhibited positivity for any of the tested bacteria (Figure 2 D).

The percentage of males among the positives samples was 68.75% (11/16) and considering the total surveyed population 7.10% (11/155); whereas, for females, the percentage among the positives samples was 31.25% (5/16) and in the total population 4.27% (5/117) (Figure 2 E-F). All 16 positive animals were adults. It is worth noting that one of the positive female *Desmodus rotundus* was identified as pregnant. The full description of the positive animals can be seen in the Table 1.

The frequency of positive samples for *Salmonella* spp., *Y. enterocolitica*, and *S. aureus* were used as dependent variables for testing logistic multivariate models. These models were not tested for *C. burnetii* and *L. monocytogenes* because of the very low frequency of positives (1 for each). Eleven animals were removed from the database because they had unidentified sex. The independent variable age was excluded because all positive animals were adults. The results of Fisher's exact test did not find more than one independent variable associated with their PCR results (*Salmonella* spp., *Y. enterocolitica*, and *S. aureus*) with a permissive or a significative *p*-value, preventing the development of a logistic model, as it is shown in Table 2.

4. Discussion

The adaptation of some bat species to urban environments is remarkable. Their ecological versatility means that these animals can exploit structures such as abandoned sites, cracks, and roofs for shelter or even adapt to small urban green areas [4,6]. Because of this synanthropic capacity, there is an increase in the chances of exposure to pathogens carried by bats for human population, either directly (handling animal rescues or trying to repel it) or through indirect contact with its secretions or excrement, and perhaps by means of domestic animals. In this sense, the investigation of the presence of zoonotic bacterial pathogens in synanthropic bats, as performed by this study, contributes to both to the literature by adding more knowledge of the bacterial species/genus carried by these animals, and to the design of public health policies to prevent or mitigate potential risks of disease transmission by bats in urban areas. However, it is important to remember that there is a gradient in bat-human interactions. For example, part of our analyzed sample came from bats collected in the cities, but also from rural areas, mining regions, tourist parks, and caves, where the bat-human contact can occur in different degrees. Only a minor portion of the samples studied came from remote areas, usually representing rare species of high biological interest.

The detection of five of the nine pathogens investigated in this study demonstrates the non-negligible presence of these agents in the studied population and the need for constant monitoring. Moreover, the detection of these pathogens in liver samples may suggest that these infectious agents were not only transiently present but possibly causing persistent infection in these animals. In fact, the immune system of bats has developed mechanisms that limit pro-inflammatory responses, making it possible for these hosts to coexist with viruses that cause serious diseases in other mammals [16]. Although for bacterial infections, the literature is not very elucidative, this mechanism could also explain the presence of potentially pathogenic bacteria in the liver of apparently healthy animals. Corroborating this hypothesis, an experimental study which inoculated bacterial antigens (lipopolysaccharides) in *Myotis myotis* showed that although there was an increase in haptoglobin levels and in the ratio of neutrophils to lymphocytes, no fever, leukocytosis, loss of body mass or a

change in the general functioning of innate immunity was detected after the challenge [17]. Likewise, albeit one study has detected *Y. enterocolitica* in dead animals, the necropsy and histopathological examination of one bat positive for *Y. enterocolitica* from Germany showed no inflammatory changes and the authors suggested a subclinical state of infection [18]. The suppression of clinical signs suggest that the immunological mechanisms limit the inflammatory responses typical of viral infections may also occur in bacterial infections. This may represent an evolutionary adaptation that justify the concern of bats to act as reservoirs of pathogens without necessarily suffering from the infection. However, a study described high mortality in bat colonies with isolation of *Y. enterocolitica*, suggesting that this pathogen could be directly associated with the death of these animals [19]. In this sense, studies that seek to understand the ability of bats to harbor bacterial pathogens and the relationship of these infections with clinical disease are still need for further progress in this field.

The enteric pathogens stood out among the others investigated bacterial agents in this study, as *Salmonella* spp. and *Y. enterocolitica* accounted for 75% (12/16) of the positive results. In terms of the frequency of agents detected and investigated, the results of this study (2.47%) were in accordance with the frequencies previously described in the literature for *Salmonella* spp., such as 1.1% (4/377) at Trinidad island (2006-2007) and 0.51% (1/196) in Brazil in 2015 [20, 21]. For *Y. enterocolitica* there are only two reports in the literature, showing frequencies of 1% (1/200) in Germany 2010 and 15% (3/20) in Georgia in 2018 [18, 22]. For *L. monocytogenes*, for the best of our knowledge, there is only one study that identified this pathogen from bat feces [1.27 % (3/236)] [23], in a frequency similar to the one find in the present study. However, recently, an experimental study was conducted to investigate the potential of *L. monocytogenes* in kidney epithelial cells of the bat *Pipistrellus nathusii ex vivo* [24], identifying not only the invasion but also the replication of this pathogen in bat kidney cells. In this sense, this research corroborates our findings in identifying this enteric pathogen outside the gastrointestinal tract. It should be noted that direct comparison between the proportions found in different studies, although valid, should be made with caution, as the

diagnostic methods, the species of bats, as well as the environment to which they are exposed were different.

The diversity of bat species in which *Salmonella* spp. and *Y. enterocolitica* were detected suggests that these pathogens can be easily transmitted among bat species. The movement of these animals within and between roosts, long-distance migrations, and interaction with other species along their routes can facilitate the transmission of these pathogens within different bat populations [25, 26]. The presence of *Y. enterocolitica* in two bats of different species, feeding habits and collection year (*D. rotundus* – hematophagous (2013) and *Molossus molossus* – aerial insectivorous (2012)), but captured in the same site, Parque Estadual do Rio Doce, in the municipality of Marliéria, Minas Gerais, and same season year, point to this interaction between species with possible horizontal pathogen transference.

Similarly, the detection of *S. aureus* in nectivorous but of different species (*Anoura caudiffer* and *A. geoffroyi*) sampled at the same place (Mata Triste) in Minduri, Minas Gerais, in the same date, reinforces this interaction between species. *Staphylococcus aureus* is commonly identified in bat feces, or oral and rectal swabs [27, 28], and there is one report of detection in a bat with wounds [29]. Since this is an opportunistic pathogen, its identification in organs is not uncommon in other animal species [30]; however, in bats, this study is preliminary and further research is necessary to understand the colonization capacity of *S. aureus* in bat organs. Regarding to *C. burnetii*, this study identified a positive sample for this agent in bats from Minas Gerais, consistent with previous findings in the Atlantic Forest [13]. Although another study did not detect this pathogen in bats [31], these results confirm its presence in different regions of Brazil. This result highlights the public health risk of *C. burnetii*, the causative agent of Q fever, a zoonosis transmissible to humans.

The negativity for *Bartonella* spp., *Rickettsia* spp., pathogenic *Leptospira* and *Pasteurella multocida* may be related to the fact that the liver is not the organ of choice for search these pathogens, although there are reports in the literature of their detection in this tissue [13]. For *Bartonella*, for

example, in the absence of blood samples, the spleen is considered the organ of choice [32]. For *Leptospira*, the kidneys and urine would be the most suitable samples[21]. In addition, another factor that may have contributed to the negative results is the lower sensitivity of conventional PCR compared to more sensitive molecular techniques, such as qPCR, in cases of biological samples that may have a lower concentration of pathogen DNA.

The use of biological collections is of great importance for scientific research, as it allows essential studies to be conducted without the need to capture or euthanize new animals in the wild. These collections preserve samples of organisms that have already been collected and catalogued, allowing studies in taxonomy, phylogeny, paleontology, biogeography, research into pathogens, ecology, education and scientific dissemination to be carried out [33]. Furthermore, by working with this material, researchers not only avoid additional impacts on biodiversity, but also benefit from ethical, conservationist and economic implications (high costs associated with collection) [34]. However, the use of samples from biological collections can represent the limitation of using only what is available in the collection, introducing the bias of not using a probabilistic sampling. Besides rendering certain analyses unfeasible when complete data are not available, this limitation also made it impossible to test the collection season variable for inclusion in the logistic multivariate model due to the relevant proportion of animals lacking this information. In fact, as the collection contains more specimens from Minas Gerais, more positives samples were detected in this state, which could wrongly suggest that there are more pathogens in bats from Minas Gerais than in other states. The same issue can be seen for other variables, such as age, sex, local of sampling, and bat species. Altogether these implications of convenience sampling (with non-representative proportions of variables) may have led to the absence of a well-adjusted model at the end of the analyses. However, regarding feeding habits, even though aerial insectivores represented the largest sample, tending towards a possible bias, all but representatives of the omnivorous trophic guild were identified as positive for at least one pathogen. This shows the diversity of pathogens present in different feeding

habits, even if the sampling is unbalanced. Future studies should conduct analyses with more samples per species to deepen the understanding of intrapopulation transmission levels.

5. Conclusion

Our results showed the presence of *Salmonella* spp., *S. aureus*, *Coxiella burnetii*, *Y. enterocolitica*, and *L. monocytogenes* in synanthropic bats from Brazil. These findings have important implications for One health approaches. The identification of pathogens in bats is crucial for the development of public policies aimed at implementing epidemiological surveillance and biosecurity strategies to reduce the potential risk of zoonotic transmission.

Conflicts of interest

The authors have no conflict of interest to declare.

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Tables

Table 1. Epidemiological information of bats from Coleção de Mamíferos da Universidade Federal de Lavras PCR-positive for potentially zoonotic bacterial pathogens, according to family, genus, species, sex, feeding habits, collection season, municipality, state, age category, and identified pathogens.

Family	Genus	Species	Sex	Feeding habit	Collection season	Municipality	State	Age category	Detected Pathogen
Phyllostomidae	*Tonatia*	*Tonatia bidens*	male	insectivorous-arthropod	NI	Santa Maria da Vitória	Bahia	Adult	*Salmonella* spp.
	Trachops	*Trachops cirrhosus*	male	Carnivorous	rainy	Marliéria	Minas Gerais	Adult	*Listeria monocytogenes*
	Desmodus	*Desmodus rotundus*	female	Hematophagous	dry	Marliéria	Minas Gerais	Adult	*Yersinia enterocolitica*
	Pygoderma	*Pygoderma bilabiatum*	male	Frugivorous	rainy	Carrancas	Minas Gerais	Adult	*Yersinia enterocolitica*
	Anoura	*Anoura geoffroyi*	male	Nectarivorous	dry	Minduri	Minas Gerais	Adult	*Salmonella* spp. and *Staphylococcus aureus*
	Anoura	*Anoura caudifer*	female	Nectarivorous	dry	Minduri	Minas Gerais	Adult	*Staphylococcus aureus*
Molossidae	*Molossus*	*Molossus aztecus*	male	(aerial) insetivorous	rainy	Marliéria	Minas Gerais	Adult	*Salmonella* spp.
	Molossus	*Molossus aztecus*	male	(aerial) insetivorous	NI	Minduri	Minas Gerais	Adult	*Yersinia enterocolitica*
	Molossus	*Molossus molossus*	female	(aerial) insetivorous	dry	Marliéria	Minas Gerais	Adult	*Yersinia enterocolitica*
	Molossus	*Molossus aztecus*	male	(aerial) insetivorous	dry	Marliéria	Minas Gerais	Adult	*Coxiella burnetii*
	Molossops	*Molossops neglectus*	male	(aerial) insetivorous	rainy	Piquete	São Paulo	Adult	*Staphylococcus aureus*
	Tadarida	*Tadarida brasiliensis*	male	(aerial) insetivorous	rainy	Caeté	Minas Gerais	Adult	*Salmonella* spp.
Emballonuridae	*Peropteryx*	*Peropteryx macrotis*	female	(aerial) insetivorous	dry	Unaí	Minas Gerais	Adult	*Salmonella* spp.
	Peropteryx	*Peropteryx macrotis*	female	(aerial) insetivorous	NI	Lavras	Minas Gerais	Adult	*Salmonella* spp.
Vespertilionidae	*Myotis*	*Myotis albescens*	male	(aerial) insetivorous	rainy	Muzambinho	Minas Gerais	Adult	*Salmonella* spp.
Mormoopidae	*Pteronotus*	*Pteronotus gymnonotus*	male	(aerial) insetivorous	dry	Parauapebas	Pará	Adult	*Yersinia enterocolitica*

NI: Not Informed

Table 2. Results of the univariate analysis using Fisher's exact test for presence of *Salmonella* spp., *Staphylococcus aureus* and *Yersinia enterocolitica* among bats from Brazil.

Pathogen	**Variable**	***p-value***
Salmonella spp.	Sex	0.70
	Family	0.19
	Feeding habit	0.49
	Genus	0.85
Staphylococcus aureus	Sex	1.00
	Family	1.00
	Feeding habit	0.13
	Genus	0.23
Yersinia enterocolitica	Sex	1.00
	Family	0.29
	Feeding habit	0.44
	Genus	0.50

Figures

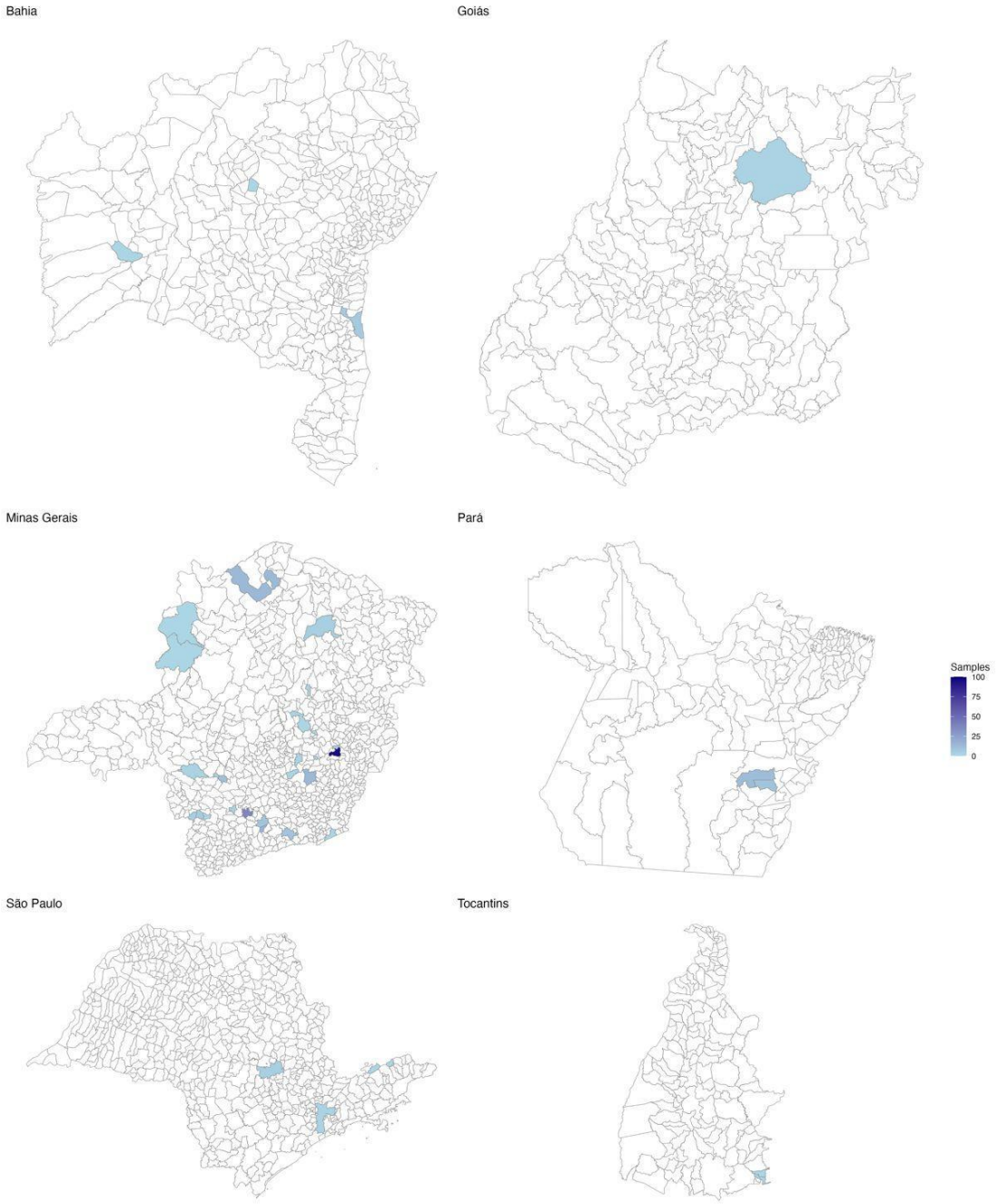
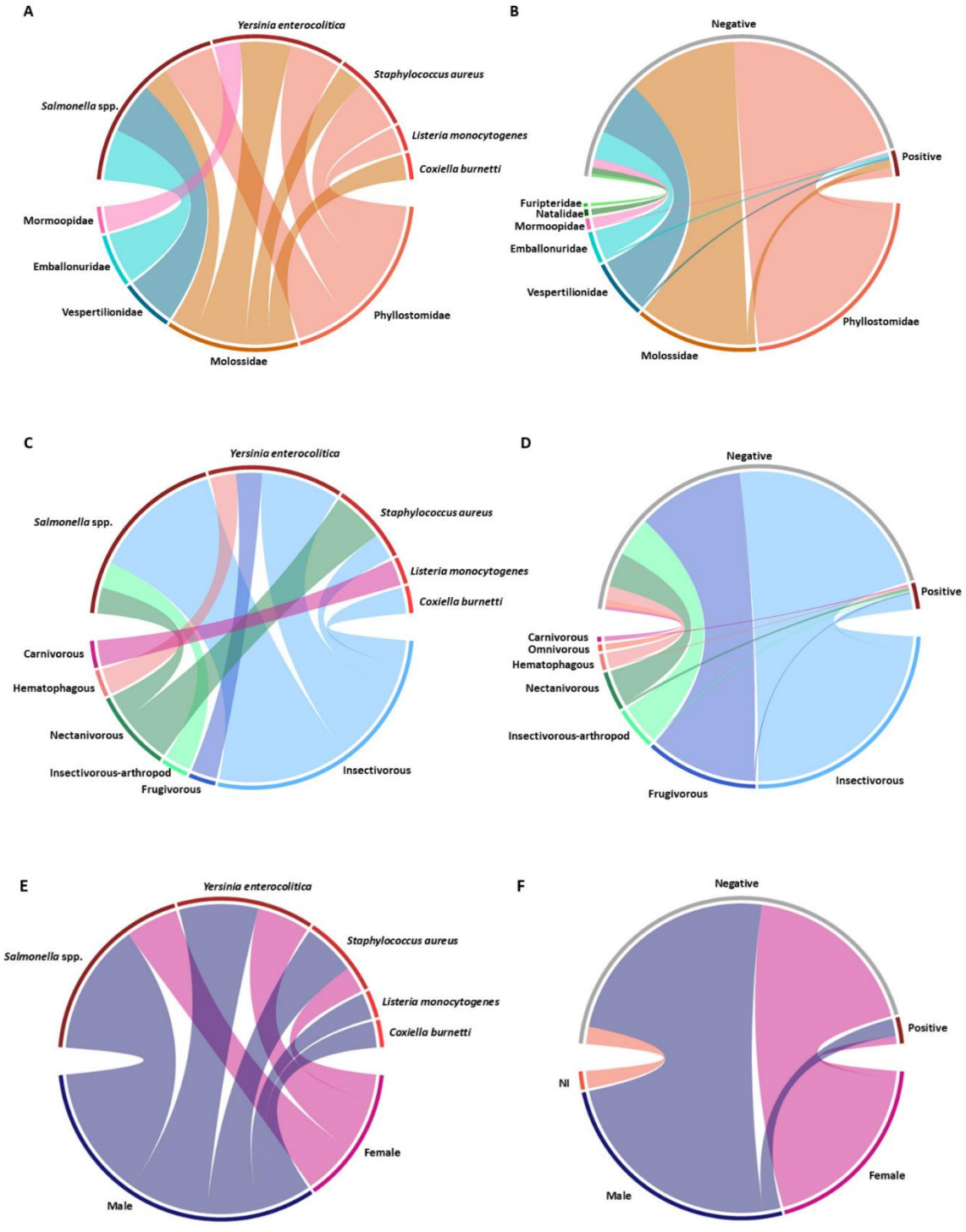


Figure 1: Distribution of species selected from Coleção de Mamíferos da Universidade Federal de Lavras, to molecularly analyze the presence of pathogens in relation to location. In Bahia: Cafarnaum (1), Ilhéus (6), and Santa Maria (1); Goiás: Niquelândia (2); Minas Gerais: Águas Vermelhas (1), Além Paraíba (1), Areado (1), Caeté (2), Carrancas (9), Coqueiral (2), Doresópolis (2), Grão Mogol (4), Itabirito (2), Januária (14), João Monlevade (4), Lavras (37), Lima Duarte (9), Mariana (14), Marliéria (95), Minduri (15), Monte Belo (5), Muzambinho (4), Pains (11), São Gonçalo do Rio Preto (2), São Roque de Minas (2), and Timóteo (3); Pará: Canaã dos Carajás (9) and Parauapebas (12); São Paulo: Campos do Jordão (1), Piquete (1), Piracicaba (4), and São Paulo (2). Tocantins: Aurora do Tocantins (1), and Lavandeira (1).



457

458 Figure 2: Frequency distribution of pathogen-positive liver samples among bats from
459 Coleção de Mamíferos da Universidade Federal de Lavras, based on bat Family, feeding
460 habit and sex. (A) Proportion of Phyllostomidae, Molossidae, Vespertilionidae,
461 Emballonuridae, and Momoopidae positive for each pathogen. (B) Proportion of different
462 bat families, negative and positive for pathogens in relation to the total number of
463 samples. (C) Proportion of carnivourous, hematophagous, nectarivorous, (gleaner)

insectivorous-arthopod, frugivorous and (aerial) insectivorous bats, positive for each pathogen. (D) Proportion of different feeding habit of bats, negative and positive for pathogens in relation to the total number of samples. (E) Proportion of male and female bats positive for each pathogen. (F) Proportion of male, female and NI (not informed) bats, negative and positive for pathogens in relation to the total number of sample.

Supplementary Material

Molecular evaluation of zoonotic bacterial pathogens in high diversity of bats from Brazil

Tables

Table S21: Characterization of all Coleção de Mamíferos da Universidade Federal de Lavras bats analyzed for zoonotic pathogens, according to family, species, feeding habit, sex, municipality, state, age and identified pathogens.

Family	Species	Feeding habit	Sex	Age category	Municipality	State	N	Pathogen
Emballonuridae	*Peropteryx macrotis*	Aerial insectivorous	Female	Adult	Lavras	Minas Gerais	1	Negative
Emballonuridae	*Peropteryx kappleri*	Aerial insectivorous	Female	Adult	Parauapebas	Pará	1	Negative
Emballonuridae	*Peropteryx kappleri*	Aerial insectivorous	Male	Adult	Parauapebas	Pará	4	Negative
Emballonuridae	*Rhynchonycteris naso*	Aerial insectivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Emballonuridae	*Peropteryx macrotis*	Aerial insectivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Emballonuridae	*Rhynchonycteris naso*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative

Emballonuridae	*Rhynchonycteris naso*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	2	*Leptospira* spp. (saprophyte)
Emballonuridae	*Rhynchonycteris naso*	Aerial insectivorous	Male	Adult	Marliéria	Minas Gerais	4	Negative
Emballonuridae	*Rhynchonycteris naso*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative
Emballonuridae	*Peropteryx macrotis*	Aerial insectivorous	Female	Adult	Lavras	Minas Gerais	1	*Salmonella* spp.
Emballonuridae	*Peropteryx macrotis*	Aerial insectivorous	Female	Adult	Unaí	Minas Gerais	1	*Salmonella* spp.
Emballonuridae	*Peropteryx macrotis*	Aerial insectivorous	Male	Adult	Lavandeira	Tocantins	1	Negative
Emballonuridae	*Peropteryx macrotis*	Aerial insectivorous	Male	Adult	Aurora do Tocantins	Tocantins	1	Negative
Furipteridae	*Furipterus horrens*	Aerial insectivorous	Female	Adult	Grão Mogol	Minas Gerais	1	Negative
Furipteridae	*Furipterus horrens*	Aerial insectivorous	NI	Adult	Canaã dos Carajás	Pará	1	Negative
Molossidae	*Promops nasutus*	Aerial insectivorous	Female	Adult	Lavras	Minas Gerais	1	Negative
Molossidae	*Eumops perotis*	Aerial insectivorous	Male	Adult	Lavras	Minas Gerais	1	Negative

Molossidae	*Eumops glaucinus*	Aerial insectivorous	Male	Adult	Lavras	Minas Gerais	1	Negative
Molossidae	*Molossus aztecus*	Aerial insectivorous	Male	Adult	Lavras	Minas Gerais	1	Negative
Molossidae	*Molossus molossus*	Aerial insectivorous	Male	Adult	Lavras	Minas Gerais	1	Negative
Molossidae	*Molossops neglectus*	Aerial insectivorous	Female	Adult	Lavras	Minas Gerais	1	Negative
Molossidae	*Eumops perotis*	Aerial insectivorous	Male	Adult	Lavras	Minas Gerais	1	Negative
Molossidae	*Eumops auripendulus*	Aerial insectivorous	Male	Young	Lima Duarte	Minas Gerais	1	Negative
Molossidae	*Eumops auripendulus*	Aerial insectivorous	Female	Adult	Lima Duarte	Minas Gerais	2	Negative
Molossidae	*Eumops auripendulus*	Aerial insectivorous	Male	Adult	Lima Duarte	Minas Gerais	1	Negative
Molossidae	*Nyctinomops laticaudatus*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative
Molossidae	*Nyctinomops laticaudatus*	Aerial insectivorous	Male	Adult	Marliéria	Minas Gerais	2	Negative
Molossidae	*Neoplatymops mattogrossensis*	Aerial insectivorous	Female	Adult	Cafarnaum	Bahia	1	Negative

Molossidae	*Molossus molossus*	Aerial insectivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Molossidae	*Molossus molossus*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative
Molossidae	*Molossus molossus*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	1	*Yersinia enterocolitica*
Molossidae	*Molossus fluminensis*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative
Molossidae	*Nyctinomops laticaudatus*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative
Molossidae	*Nyctinomops laticaudatus*	Aerial insectivorous	NI	Adult	Marliéria	Minas Gerais	1	Negative
Molossidae	*Molossus aztecus*	Aerial insectivorous	Male	Adult	Marliéria	Minas Gerais	8	Negative
Molossidae	*Molossus aztecus*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	3	Negative
Molossidae	*Molossus fluminensis*	Aerial insectivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Molossidae	*Molossus aztecus*	Aerial insectivorous	Male	Adult	Marliéria	Minas Gerais	1	*Coxiella burnetti*
Molossidae	*Molossus fluminensis*	Aerial insectivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative

Molossidae	*Molossus molossus*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative
Molossidae	*Promops nasutus*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	2	Negative
Molossidae	*Molossus aztecus*	Aerial insectivorous	Male	Adult	Marliéria	Minas Gerais	1	*Salmonella* spp.
Molossidae	*Molossops temminckii*	Aerial insectivorous	Male	Adult	Niquelândia	Goiás	1	Negative
Molossidae	*Molossops temminckii*	Aerial insectivorous	Male	Adult	Januária	Minas Gerais	1	Negative
Molossidae	*Tadarida brasiliensis*	Aerial insectivorous	Male	Adult	Lavras	Minas Gerais	3	Negative
Molossidae	*Tadarida brasiliensis*	Aerial insectivorous	Female	Adult	Lavras	Minas Gerais	12	Negative
Molossidae	*Tadarida brasiliensis*	Aerial insectivorous	Female	Adult	Lavras	Minas Gerais	1	Negative
Molossidae	*Tadarida brasiliensis*	Aerial insectivorous	Male	Adult	Lavras	Minas Gerais	3	Negative
Molossidae	*Cynomops planirostris*	Aerial insectivorous	Male	Adult	Lavras	Minas Gerais	1	Negative
Molossidae	*Molossus aztecus*	Aerial insectivorous	Male	Adult	Itabirito	Minas Gerais	1	Negative

Molossidae	*Cynomops planirostris*	Aerial insectivorous	Male	Adult	Lavras	Minas Gerais	1	Negative
Molossidae	*Tadarida brasiliensis*	Aerial insectivorous	Male	Adult	Campos do Jordão	São Paulo	1	Negative
Molossidae	*Tadarida brasiliensis*	Aerial insectivorous	Male	Adult	Caeté	Minas Gerais	1	*Salmonella* spp.
Molossidae	*Molossops neglectus*	Aerial insectivorous	Male	Adult	Piquete	São Paulo	1	*Staphylococcus aureus*
Molossidae	*Tadarida brasiliensis*	Aerial insectivorous	Male	Adult	Mariana	Minas Gerais	1	Negative
Molossidae	*Molossops temminckii*	Aerial insectivorous	Female	Adult	Januária	Minas Gerais	1	Negative
Molossidae	*Molossops temminckii*	Aerial insectivorous	Male	Adult	Januária	Minas Gerais	1	Negative
Molossidae	*Molossus molossus*	Aerial insectivorous	Male	Adult	Muzambinho	Minas Gerais	1	Negative
Molossidae	*Molossops temminckii*	Aerial insectivorous	Female	Adult	São Gonçalo do Rio Preto	Minas Gerais	1	Negative
Molossidae	*Molossops neglectus*	Aerial insectivorous	Female	Adult	Areado	Minas Gerais	1	Negative
Molossidae	*Molossops neglectus*	Aerial insectivorous	Female	Adult	Muzambinho	Minas Gerais	1	Negative

Molossidae	*Nyctinomops aurispinosus*	Aerial insectivorous	Female	Adult	São Paulo	São Paulo	1	Negative
Molossidae	*Molossus aztecus*	Aerial insectivorous	Male	Adult	Minduri	Minas Gerais	1	*Yersinia enterocolitica*
Molossidae	*Molossus aztecus*	Aerial insectivorous	Female	Adult	Minduri	Minas Gerais	1	Negative
Molossidae	*Molossops temminckii*	Aerial insectivorous	Male	Adult	Pains	Minas Gerais	1	Negative
Mormoopidae	*Pteronotus gymnonotus*	Aerial insectivorous	Male	Adult	Parauapebas	Pará	1	*Yersinia enterocolitica*
Mormoopidae	*Pteronotus personatus*	Aerial insectivorous	Male	Adult	Parauapebas	Pará	6	Negative
Natalidae	*Natalus macrourus*	Aerial insectivorous	Male	Adult	Januária	Minas Gerais	1	Negative
Natalidae	*Natalus macrourus*	Aerial insectivorous	NI	Adult	Canaã dos Carajás	Pará	1	Negative
Natalidae	*Natalus macrourus*	Aerial insectivorous	Male	Adult	Canaã dos Carajás	Pará	1	Negative
Natalidae	*Natalus macrourus*	Aerial insectivorous	Male	Adult	Januária	Minas Gerais	1	Negative
Phyllostomidae	*Platyrrhinus lineatus*	Frugivorous	NI	Adult	São Roque de Minas	Minas Gerais	1	Negative

Phyllostomidae	*Platyrrhinus lineatus*	Frugivorous	Female	Adult	São Roque de Minas	Minas Gerais	1	Negative
Phyllostomidae	*Carollia perspicillata*	Frugivorous	Male	Adult	Minduri	Minas Gerais	2	Negative
Phyllostomidae	*Chrotopterus auritus*	Carnivorous	Male	Adult	Minduri	Minas Gerais	1	Negative
Phyllostomidae	*Sturnira lilium*	Frugivorous	Female	Adult	Coqueiral	Minas Gerais	1	Negative
Phyllostomidae	*Sturnira lilium*	Frugivorous	Male	Adult	Coqueiral	Minas Gerais	1	Negative
Phyllostomidae	*Carollia perspicillata*	Frugivorous	Female	Adult	Minduri	Minas Gerais	2	Negative
Phyllostomidae	*Carollia brevicauda*	Frugivorous	Female	Adult	Minduri	Minas Gerais	1	Negative
Phyllostomidae	*Pygoderma bilabiatum*	Frugivorous	Male	Adult	Minduri	Minas Gerais	1	Negative
Phyllostomidae	*Artibeus fimbriatus*	Frugivorous	Male	Adult	Lavras	Minas Gerais	1	Negative
Phyllostomidae	*Glossophaga soricina*	Nectarivorous	Female	Adult	Lavras	Minas Gerais	1	Negative
Phyllostomidae	*Platyrrhinus lineatus*	Frugivorous	Female	Adult	Lavras	Minas Gerais	1	Negative

Phyllostomidae	*Phylloderma stenops*	Gleaner insectivorous-arthropods	Female	Adult	Januária	Minas Gerais	1	Negative
Phyllostomidae	*Platyrrhinus recifinus*	Frugivorous	Male	Adult	João Monlevade	Minas Gerais	1	Negative
Phyllostomidae	*Chiroderma doriae*	Frugivorous	Female	Adult	João Monlevade	Minas Gerais	1	Negative
Phyllostomidae	*Trachops cirrhosus*	Carnivorous	Male	Adult	Marliéria	Minas Gerais	1	*Listeria monocytogenes*
Phyllostomidae	*Glossophaga soricina*	Nectarivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Tonatia bidens*	Gleaner insectivorous-arthropods	Male	Adult	Santa Maria da Vitória	Bahia	1	*Salmonella* spp.
Phyllostomidae	*Sturnira lilium*	Frugivorous	Male	Adult	Lima Duarte	Minas Gerais	3	Negative
Phyllostomidae	*Desmodus rotundus*	Hematophagous	Male	Adult	Lima Duarte	Minas Gerais	2	Negative
Phyllostomidae	*Rhinophylla pumilio*	Frugivorous	Male	Adult	Ilhéus	Bahia	1	Negative
Phyllostomidae	*Desmodus rotundus*	Hematophagous	Male	Adult	Ilhéus	Bahia	1	Negative
Phyllostomidae	*Desmodus rotundus*	Hematophagous	Male	Adult	Grão Mogol	Minas Gerais	1	Negative

Phyllostomidae	*Rhinophylla pumilio*	Frugivorous	Female	Adult	Ilhéus	Bahia	1	Negative
Phyllostomidae	*Artibeus cinereus*	Frugivorous	Female	Adult	Grão Mogol	Minas Gerais	1	Negative
Phyllostomidae	*Desmodus rotundus*	Hematophagous	Male	Adult	Grão Mogol	Minas Gerais	1	Negative
Phyllostomidae	*Artibeus cinereus*	Frugivorous	Male	Adult	Ilhéus	Bahia	1	Negative
Phyllostomidae	*Platyrrhinus recifinus*	Frugivorous	Male	Adult	Ilhéus	Bahia	2	Negative
Phyllostomidae	*Pygoderma bilabiatum*	Frugivorous	Female	Adult	Águas Vermelhas	Minas Gerais	1	Negative
Phyllostomidae	*Desmodus rotundus*	Hematophagous	Female	Adult	Carrancas	Minas Gerais	1	Negative
Phyllostomidae	*Pygoderma bilabiatum*	Frugivorous	Male	Adult	Carrancas	Minas Gerais	1	*Yersinia enterocolitica*
Phyllostomidae	*Sturnira lilium*	Frugivorous	Male	Adult	Carrancas	Minas Gerais	1	Negative
Phyllostomidae	*Anoura caudifer*	Nectarivorous	Male	Young	Carrancas	Minas Gerais	1	Negative
Phyllostomidae	*Platyrrhinus recifinus*	Frugivorous	Male	Young	Carrancas	Minas Gerais	1	Negative
Phyllostomidae	*Anoura caudifer*	Nectarivorous	Male	Adult	Carrancas	Minas Gerais	1	Negative

Phyllostomidae	*Platyrrhinus lineatus*	Frugivorous	Female	Adult	Carrancas	Minas Gerais	1	Negative
Phyllostomidae	*Desmodus rotundus*	Hematophagous	Female	Adult	Carrancas	Minas Gerais	1	Negative
Phyllostomidae	*Sturnira lilium*	Frugivorous	Female	Young	Carrancas	Minas Gerais	1	Negative
Phyllostomidae	*Vampyressa pusilla*	Frugivorous	Female	Adult	Januária	Minas Gerais	1	Negative
Phyllostomidae	*Lionycteris spurrelli*	Nectarivorous	Male	Adult	Januária	Minas Gerais	1	Negative
Phyllostomidae	*Lonchophylla dekeyseri*	Nectarivorous	Male	Adult	Januária	Minas Gerais	1	Negative
Phyllostomidae	*Lionycteris spurrelli*	Nectarivorous	Female	Adult	Januária	Minas Gerais	1	Negative
Phyllostomidae	*Macrophyllum macrophyllum*	Gleaner insectivorous-arthropods	Male	Adult	Timóteo	Minas Gerais	3	Negative
Phyllostomidae	*Lonchophylla mordax*	Nectarivorous	Female	Adult	Januária	Minas Gerais	1	Negative
Phyllostomidae	*Mimon bennettii*	Gleaner insectivorous-arthropods	Male	Adult	Januária	Minas Gerais	1	Negative

Phyllostomidae	*Artibeus planirostris*	Frugivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Artibeus lituratus*	Frugivorous	Male	Adult	Marliéria	Minas Gerais	6	Negative
Phyllostomidae	*Artibeus obscurus*	Frugivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Artibeus planirostris*	Frugivorous	Female	Adult	Marliéria	Minas Gerais	4	Negative
Phyllostomidae	*Micronycteris schmidtorum*	Gleaner insectivorous-arthropods	Female	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Artibeus obscurus*	Frugivorous	Male	Adult	Marliéria	Minas Gerais	5	Negative
Phyllostomidae	*Platyrrhinus recifinus*	Frugivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Chiroderma villosum*	Frugivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Chiroderma villosum*	Frugivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Trachops cirrhosus*	Carnivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Sturnira lilium*	Frugivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative

Phyllostomidae	*Glossophaga soricina*	Nectarivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Artibeus lituratus*	Frugivorous	Female	Adult	Marliéria	Minas Gerais	2	Negative
Phyllostomidae	*Artibeus planirostris*	Frugivorous	Male	Young	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Glossophaga soricina*	Nectarivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Micronycteris minuta*	Gleaner insectivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Dryadonycteris capixaba*	Nectarivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Platyrrhinus recifinus*	Frugivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Desmodus rotundus*	Hematophagous	Female	Adult	Marliéria	Minas Gerais	1	*Yersinia enterocolitica*
Phyllostomidae	*Trinycteris nicefori*	Gleaner insectivorous-arthropods	Male	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Glossophaga soricina*	Nectarivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Anoura caudifer*	Nectarivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative

Phyllostomidae	*Micronycteris minuta*	Gleaner insectivorous-arthropods	Male	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Micronycteris minuta*	Gleaner insectivorous-arthropods	Female	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Glossophaga soricina*	Nectarivorous	Male	Adult	Marliéria	Minas Gerais	3	Negative
Phyllostomidae	*Glossophaga soricina*	Nectarivorous	Female	Adult	Marliéria	Minas Gerais	2	Negative
Phyllostomidae	*Uroderma magnirostrum*	Frugivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Gardnerycteris crenulatum*	Gleaner insectivorous-arthropods	Female	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Vampyressa pusilla*	Frugivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Phyllostomidae	*Macrophyllum macrophyllum*	Gleaner insectivorous-arthropods	Female	Adult	Marliéria	Minas Gerais	2	Negative
Phyllostomidae	*Micronycteris sanborni*	Gleaner insectivorous-arthropods	Female	Adult	Niquelândia	Goiás	1	Negative

Phyllostomidae	*Vampyressa pusilla*	Frugivorous	Female	Adult	Monte Belo	Minas Gerais	1	Negative
Phyllostomidae	*Vampyressa pusilla*	Frugivorous	Male	Adult	Caeté	Minas Gerais	1	Negative
Phyllostomidae	*Lonchorhina aurita*	Gleaner insectivorous-arthropods	NI	Adult	Canaã dos Carajás	Pará	1	Negative
Phyllostomidae	*Phyllostomus latifolius*	Omnivorous	NI	Adult	Canaã dos Carajás	Pará	1	Negative
Phyllostomidae	*Anoura caudifer*	Nectarivorous	NI	Adult	Canaã dos Carajás	Pará	1	Negative
Phyllostomidae	*Phyllostomus hastatus*	Omnivorous	NI	Adult	Canaã dos Carajás	Pará	1	Negative
Phyllostomidae	*Phyllostomus latifolius*	Omnivorous	NI	Adult	Canaã dos Carajás	Pará	2	Negative
Phyllostomidae	*Diphylla ecaudata*	Hematophagous	Female	Adult	Januária	Minas Gerais	1	Negative
Phyllostomidae	*Diphylla ecaudata*	Hematophagous	Female	Young	Januária	Minas Gerais	1	Negative
Phyllostomidae	*Anoura geoffroyi*	Nectarivorous	Male	Adult	Minduri	Minas Gerais	2	Negative
Phyllostomidae	*Anoura geoffroyi*	Nectarivorous	Male	Adult	Minduri	Minas Gerais	1	*Salmonella* spp. and *Staphylococcus aureus*
Phyllostomidae	*Anoura caudifer*	Nectarivorous	Female	Adult	Minduri	Minas Gerais	1	*Staphylococcus aureus*

Phyllostomidae	*Mimon bennettii*	Gleaner insectivorous-arthropods	Male	Adult	Paracatu	Minas Gerais	1	Negative
Phyllostomidae	*Micronycteris microtis*	Gleaner insectivorous-arthropods	Female	Adult	Monte Belo	Minas Gerais	1	Negative
Phyllostomidae	*Mimon bennettii*	Gleaner insectivorous-arthropods	Male	Adult	São Gonçalo do Rio Preto	Minas Gerais	1	Negative
Phyllostomidae	*Mimon bennettii*	Gleaner insectivorous-arthropods	Female	Adult	Mariana	Minas Gerais	1	Negative
Phyllostomidae	*Micronycteris megalotis*	Gleaner insectivorous-arthropods	Male	Adult	Mariana	Minas Gerais	1	Negative
Phyllostomidae	*Micronycteris microtis*	Gleaner insectivorous-arthropods	Male	Adult	Mariana	Minas Gerais	1	Negative
Phyllostomidae	*Platyrrhinus recifinus*	Frugivorous	Female	Adult	NI	NI	1	Negative
Phyllostomidae	*Glyphonycteris sylvestris*	Gleaner insectivorous-arthropods	Male	Adult	Mariana	Minas Gerais	1	Negative
Phyllostomidae	*Chiroderma doriae*	Frugivorous	Female	Adult	Mariana	Minas Gerais	1	Negative

Phyllostomidae	*Platyrrhinus lineatus*	Frugivorous	Male	Adult	Pains	Minas Gerais	1	Negative
Phyllostomidae	*Carollia perspicillata*	Frugivorous	Male	Adult	Pains	Minas Gerais	1	Negative
Phyllostomidae	*Artibeus planirostris*	Frugivorous	Male	Adult	Pains	Minas Gerais	1	Negative
Phyllostomidae	*Tonatia bidens*	Gleaner insectivorous-arthropods	Male	Adult	Pains	Minas Gerais	2	Negative
Phyllostomidae	*Carollia perspicillata*	Frugivorous	Female	Adult	Pains	Minas Gerais	1	Negative
Phyllostomidae	*Platyrrhinus lineatus*	Frugivorous	Male	Adult	Pains	Minas Gerais	1	Negative
Phyllostomidae	*Platyrrhinus recifinus*	Frugivorous	Male	Adult	Além Paraíba	Minas Gerais	1	Negative
Phyllostomidae	*Artibeus planirostris*	Frugivorous	Male	Adult	Doresópolis	Minas Gerais	1	Negative
Phyllostomidae	*Platyrrhinus lineatus*	Frugivorous	Female	Adult	Pains	Minas Gerais	1	Negative
Phyllostomidae	*Chiroderma villosum*	Frugivorous	Female	Adult	Pains	Minas Gerais	1	Negative

Phyllostomidae	*Glyphonycteris behnii*	Gleaner insectivorous-arthropods	Female	Adult	Doresópolis	Minas Gerais	1	Negative
Phyllostomidae	*Glossophaga soricina*	Nectarivorous	Male	Adult	Pains	Minas Gerais	1	Negative
Vespertilionidae	*Histiotus velatus*	Aerial insectivorous	Female	Adult	Lavras	Minas Gerais	2	Negative
Vespertilionidae	*Myotis riparius*	Aerial insectivorous	Female	Adult	Lavras	Minas Gerais	1	Negative
Vespertilionidae	*Neoeptesicus brasiliensis*	Aerial insectivorous	Female	Adult	João Monlevade	Minas Gerais	4	Negative
Vespertilionidae	*Histiotus velatus*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	2	Negative
Vespertilionidae	*Histiotus velatus*	Aerial insectivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Vespertilionidae	*Myotis levis*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative
Vespertilionidae	*Myotis levis*	Aerial insectivorous	Male	Adult	Marliéria	Minas Gerais	1	Negative
Vespertilionidae	*Myotis albescens*	Aerial insectivorous	Male	Adult	Marliéria	Minas Gerais	2	Negative
Vespertilionidae	*Myotis albescens*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	1	Negative

Vespertilionidae	*Myotis ruber*	Aerial insectivorous	Female	Adult	Muzambinho	Minas Gerais	1	Negative
Vespertilionidae	*Myotis albescens*	Aerial insectivorous	Male	Adult	Muzambinho	Minas Gerais	1	*Salmonella* spp.
Vespertilionidae	*Myotis nigricans*	Aerial insectivorous	Female	Adult	Monte Belo	Minas Gerais	3	Negative
Vespertilionidae	*Neoeptesicus furinalis*	Aerial insectivorous	Female	Adult	Mariana	Minas Gerais	1	Negative
Vespertilionidae	*Myotis levis*	Aerial insectivorous	Male	Adult	Mariana	Minas Gerais	2	Negative
Vespertilionidae	*Myotis albescens*	Aerial insectivorous	Female	Adult	Mariana	Minas Gerais	1	Negative
Vespertilionidae	*Myotis izecksohni*	Aerial insectivorous	Female	Adult	Mariana	Minas Gerais	1	Negative
Vespertilionidae	*Myotis nigricans*	Aerial insectivorous	Male	Adult	Mariana	Minas Gerais	1	Negative
Vespertilionidae	*Myotis ruber*	Aerial insectivorous	Male	Adult	Mariana	Minas Gerais	1	Negative
Vespertilionidae	*Myotis* sp.	Aerial insectivorous	Female	Adult	Lavras	Minas Gerais	1	Negative
Vespertilionidae	*Histiotus velatus*	Aerial insectivorous	Female	Adult	Minduri	Minas Gerais	1	Negative

Vespertilionidae	*Myotis riparius*	Aerial insectivorous	NI	Adult	Mariana	Minas Gerais	1	Negative
Vespertilionidae	*Lasiurus cinereus*	Aerial insectivorous	Female	Adult	Itabirito	Minas Gerais	1	Negative
Vespertilionidae	*Neoeptesicus furinalis*	Aerial insectivorous	Male	Adult	Minduri	Minas Gerais	1	Negative
Vespertilionidae	*Lasiurus cinereus*	Aerial insectivorous	Male	Adult	Piracicaba	São Paulo	3	Negative
Vespertilionidae	*Lasiurus blossevillii*	Aerial insectivorous	Male	Adult	São Paulo	São Paulo	1	Negative
Vespertilionidae	*Lasiurus blossevillii*	Aerial insectivorous	Female	Adult	Piracicaba	São Paulo	1	Negative

Table S2: Description of the primers used for research on each gene related to the zoonotic bacteria of interest at PCR from liver samples collected from bats in Brazil.

Pathogen	Gene	Primer direction	Primers sequences (5'-3')	PCR product size (bp)	Reference	Reference strain used as positive control
Coxiella burnetii	*IS1111*	F	TATGTATCCACCGTAGCCAGTC	686	Willems et. al, 1993; Lorenz et al., 1998 [1]	*Coxiella burnetii* CB1 (LISASC-UFLA collection)
		R	CCCAACAACACCTCCTTATTC			
Listeria monocytogenes	*hlyA*	F	AAATCTGTCTCAGGYGATGT	103	Barbau-Piednoir et al., 2013 [2]	*Listeria monocytogenes* ATCC 15313
		R	CGATGATTTGAACTTCATCTTTTGC			
Pasteurella multocida	*KMT1*	F	ATCCGCTATTTACCCAGTGG	457	Abbas et. al, 2018 [3]	*Pasteurella multocida* ATCC 12946
		R	GCTGTAAACGAACTCGCCAC			
Yersinia enterocolitica	*ail*	F	TAATGTGTACGCTGCGAG	351	Thoerner et al., 2003 [4]	*Yersinia enterocolitica* O:9 CLIST 3445 - YE 383
		R	GACGTCTTACTTGCACTG			
Rickettsia spp.	*gtlA*	F	GAGAGAAAATTATATCCAAATGTTGAT	147	Labruna et al. 2004 [5]	*Rickettsia vini* VPS-USP
		R	AGGGTCTTCGTGCATTTCTT			
Bartonella spp.	*gltA*	F	GCTATGTCTGCVTTCTATCAYGA	731	Rozental et al. 2017 [6]	*Bartonella henselae* Vector-Borne Bioagents Laboratory (VBBL)
		R	AGAACAGTAAACATTTCNGTHGG			
Salmonella spp.	*ompC*	F	ACCGCTAACGCTCGCCTGTAT	159	Kwang et al. 1996 [7]	*Salmonella* Typhimurium ATCC 14028
		R	AGAGGTGGACGGGTTGCTGCCGTT			
Leptospira spp.		F	GGAACTGAGACACGGTCCAT	430		

	16S rRNA	R	GCCTCAGCGTCAGTTTTAGG			
	LipL32	F	AAGAATGTCGGCGATTATGC	279	Tansuphasiri et al., 2006 [8]	*Leptospira interrogans* serovar Hardjo-prajitno LZB-USP
		R	CCAACAGATGCAACGAAAGA			
Staphylococcus aureus	*nuc*	F	AGTTCAGCAAATGCATCACA	400	Cremonesi et al., 2005 [9]	*Staphylococcus aureus* ATCC 25923
		R	TAGCCAAGCCTTGACGAACT			

Abbreviations: LISASC: Laboratórios Integrados de Sanidade Animal e Saúde Coletiva - Universidade federal de Lavras; ATCC: American Type Culture Collection; VPS-USP: Departamento de Medicina Veterinária Preventiva e Saúde Animal - Universidade de São Paulo; LZB-USP: Laboratório de Zoonoses Bacterianas - Universidade de São Paulo.

Table S3: Characterization of four samples excluded of Coleção de Mamíferos da Universidade Federal de Lavras bats according to family, species, feeding habit, sex, municipality, state, age, DNA concentration and relation A26/A280.

Family	Species	Feeding habit	Sex	Age category	Municipality	Statek	DNA concentration *	A260/A280
Molossidae	*Tadarida brasilienses*	Aerial insectivorous	Female	Adult	Lavras	Minas Gerais	6.4	1.11
Molossidae	*Molossus aztecus*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	-1.2	-0.41
Vespertiolionidae	*Myotis izecksohni*	Aerial insectivorous	Female	Adult	Marliéria	Minas Gerais	19.8	1.51
Phyllostomidae	*Glossophaga soricina*	Nectarivorous	Male	Adult	Marliéria	Minas Gerais	25.0	1.22

*****ng/ µL

Figures

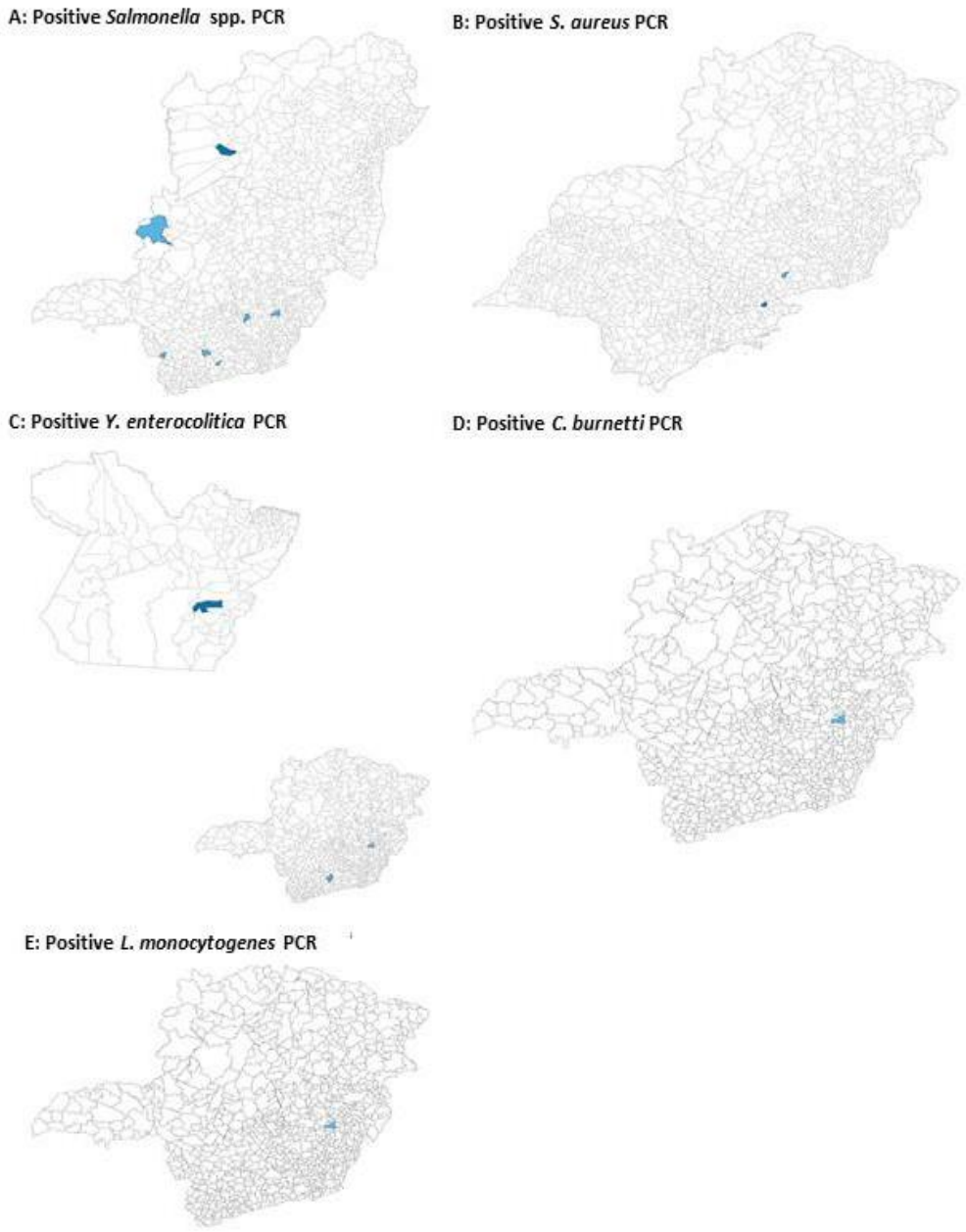


Figure S1: Location of the specimens by pathogen detected. A) Location of specimens positive for *Salmonella* spp. in the PCR by municipality Bahia: Santa Maria da Vitória (1); Minas Gerais: Caeté (1), Lavras (1), Marliéria (1), Minduri (1), Muzambinho (1) and Unaí (1). B) Location of specimens positive for *S. aureus* in the PCR by São Paulo: Piquete (1); Minas Gerais: Minduri (1). C) Location of specimens positive for *Y. enterocolitica* in the PCR by Pará: Parauapebas (1); Minas Gerais: Carrancas(1), Marliéria (2), Minduri (1). D) Location of specimens positive for *Coxiella burnetti*. in the PCR by Minas Gerais: Marliéria (1). E) Location of specimens positive for *L. monocytogenes* in the PCR by Minas Gerais: Marliéria (1).

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ARTICLE 2

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Detection of zoonotic bacterial pathogens in bats from vereda ecosystems in the Brazilian Cerrado

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ABSTRACT

Bats can harbor a wide diversity of zoonotic bactéria, yet information from Neotropics remains limited. This study aimed to investigate the ocurrence of zoonotic bacterial pathogens (*Salmonella* spp., *Leptospira* spp., *Bartonella* spp., *Yersinia enterocolitica*, *Staphylococcus aureus*, *Rickettsia* spp., *Pasteurella multocida*, *Coxiella burnetii*, *Brucella* spp., *C. psittaci* and *Listeria monocytogenes*) in bats sampled in areas of veredas, in the Brazilian Cerrado. DNA was extracted from blood, spleen and liver samples from 172 bats and screned for the presence of the described pathogens using PCR and qPCR techniques. Overall, 18.0% (31/172) bats positive for at least one pathogen. The most frequently detected agents were *Brucella* spp. 5.8% (10/172), followed by *L. monocytogenes*, *Y. enterocolitica* and *Salmonella* spp. (3.5% each), *C. burnetii* and *Bartonella* spp. (1.2% each) and *S. aureus* (0.6%). also detected in lower frequencies. Interaction network analyses showed differences between preserved and degraded veredas. The degraded vereda exhibited higher modularity than expected by null models, indicating a more compartmentalized host–pathogen interaction structure, whereas the preserved vereda showed a more concentrated network dominated by Phyllostomidae bats and fewer bacterial taxa. These findings reinforce the potential role of bats in the maintenance and circulation of zoonotic bacterial pathogens in wildlife populations. The results reinforce the importance of integrating ecological approaches into wildlife disease surveillance within the One Health framework.

Keywords: Chiroptera; Bacterial pathogens; Brazilian Cerrado; Molecular detection; One Health; Wildlife surveillance

INTRODUCTION

Habitat degradation can alter wildlife communities and reshape host–pathogen interactions, potentially influencing the circulation and maintenance of zoonotic agents in natural ecosystems (Gibb et al. 2020, Pei et al. 2025). Understanding how environmental disturbance affects pathogen dynamics in wildlife has become increasingly important within the One Health framework, particularly in biodiversity-rich tropical regions undergoing rapid environmental change (Kurtieva et al. 2025). Among wild animals, bats (order Chiroptera) stand out as important reservoirs of a wide diversity of microorganisms, including viruses, bacteria, fungi, and protozoa, some of which are associated with diseases in humans and other animals (Mühldorfer 2013, Beltz 2017, Vieira et al. 2022, Ferreira et al. 2024). Their high mobility, social behavior, longevity, and ability to occupy diverse ecological niches may facilitate the maintenance and circulation of pathogens across ecosystems (Calisher et al. 2006, Dhivahar et al. 2023).

The association between bats and zoonotic pathogens has been recognized for more than a century, since the first identification of a Lyssavirus in bats (Carini 1911). In recent decades, epidemics and pandemics, including coronavirus disease 2019 (COVID-19), have reinforced the role of these animals in the dynamics of infectious diseases, particularly in the context of emerging pathogens (Calisher et al. 2006, Smith and Wang 2013, Wacharapluesadee et al. 2021). Although most studies have focused on viral pathogens, growing evidence indicates that bats also harbor a significant diversity of bacteria with zoonotic potential, highlighting the need for further investigation of these microorganisms (Ferreira et al. 2021, Szentivanyi et al. 2023, Ferreira et al. 2024).

Indeed, bats exhibit several ecological and biological traits that favor their role in the maintenance and circulation of microorganisms (Calisher et al. 2006, Beltz 2017, Dhivahar et al. 2023). They represent one of the most diverse orders of mammals and display a wide range of feeding habits, including carnivorous, frugivorous, nectarivorous, insectivorous, and hematophagous species (Kunz et al. 2011). In addition, their broad geographic distribution, social behavior, longevity, and ability to occupy diverse ecological niches contribute to the maintenance and dissemination of microorganisms, including potential pathogens (Mühldorfer 2013, Brook and Dobson 2015, Ferreira et al. 2024).

However, while some characteristics may facilitate their involvement in the transmission cycles of zoonotic agents, bats also perform essential ecological functions,

such as insect population control, pollination, and seed dispersal, contributing directly to biodiversity maintenance and ecosystem stability (Teeling et al. 2009, Kunz et al. 2011). This dual role underscores the complexity of bats within a One Health framework, highlighting the need for an integrated approach that considers both the risks and benefits associated with these animals.

Furthermore, understanding how environmental conditions influence pathogen circulation in bat populations is essential, as these interactions may vary across ecosystems and biomes. In this context, Brazil harbors a wide diversity of biomes, including the Amazon, Atlantic Forest, Cerrado, Caatinga, Pantanal, and Pampa, each characterized by distinct climatic and ecological conditions that can shape host–pathogen dynamics (IBGE, 2019). Among these, the Cerrado stands out as one of the most biodiverse savanna regions in the world, supporting a rich and diverse bat fauna (Ribeiro et al. 2008).

Within the Cerrado, veredas represent unique wetland ecosystems that function as ecological corridors, connecting vegetation fragments and promoting biodiversity (Drummond et al. 2005, Ribeiro et al. 2008). These environments provide important resources such as water, shelter, and food availability, which support highly diverse bat assemblages (Ribeiro et al. 2008, Kunz et al. 2011). However, anthropogenic pressures, including agricultural expansion and habitat fragmentation, may alter the ecological dynamics of these populations and influence pathogen circulation, with potential implications for environmental and public health (Meirelles et al. 2004, Bahia et al. 2009).

Despite their epidemiological importance, bats also provide essential ecological functions, including pollination, seed dispersal, and insect population control, contributing directly to ecosystem functioning and biodiversity maintenance (Kunz et al. 2011, Ramírez-Fráncel et al. 2022). Therefore, understanding pathogen circulation in bat populations requires an ecological perspective that integrates host diversity, environmental conditions, and species interactions (Zemanová et al. 2019). In this context, ecological network approaches have emerged as powerful tools for investigating host–pathogen relationships, allowing the identification of interaction patterns, modularity, and potential effects of environmental disturbance on pathogen circulation dynamics (Alcantara et al. 2023). In this scenario, studies focused on the detection of bacterial pathogens are critical to elucidate the role of bats as reservoirs and the influence of ecological factors on these processes. Therefore, this study aimed to investigate the

occurrence of zoonotic bacterial pathogens in bats sampled from preserved and degraded vereda ecosystems in the Brazilian Cerrado using PCR-based molecular techniques. In addition, complementary network analyses were performed to explore host–pathogen interaction patterns in these environments.

MATERIAL AND METHODS

Ethics statement

The procedures were carried out in accordance with the ethical principles of animal experimentation (process number 237 of the ethics committee on experimentation and animal welfare of the Universidade Estadual de Montes Claros), and the collection license was provided by the Sistema de Autorização e Informação em Biodiversidade (SISBIO 19037-1). The license for authorization and carrying out scientific research in the State of Minas Gerais was provided by the Instituto Estadual de Florestas (IEF), through registration number 056/2021.

Study area and sampling

Sampling was conducted in two areas included in the Long-Term Ecological Research Program PELD-Veredas, located in the northern region of Minas Gerais, within the Brazilian Cerrado (Figure 1). The first area was the Reserva Particular do Patrimônio Natural Porto Cajueiro (Porto Cajueiro Private Natural Heritage Reserve, RPPN Porto Cajueiro), located within the Área de Proteção Ambiental Cochá e Gibão (Cochá and Gibão Environmental Protection Area), in the municipality of Januária. This reserve includes well-preserved vereda ecosystems, characterized by hygrophilous vegetation associated with permanently or seasonally waterlogged soils and dominated by the palm *Mauritia flexuosa*. These environments play a key ecological role in maintaining hydrological balance, acting as natural water regulators and supporting high biodiversity (Ávila et al. 2016, Nunes et al. 2022).

The second area was the Parque Estadual Veredas do Peruaçu (Veredas do Peruaçu State Park, PE Veredas do Peruaçu), located between the municipalities of Januária and Cônego Marinho. In contrast to the RPPN Porto Cajueiro, veredas in this region show signs of environmental degradation, particularly associated with the drying

process driven by land-use changes and climate variability (Araújo et al. 2024). This process leads to reduced groundwater levels, alterations in vegetation structure, and the encroachment of typical savanna species into previously wetter environments, resulting in changes in community composition and ecological interactions (de Araújo and Silveira 2024).

Two sampling campaigns were carried out in each study area. The first campaign took place in April 2023 (rainy season), and the second in October 2023 (dry season). In each area, polyester mist nets (9 × 3 m, 20 mm mesh) were set in the understory, spaced 800 m to 1 km apart. Nets were positioned along pre-existing trails, forest clearings, or near flowering and fruiting plants (Kurta and Kunz 1988, Novaes and Laurindo 2014), and remained open for five hours after sunset (6:00 p.m. to 11:00 p.m.). Sampling effort was calculated according to (Straube and Bianconi 2002). Captured bats were placed in cotton bags and subsequently processed (Tavares et al. 2021). All researchers used appropriate personal and collective protective equipment to ensure biosafety. Individuals were identified based on morphological characteristics (Reis et al. 2017, Díaz et al. 2021), with *Myotis* and *Molossus* species identified following Novaes et al. 2022 and Díaz, Solari et al. 2021, respectively. Taxonomic classification followed Garbino et al. (2020), except for the genus *Neoeptesicus*, which followed Cláudio et al. 2023. For each individual, sex, age class, reproductive status, body mass, and forearm length were recorded. Age was determined based on the ossification of phalangeal epiphyses (Anthony 1988). Reproductive status was assessed by external examination, classifying females as pregnant, lactating, post-lactating, or non-reproductive, and males as scrotal or non-scrotal. Pregnant or lactating females, as well as those carrying young, were released. The remaining individuals were sampled for pathogen bioprospecting. The animals were anesthetized with ketamine hydrochloride intramuscularly in the pectoral muscle for blood sample extraction by cardiac puncture. Subsequently, euthanasia was performed with an overdose of anesthetic, and fragments of spleen and liver were collected from each bat. The samples were stored with RNAlater® (Qiagen, Germany) and preserved at -20 °C.

DNA extraction

The DNA of the samples was extracted using the “High Pure PCR Tamplate Preparation Kit” (Roche®, Germany), following the manufacturer's standards. To verify

the quality and concentration of the extracted DNA, samples were analyzed in a spectrophotometer (Nanodrop®, Thermo Fisher Scientific, USA). Low-quality samples that could not be re-extracted were excluded from the analysis. The remaining samples were stored at -20°C.

Molecular detection of pathogens

Conventional PCR assays were used to screen samples for *Salmonella* spp. (*ompC*), *Leptospira* spp. (*16S rRNA* and *lipL32*), *Bartonella* spp. (*gltA*), *Yersinia enterocolitica* (*ail*), *Staphylococcus aureus* (*nuc*), *Rickettsia* spp. (*gltA*), *Pasteurella multocida* (*kmt1*), and *Coxiella burnetii* (*IS1111*). Real-time quantitative PCR (qPCR) assays were performed to detect *Listeria monocytogenes* (*hlyA*), *Chlamydia psittaci* (*envB*), and *Brucella* spp. (*IS711*). Details of primers, positive controls, amplicon sizes, and references are provided in Table 1. Ultrapure sterile water was used as a negative control in all reactions.

The conventional PCR were carried out in a standard thermal cycler T960 touch (Heal Force, China) and the products were visualized by electrophoresis on 1.5% agarose gels stained with (0.5 mg/mL) ethidium bromide, using Tris–borate–EDTA (TBE) buffer (89 mM Tris base, 89 mM boric acid, and 2 mM EDTA, pH 8.0). A 100 bp DNA ladder (Sinapse Inc., São Paulo, Brazil) was included as a molecular marker in all assays. Gel images were captured using a transilluminator (L-Pix Chemi Photo Digitizer, Loccus Biotecnologia, Brazil) for subsequent analysis.

For real-time PCR, reactions were prepared using the SYBR Green qPCR Master Mix (Cellco, Brazil) for *Listeria monocitogenes*, Green Master qPCR Master Mix (Cellco, Brazil) for *C. psittaci*, and qPCR Probes Master (Cellco, Brazil) for *Brucella* spp.. The run was performed on the CFX OPUS 96 system (Bio-Rad, EUA).

Statistical analyses

A multivariable logistic regression model was used to investigate the association between independent variables (family, genus, species, feeding habit, sampling season, sex, age, and collection site) and the outcome (PCR results for selected bacterial pathogens), aiming to identify factors potentially associated with pathogen occurrence

(Dohoo et al. 2009). Initially, Fisher's exact test was applied to assess the association between the dependent variable (PCR results) and each independent variable. Variables with a p-value ≤ 0.20 were selected for inclusion in the multivariable logistic regression model (Dohoo et al. 2009). The fitting of the models was assessed by considering the likelihood ratio of Fisher test result and its p-value, comparing the adjust of the complete model to its null model, being considered significant when p-value was < 0.05 (Dohoo et al. 2009). Model performance was assessed based on predictive ability (sensitivity and specificity) and goodness-of-fit using the Pearson chi-square (χ^2) test, when applicable (Dohoo et al. 2009). Statistical analyses were performed using Stata version 19.

Network analyses

Interaction networks between bats and bacterial taxa were constructed separately for each study area using a binary bipartite matrix, in which rows represented bat species and columns represented bacterial taxa. A matrix was constructed for each protected area, with one matrix for the RPPN Porto Cajueiro and another for the PE Veredas do Peruaçu. At the network level, we quantified specialization using the complementary specialization index H2′, which is robust to differences in network size and sampling effort (Blüthgen et al. 2006). Modularity was assessed using a likelihood-based algorithm implemented in the function *computeModules*, which identifies compartments of tightly interacting species within bipartite networks (Dormann and Strauss 2014). To evaluate whether the observed network structure differed from random expectations, we generated 500 null models using the swap algorithm (*swap.web*), which preserves species degree (i.e., the number of interactions per species) while randomizing interaction identities. This method is particularly appropriate for binary networks because it controls for differences in interaction breadth without assuming abundance distributions (Dormann et al. 2009). For each network descriptor (H2′ and modularity), observed values were compared to null distributions, and statistical significance was assessed using two-tailed permutation tests based on the proportion of null values as extreme as or more extreme than the observed metric. In addition, we calculated basic structural properties of each network, including the richness of bat species (S_hosts), richness of bacterial taxa (S_parasites), total network size (S_total), and the number of realized interactions (links, L), following standard practices in ecological network analysis (Dunne et al. 2002).

A multilayer Sankey diagram was constructed for each area to represent bat–parasite interactions across hierarchical variables, including sampling season (rainy and dry), bat family, bat species, biological sample type (spleen and blood), and detected bacterial taxa. Interaction records were aggregated and converted into flow frequencies, which were used to define the width of connections between nodes. This approach allowed the visualization of the relative contribution of each category and the identification of dominant interaction pathways across ecological and sampling dimensions. This figure and the others were created using the R software (version 4.3.3).

RESULTS

A total of 172 bats were captured, representing 3 families, 12 genera, and 17 species. Among the families, Phyllostomidae was the most represented [79.7% (137/172)], followed by Molossidae [15.1% (26/172)] and Vespertilionidae [5.2% (9/172)]. At the genus level, the most abundant were *Platyrrhinus* [41.9% (72/172)], *Glossophaga* [20.3% (35/172)], *Artibeus* [11.6% (20/172)], *Molossops* [9.9% (17/172)], *Molossus* [5.2% (9/172)], *Histiotus* [2.9% (5/172)], *Carollia* [2.3% (4/172)], and *Gardnerycteris* [1.7% (3/172)], while *Lophostoma*, *Myotis*, and *Neoeptesicus* were each represented by a single individual [0.6% (1/172)]. At the species level, *Platyrrhinus lineatus* was the most frequent species [41.3% (71/172)], followed by *Glossophaga soricina* [20.3% (35/172)], *Molossops temminckii* [9.9% (17/172)] and *Artibeus lituratus* [6.4% (11/172)]. Figure 2 shows the distribution of all species and their respective frequences.

Most individuals were males [64.5% (111/172)] and adults [97.1% (167/172)]. Most bats were captured in the RPPN Porto Cajueiro [66.7% (115/172)] whereas the remaining individual were capture in in PE Vereda Peruaçu [33.1% (57/172)]. Similar number of individuals were captured during the dry season [51.2% (88/172)] and the rainy season [48.8% (84/172)]. Most bats were frugivorous [55.8% (96/172)], followed by insectivorous [23.3% (40/172)] and nectarivorous species [20.3% (35/172)], while only one bat was classified as omnivorous [0.6% (1/172)]. Supplementary Material Table S1 provides detailed information for all captured bats.

From 172 bats, a total of 313 biological samples were obtained, including blood [50.6% (87/172)], spleen [84.9% (146/172)], and liver [46.5% (80/172)]. Only one individual provide all three sample types [0.6% (1/172)]. Figure 3 presents a Venn

diagram illustrating the distribution and overlap of biological sample types (blood, spleen, and liver) among sampled individuals. Supplementary Table S1 summarizes the results for all analyzed specimens.

A total of 31 bats tested positive for at least one bacterial pathogen, representing 18.0% (31/172) of all sampled individuals. Among the positive bats, most belonged to the family Phyllostomidae [80.6% (25/31)], followed by Molossidae [16.1% (5/31)], and a single specimen of Vespertilionidae [3.2% (1/31)], corresponding 14.5%, 2.9%, and 0.6% of the total 172 bats sampled, respectively. At the genus level, pathogens were detected in 9 out of 11 genera [81.8% (9/11)], including *Platyrrhinus* [32.3% (10/31)], *Glossophaga* [29.0% (9/31)], *Molossops* [12.9% (4/31)], *Artibeus* [9.7% (3/31)], and *Carollia*, *Gardnerycteris*, *Histiotus*, *Molossus*, and *Phyllostomus* [3.2% (1/31)] each. At the species level, pathogens were detected in 11 out of 17 species [64.7% (11/17)]. The most frequently detected species were *Platyrrhinus lineatus* [32.3% (10/31)] and *Glossophaga soricina* [29.0% (9/31)], followed by *Molossops temminckii* [12.9% (4/31)], *Artibeus lituratus* [6.5% (2/31)], and *Artibeus cinereus*, *Carollia brevicauda*, *Gardnerycteris crenulatum*, *Histiotus diaphanopterus*, *Molossus molossus*, and *Phyllostomus discolor* [3.2% (1/31)] each.

Most positive individuals were males [58.1% (18/31)] and adults [96.8% (30/31)]. Positive bats were detected at two sampling areas, with most captured in RPPN Porto Cajueiro [54.8% (17/31)] followed by PE Vereda Peruaçu [45.2% (14/31)]. Regarding seasonality, the number of positive individuals was similar between the dry and rainy seasons [54.8% (17/31)]. In relation to feeding habits, most were frugivorous [45.2% (14/31)], followed by nectarivorous [29.0% (9/31)] and insectivorous species [22.6% (7/31)], while only one positive bat was classified as omnivorous [3.2% (1/31)].

Regarding pathogen detection, 63.6% of the targeted pathogens were identified [63.6% (7/11)]. The most frequently detected agent was *Brucella* spp. [5.8% (10/172)], followed by *L. monocytogenes*, *Y. enterocolitica* and *Salmonella* spp. [3.5% (6/172)] each, *C. burnetii* and *Bartonella* spp. [1.2% (2/172)] each, and *S. aureus* [0.6% (1/172)]. The Figure 4 summarizes the characteristics of the positive individuals. Among positive bats, two individuals presented coinfections involving different pathogens. In addition, in two individuals, the same pathogen (*Y. enterocolitica*) was detected in more than one biological sample. Therefore, the total number of pathogen detections exceeds the number

of positive individuals, as some detections corresponded to coinfections or repeated detection of the same pathogen in different issues. Epidemiological data for these individuals are presented in Table 2.

The frequencies of positive samples for *Salmonella* spp., *Y. enterocolitica*, *Brucella* spp., *C. burnetti*, *L. monocitogenes* and *Bartonella* spp. were used as dependent variables in logistic multivariate models. These models were not tested for *S.* aureus because of the very low frequency of positives (1 for each). However, Fisher's exact test identified at most one independent variable associated with the majority of the tested pathogens ($p \leq 0.20$), which precluded the construction of reliable multivariable models. For *Bartonella* spp., the variables species and genus were associated ($p \leq 0.20$); however, due to collinearity between these variables, fitting a multivariable model including both was not appropriate. These results are presented in Table 3.

Interaction networks differed between the two veredas in both composition and structural organization (Table 4). The RPPN Porto Cajueiro network comprised seven bat species and four bacterial taxa (S_total = 11), while the PE Veredas do Peruaçu network included six bat species and seven bacterial taxa (S_total = 13). Despite these differences in richness, both networks exhibited the same number of realized interactions (L = 11), with RPPN Porto Cajueiro showing higher host richness and PE Veredas do Peruaçu higher parasite richness.

Modularity analyses revealed contrasting patterns between areas (Table 4). In RPPN Porto Cajueiro, the observed modularity (Q = 0.413) was virtually identical to that expected under the null model (mean = 0.412; p = 1.000). In contrast, the PE Veredas do Peruaçu network exhibited higher modularity (Q = 0.595), significantly greater than the null expectation (mean = 0.541; p = 0.012), suggesting a more compartmentalized interaction structure. The network of the PE Veredas do Peruaçu exhibited a more diverse and distributed pattern, involving multiple bat families, tissues, and a greater variety of bacterial taxa (Figure 4). In contrast, the RPPN Porto Cajueiro showed a more concentrated network, dominated by Phyllostomidae, with interactions mainly associated with spleen samples and lower parasite diversity.

DISCUSSION

Understanding the role of bats as reservoirs of zoonotic bacteria is essential for characterizing the dynamics of infectious diseases at the wildlife-human interface

between wildlife and humans. From a One Health perspective, this interface is intrinsically linked to environmental interactions, which play a fundamental role in the transmission and maintenance of pathogens (Shaheen 2022). In this context, the vereda ecosystems represent unique wetlands in the Brazilian Cerrado, characterized by high biodiversity, ecological connectivity, and resource availability (Tubelis 2009, Alencar and Ferreira 2016, Alves et al. 2026). However, environmental degradation may alter these ecological interactions and potentially influence pathogen circulation dynamics in wildlife populations. These conditions can favor interactions between hosts, pathogens, and the environment, potentially influencing the circulation of microorganisms. Therefore, investigating the occurrence of bacterial pathogens in bats inhabiting these ecosystems is essential to assess the potential risks to public and environmental health.

Indeed, the detection of multiple pathogens in bats sampled in both the PE Veredas do Peruaçu and RPPN Porto Cajueiro, including *Brucella* spp., *L. monocytogenes*, *Y. enterocolitica*, *Salmonella* spp., *Bartonella* spp., *C. burnetii* and *S. aureus*, underscores the potential of bat populations inhabiting these regions to harbor zoonotic bacterial pathogens. Furthermore, the overall positivity rate of 18.0%, together with findings reported in previous studies (Ferreira et al. 2021, Ferreira et al. 2024, Ferreira et al. 2025), reinforces the growing evidence that bats can play an important role in maintaining bacterial agents. These findings contribute to a better understanding of the role of the role of bats in the epidemiological cycle of zoonotic diseases and emphasize the importance of wildlife surveillance within a One Health framework.

Among the detected pathogens, *Brucella* spp. showed the highest frequency, being identified in different bat species and sampling sites. The role of bats in the epidemiology of brucellosis remains poorly understood. However, evidence of *Brucella* detection of in bats has already been reported in different geographic regions, such as Georgia, Costa Rica and Brazil, suggesting that these animals may play an important role in the maintenance of *Brucella* species in wildlife environments (Bai et al. 2017, Hernández-Mora et al. 2023, Silva et al. 2023). In Brazil, antigenic and molecular evidence of *Brucella* spp. was already reported in *Artibeus lituratus* and *Glossophaga soricina*, the same species detected in the present study, although with detection occurred epididymal samples (Silva et al. 2023). Likewise, a molecular survey conducted in Georgia detected *Brucella* DNA in spleen samples, similarly to the predominant sample type identified in the present study, although involving different bat species (Bai et al. 2017). In this

context, the detection of *Brucella* DNA in internal organs may suggest systemic dissemination associated with inflammatory manifestations in these animals. Although no clinical or pathological alterations were evaluated in the present study, previous reports have described reproductive lesions associated with Brucella infection in these animals, including placentitis and epididymo-orchitis. epididimo-orquite (Hernández-Mora, Chacón-Díaz et al. 2023, Silva et al. 2023). These findings raise concerns regarding the potential role of bats in the epidemiological cycle of brucellosis and reinforce the need to consider wildlife in surveillance and control programs targeting this disease.

The detection of enteric pathogens such as *Salmonella* spp., *Y. enterocolitica*, and *L. monocytogens* was another noteworthy finding, particularly because these agents were detected in tissues different from their preferential colonization sites, including blood and spleen samples. The detection of these agents in the present study corroborates previous reports in the literature and reinforces the ability of bats to harbor these bacterial agents (Mühldorfer et al. 2010, Ferreiraet al. 2021, Ferreira et al. 2025). In fact, a recent study also conducted on bats from Minas Gerais in 2025, reported the detection of these pathogens in liver samples from different bat species with feeding habits similar to those identified in the present study, especially insectivorous and frugivorous species (Ferreira et al. 2025). Once again, it is important to emphasize that the detection of these pathogens outside their preferred colonization sites, particularly in blood and spleen samples, may suggests bacteremia with the possibility of systemic infection, supporting the potential importance of bats for public health in the epidemiological cycle of diseases caused by these pathogens.

Particularly for *Y. enterocolitica* the pathogen was detected in both blood and spleen samples from the same individual in two specimens, one *G. soricina* and one *P. lineatus*, representing an interesting finding. All animals included in this study were captured during active flight using mist nets, suggesting that these animals were in good health condition, since flight is an high energetically demanding activity for bats. In this context, an important point raised by (Dhivahar et al. 2023) is the remarkable ability of bats to modulate their immune system without exhibiting overt clinical manifestations of disease. This characteristic has been highlighted in several studies (Subudhi et al. 2019, Banerjee et al. 2020, Seltmann et al. 2022, Dhivahar et al. 2023), which suggest reduced production of pro-inflammatory cytokines, such as TNF-α, leading to suppression of inflammatory responses. Because flight requires elevated metabolic rates and

consequently generates high levels of reactive oxygen species, immune suppression may represent an adaptive strategy to reduce oxidative stress and maintain physiological balance, given that inflammatory responses are energetically costly.

Another interesting finding involving enteric pathogens was the occurrence of coinfection with *Brucella* spp. in two individuals of *G. soricina*. In one individual, *Y. enterocolitica* was also detected in the spleen; whereas in other individual, *Brucella* spp. was detected in the blood and *L. monocytogenes* in the spleen (Table 2). Considering the feeding behavior of *G. soricina*, a primarly nectarivorous species with opportunistic habits, it is plausible that different food sources may represent routes of exposure to multiple pathogens derived from potentially contaminated shared resources.

In relation to the other detected pathogens, *C. burnetii*, *Bartonella* spp., and *S. aureus*, were also observed. Although detected at low frequencies the presence of theses pathogens is still epidemiologically relevant, especially considering the zoonotic potential associated with these bacterial agents. The detection of *C. burnetii* in the present study adds to previous reports from Minas Gerais, confirming the presence of this pathogen in bats from this Brazilian state (Ferreira et al. 2025). Likewise, the detection of *Bartonella* spp. corroborates previous studies that have already described this pathogen in liver and spleen samples from different bats in different Brazilian states (Ferreira et al. 2018, Andre et al. 2019). Regarding *S. aureus*, this represents the second report of the detection of this pathogen in bat liver samples, contributing to the understanding of the ability of this opportunistic pathogen to colonize bat organs. These findings reinforce the diversity of zoonotic bacterial agents that may circulate in bat populations and highlight the importance of continuous surveillance of wildlife species.

The non-detection of pathogens such as *Leptospira* spp., *Rickettsia* spp., *C. psittaci*, and *P. multocida* may suggest the absence or low circulation of these pathogens in the studied regions. However, this interpretation should be made with caution, since other sample types are considered more suitable for the detection of these pathogens, such as kidney and urine samples for *Leptospira* spp., and respiratory tract samples for *C. psittaci* and *P. multocida*, which are commonly associated with respiratory colonization and infection. (Ferreira et al. 2021, Yehia et al. 2023, Smallman et al. 2024). In addition, another factor that may have contributed to the negative results obtained by conventional PCR is the lower sensitivity of this technique compared to more sensitive molecular

approaches, such as qPCR, particularly in biological samples with potentially low concentrations of pathogen DNA.

Unfortunately, there were no statistical associations that allowed further analyses using a multivariable logistic model. Although relevant, the low detection frequency for each pathogen and the limited number of sampled animals may have contributed to the impossibility of advancing with the multivariable logistic analysis, representing a limitation of the present study. On the other hand, it is important to highlight that studies involving wild animals face ethical, conservation, and economic implications, including the high costs associated with field sampling, which often limit the inclusion of a large number of animals (Plowright et al. 2019). Even so, these studies remain extremely valuable, especially for advancing the understanding of pathogen circulation and wildlife participation within the One Health context.

Despite these limitations, the network analyses provided important insights into the ecological interactions between bats and bacterial pathogens in the studied vereda ecosystems. The interaction networks differed between the preserved and degraded veredas, suggesting that local environmental characteristics may influence the circulation and organization of host–pathogen interactions. While the RPPN Porto Cajueiro network exhibited a more concentrated interaction pattern, mainly associated with Phyllostomidae and spleen samples, the PE Veredas do Peruaçu network showed a more distributed and compartmentalized structure, involving multiple bat families, tissues, and a greater diversity of bacterial taxa. In addition, the significantly higher modularity observed in the degraded vereda of PE Veredas do Peruaçu, together with the greater richness of bacterial taxa, may suggest that environmental disturbance influences the organization and compartmentalization of host–pathogen interactions. Together, these findings reinforce the complexity of pathogen circulation dynamics in wildlife populations and highlight the importance of integrating ecological approaches into One Health surveillance studies.

CONCLUSION

This study demonstrate the occurrence of zoonotic bacterial pathogens in bats inhabiting vereda ecosystems in Minas Gerais, Brazil, reinforcing the potential role of these animals in the maintenance and circulation of bacterial agents of public health importance. In addition, the differences observed between preserved and degraded veredas suggest that environmental characteristics may influence host–pathogen

interaction dynamics in wildlife populations. By combining molecular screening with ecological network analyses, this study expands current knowledge on bacterial circulation in Neotropical bats and highlights the value of integrating ecological approaches into wildlife disease surveillance. Together, these findings contribute to the understanding of bacterial pathogen circulation in bats and highlight the importance of integrating ecological approaches and wildlife surveillance within the One Health framework.

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STATEMENTS & DECLARATIONS

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COMPETING INTERESTS

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

ACRF: Conceived and designed the study, analyzed data, and wrote the manuscript. BAA, LFC, MESTC, LAG, MCJA and BA: performed research and analyzed data. WSA: analyzed data and supervised the writing of the manuscript. YRFN, RLMN, TMV and EMSD: designed the research and supervised the writing of the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY

All data supporting the findings of this study are available within the article and its Supplementary Information files.

ETHICS APPROVAL

All procedures were approved by the Ethics Committee on Animal Experimentation and Welfare of the Universidade Estadual de Montes Claros (process no. 237). Collection and research activities were authorized by SISBIO (19037-1) and by the Instituto Estadual de Florestas (IEF; registration no. 056/2021).

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FIGURES

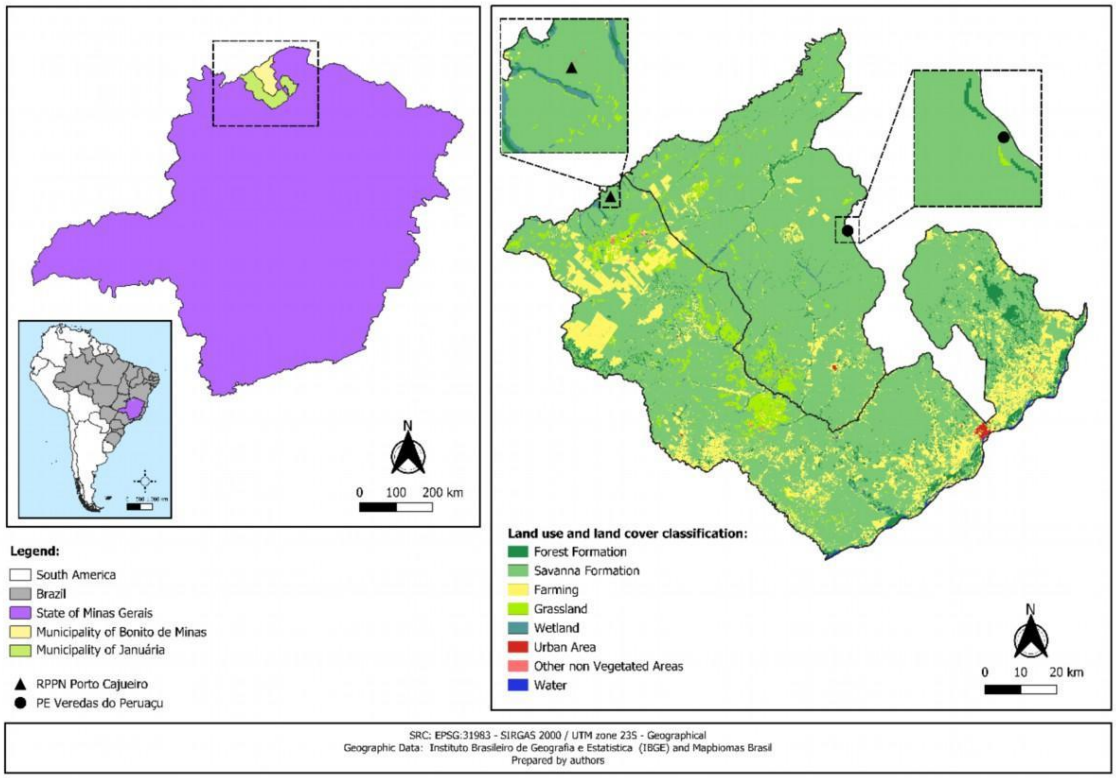
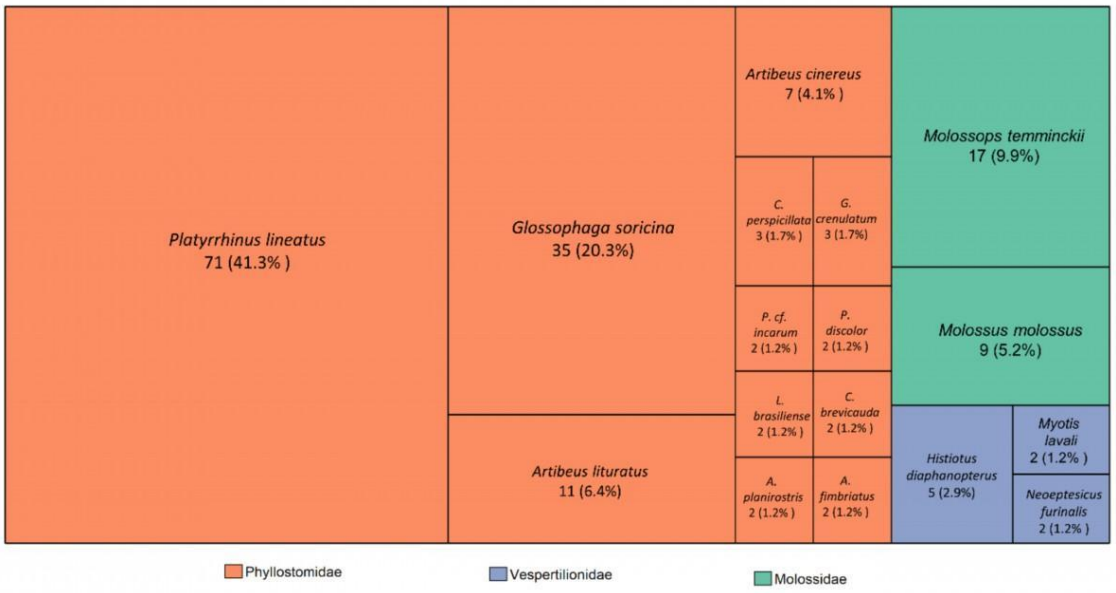


Figure 1: Location of the study areas within the Brazilian Cerrado in northern Minas Gerais, Brazil. Sampling was conducted in two vereda ecosystems: the preserved vereda located in the Reserva Particular do Patrimônio Natural Porto Cajueiro (RPPN Porto Cajueiro), municipality of Januária, and the environmentally degraded vereda located in the Parque Estadual Veredas do Peruaçu (PE Veredas do Peruaçu), between the municipalities of Januária and Cônego Marinho.



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753 **Figure 2:** Distribution of bat species captured in the study area. Bars represent the
754 number of individuals per species, grouped by family, with colors indicating the
755 corresponding taxonomic families.

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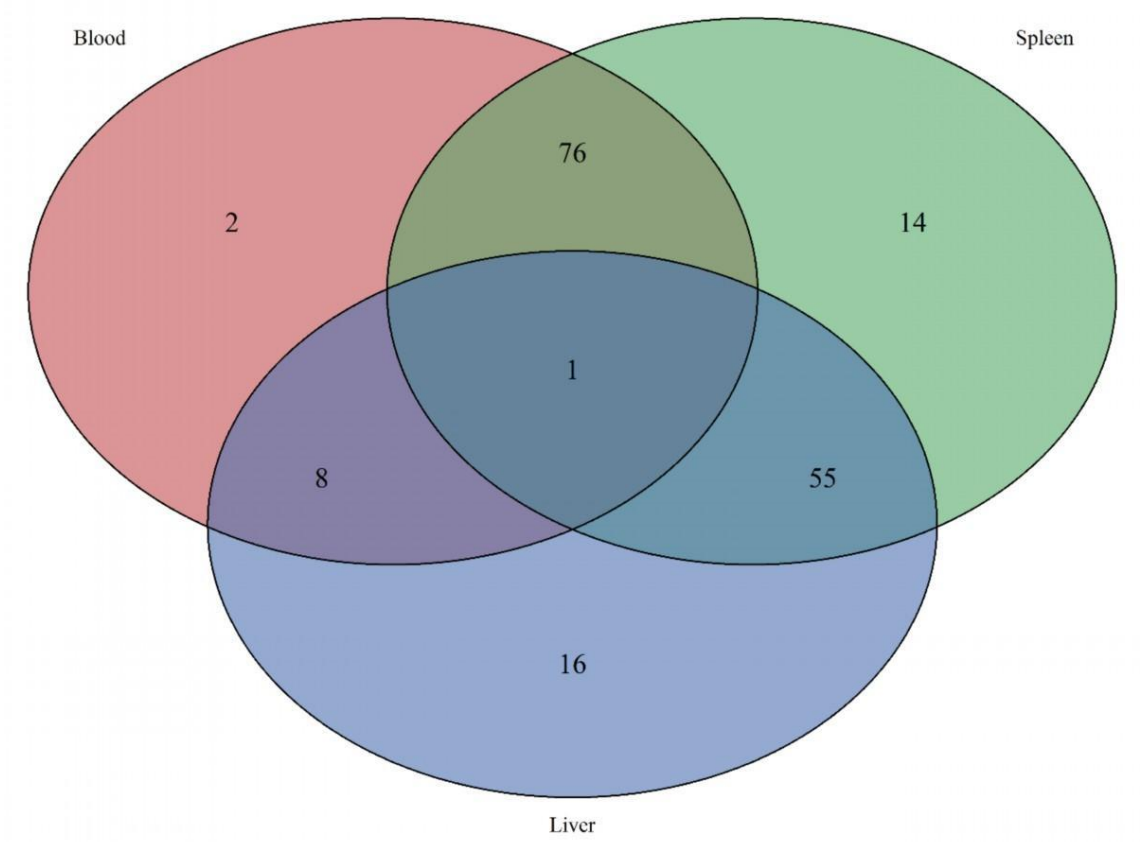

Figure 3: Venn diagram illustrating the distribution and overlap of biological sample types (blood, spleen, and liver) obtained from bats. The diagram shows the number of individuals with each sample type and their combinations.

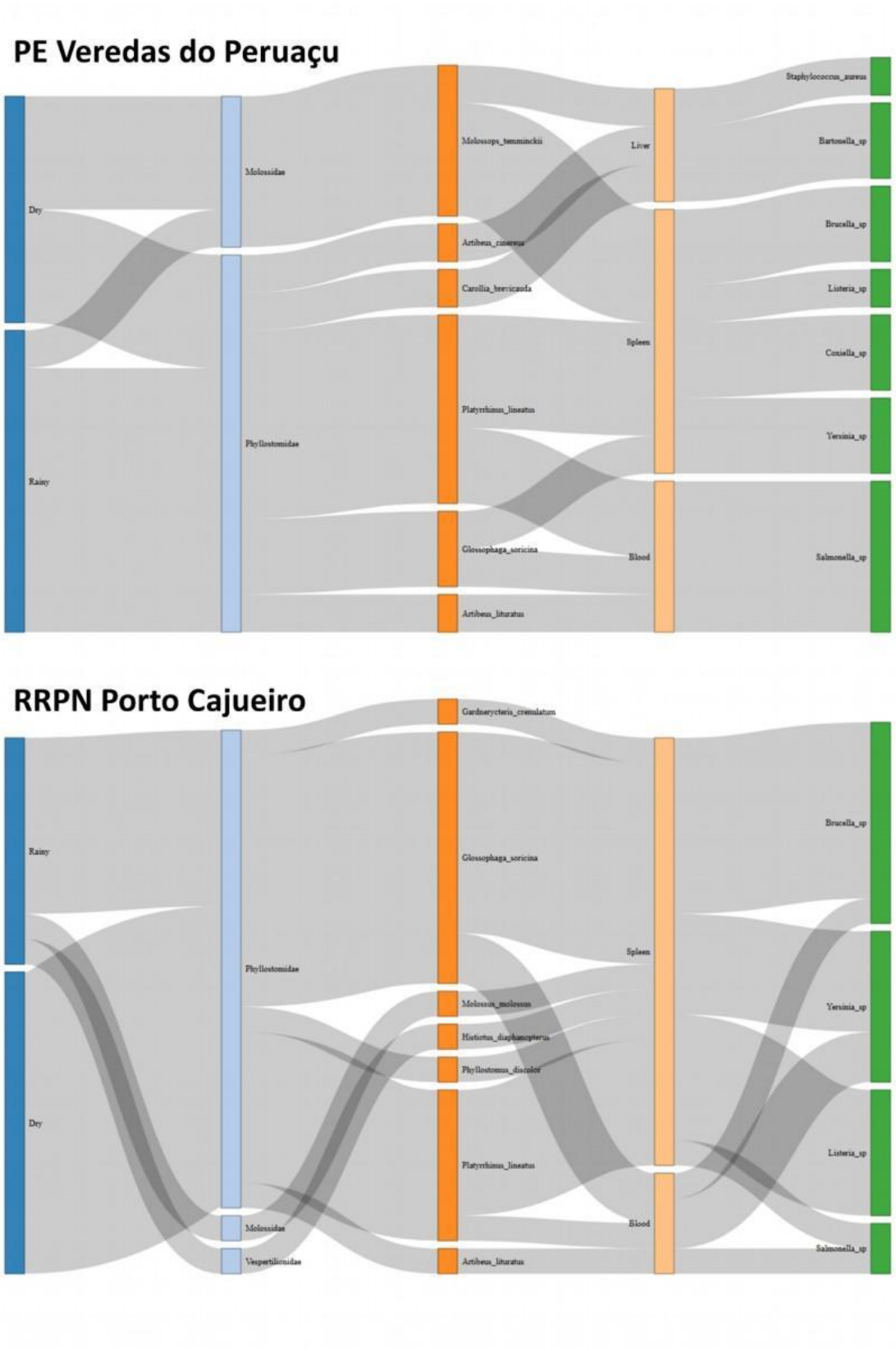

Figure 4: Multilayer Sankey diagram illustrating bat–parasite interactions in two veredas in Brazil. Flows represent interaction frequencies linking sampling season (rainy and dry), bat family, bat species, biological sample type (spleen and blood), and detected bacterial taxa. The width of each flow is proportional to the number of recorded interactions,

highlighting dominant pathways of host–parasite associations and the relative contribution of each category.

TABLES

Table 1: Description of the primers used for research on each gene related to the zoonotic bacteria of interest at PCR from liver samples collected from Brazilian Cerrado bats.

Pathogen	Gene	Primer direction	Primers sequences (5'-3')	PCR product size (bp)	Reference	Strain used as positive control
Coxiella burnetii	*IS1111*	F	TATGTATCCACCGTAGCCAGTC	686	(Willems et al. 1993)	*Coxiella burnetii* CB1 (LISASC-UFLA collection)
		R	CCCAACAACACCTCCTTATTC			
Listeria monocytogenes	*hlyA*	F	AAATCTGTCTCAGGYGATGT	103	(Barbau-Piednoir et al. 2013)	*Listeria monocytogenes* ATCC 15313
		R	CGATGATTTGAACTTCATCTTTTGC			
Pasteurella multocida	*KMT1*	F	ATCCGCTATTTACCCAGTGG	457	(Abbas et al. 2018)	*Pasteurella multocida* ATCC 12946
		R	GCTGTAAACGAACTCGCCAC			
Yersinia enterocolitica	*ail*	F	TAATGTGTACGCTGCGAG	351	(Thoerner et al. 2003)	*Yersinia enterocolitica* O:9 CLIST 3445 - YE 383
		R	GACGTCTTACTTGCACTG			
Rickettsia spp.	*gtlA*	F	GAGAGAAAATTATATCCAAATGTTGAT	147	(Labruna et al. 2004)	*Rickettsia vini* VPS-USP
		R	AGGGTCTTCGTGCATTTCTT			
Bartonella spp.	*gltA*	F	GCTATGTCTGCVTTCTATCAYGA	731	(Rozental et al. 2017)	*Bartonella henselae* Vector-Borne Bioagents Laboratory (VBBL)
		R	AGAACAGTAAACATTTCNGTHGG			
Salmonella spp.	*ompC*	F	ACCGCTAACGCTCGCCTGTAT	159	(Kwang et al. 1996)	*Salmonella* Typhimurium ATCC 14028
		R	AGAGGTGGACGGGTTGCTGCCGTT			

Leptospira spp.	*16S rRNA*	F	GGAACTGAGACACGGTCCAT	430	(Tansuphasiri et al. 2006)	*Leptospira interrogans* serovar Hardjo-prajitno LZB-USP
		R	GCCTCAGCGTCAGTTTTAGG			
	LipL32	F	AAGAATGTCGGCGATTATGC	279		
		R	CCAACAGATGCAACGAAAGA			
Staphylococcus aureus	*nuc*	F	AGTTCAGCAAATGCATCACA	400	(Cremonesi et al. 2005)	*Staphylococcus aureus* ATCC 25923
		R	TAGCCAAGCCTTGACGAACT			
Brucella spp.	*IS711*	F	CGCTCGCGCGGTGGAT	178	(Bounaadja et al. 2009)	*Brucella abortus* 544 (ATCC 23448)
		R	CTTGAAGCTTGCGGACAGTCACC			

Chlamydia psittaci	*envB*	F	AACCTCGGATAGCAAATTAATCTGG	152	(Okuda et al. 2011)	*Chlamydia psittaci Nobivac Feline 1 HCPCh*
		R	ATTTGGTATAAGAGCGAAGTTCTGG			

Abbreviations: LISASC: Laboratórios Integrados de Sanidade Animal e Saúde Coletiva - Universidade federal de Lavras; ATCC: American Type Culture Collection; VPS-USP: Departamento de Medicina Veterinária Preventiva e Saúde Animal - Universidade de São Paulo; LZB-USP: Laboratório de Zoonoses Bacterianas - Universidade de São Paulo.

776 **Table 2:** Epidemiological characteristics of bats with coinfections or repeated detection of the same pathogen, including species, sex, age,
777 sampling location and season, and detected pathogens with respective biological samples.

Family	Specie	Sex	Age category	Colletion Location	Colletion season	Detected Pathogen and biological sample
Phyllostomidae	*Glossophaga soricina*	Male	Adult	RPPN Porto Cajueiro	Rainy	*Y. enterocolitica* (spleen) and *Brucella* spp. (spleen)
	Glossophaga soricina	Female	Adult	RPPN Porto Cajueiro	Dry	*Y. enterocolitica* (spleen and blood)
	Platyrrhinus lineatus	Male	Adult	RPPN Porto Cajueiro	Dry	*Y. enterocolitica* (spleen and blood)
	Glossophaga soricina	Female	Adult	RPPN Porto Cajueiro	Dry	*Brucella* spp. (blood) and *L. monocitogenes* (spleen)

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Table 3: Results of the univariate analysis using Fisher's exact test for each detected pathogen among bats from Brazil. Only pathogens with at least one positive sample were included.

Pathogen	**Positive / Total (%)**	**Variable**	***p-value***
***Bartonella* spp.**	2/172 (1.2%)	Species	0.013
		Genus	0.082
Y. enterocolitica	6/172 (3.49%)	Feeding habit	0.168
***Brucella* spp.**	10/172 (5.8%)	Feeding habit	0.033
L. monocytogenes	6/172 (3.49%)	Feeding habit	0.094

Table 4. Structural properties of bat–parasite interaction networks in two veredas in northern Minas Gerais, Brazil. Values include observed modularity (Q), mean modularity under null models (Null Q), associated p-values, species richness (hosts and parasites), total network size, and number of interactions (links).

Metric	**RPPN Porto Cajueiro (preserved vereda)**	**PE Veredas do Peruaçu (degraded vereda)**
Modularity (Q)	0.413	0.595
Null Modularity Q (mean)	0.412	0.541
Modularity p-value	1.000	0.012
S_hosts	7	6
S_parasites	4	7
S_total	11	13
Links (L)	11	11

Supplementary Material

Detection of zoonotic bacterial pathogens in bats from vereda ecosystems in the Brazilian Cerrado

Table S1: Characterization of all bats analyzed for zoonotic bacterial pathogens from vereda ecosystems of the Brazilian Cerrado according to family, species, feeding habit, sex, sampling site, age, and detected pathogens.

Specie	Genus	Family	Feeding habits	Sex	Age	Place	Season	Samples	Pathogen	N
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	F	Adult	PE Vereda Peruaçu	Rainy	blood, liver	Negative	1
Molossops temminckii	Molossops	Molossidae	Insectivorous	M	Adult	PE Vereda Peruaçu	Rainy	blood, liver	*Brucella* spp. (blood), S. *aureus* (liver)	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frutivorous	M	Adult	PE Vereda Peruaçu	Rainy	blood, spleen, liver	Negative	1
Carollia perspicillata	Carollia	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Rainy	liver	Negative	1

Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	F	Adult	PE Vereda Peruaçu	Rainy	blood, liver	Negative	2
Carollia brevicauda	Carollia	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Rainy	blood, liver	*Bartonella* spp. (liver)	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Rainy	blood, liver	*Salmonella* spp. (blood)	1
Artibeus cinereus	Artibeus	Phyllostomidae	Frugivorous	F	Adult	PE Vereda Peruaçu	Rainy	blood, liver	*Bartonella* spp. (liver)	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Rainy	blood	Negative	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Rainy	blood, liver	Negative	1
Artibeus cinereus	Artibeus	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Rainy	liver	Negative	2
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	F	Adult	PE Vereda Peruaçu	Rainy	liver	Negative	2

Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Rainy	liver	Negative	2
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	F	Adult	PE Vereda Peruaçu	Rainy	spleen, liver	Negative	1
Artibeus cinereus	Artibeus	Phyllostomidae	Frugivorous	F	Adult	PE Vereda Peruaçu	Rainy	spleen, liver	Negative	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	F	Adult	PE Vereda Peruaçu	Rainy	spleen	*Yersinia enterocolitica* (spleen)	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	F	Adult	PE Vereda Peruaçu	Rainy	spleen, liver	Negative	3
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Rainy	spleen, liver	*Coxiella burnetti* (spleen)	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Rainy	spleen, liver	*Yersinia enterocolitica* (spleen)	1
Molossops temminckii	Molossops	Molossidae	Insectivorous	M	Adult	PE Vereda Peruaçu	Rainy	spleen, liver	Negative	1

Molossops temminckii	Molossops	Molossidae	Insectivorous	F	Young	PE Vereda Peruaçu	Rainy	spleen, liver	Negative	1
Gardnerycteris crenulatum	Gardnerycteris	Phyllostomidae	Insectivorous	M	Adult	PE Vereda Peruaçu	Rainy	spleen	Negative	1
Molossops temminckii	Molossops	Molossidae	Insectivorous	F	Adult	PE Vereda Peruaçu	Rainy	liver	Negative	2
Platyrrhinus cf. incarum	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Rainy	spleen	Negative	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Rainy	spleen, liver	Negative	1
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	F	Adult	PE Vereda Peruaçu	Rainy	spleen, liver	*Coxiella burnetti* (spleen)	1
Artibeus cinereus	Artibeus	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Rainy	spleen, liver	Negative	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	RPPN Porto Cajueiro	Rainy	liver	Negative	1

Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	F	Adult	RPPN Porto Cajueiro	Rainy	liver	Negative	2
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	F	Adult	RPPN Porto Cajueiro	Rainy	liver	Negative	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	F	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	Negative	3
Lophostoma brasiliense	Lophostoma	Phyllostomidae	Insectivorous	F	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	Negative	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frutivorous	M	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	Negative	4
Artibeus cinereus	Artibeus	Phyllostomidae	Frugivorous	F	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	Negative	1
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	F	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	Negative	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	*Brucella* spp. (spleen)	2

Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	M	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	*Yersinia enterocolitica* (spleen), *Brucella* spp. (spleen)	1
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	F	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	Yersinia (spleen)	1
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	M	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	Negative	12
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	M	Youn g	RPPN Porto Cajueiro	Rainy	spleen, liver	Negative	1
Phyllostomus discolor	Phyllostomu s	Phyllostomidae	Omnivorous	F	Youn g	RPPN Porto Cajueiro	Rainy	spleen, liver	*Brucella* spp. (spleen)	1
Artibeus planirostris	Artibeus	Phyllostomidae	Frugivorous	M	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	Negative	1
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	M	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	*Brucella* spp. (spleen)	1

Artibeus lituratus	Artibeus	Phyllostomidae	Frugivorous	M	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	Negative	1
Myotis lavali	Myotis	Vespertilionidae	Insectivorous	F	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	Negative	1
Artibeus cinereus	Artibeus	Phyllostomidae	Frugivorous	M	Adult	RPPN Porto Cajueiro	Rainy	liver	Negative	1
Artibeus lituratus	Artibeus	Phyllostomidae	Frugivorous	F	Adult	RPPN Porto Cajueiro	Rainy	liver	Negative	1
Histiotus diaphanopterus	Histiotus	Vespertilionidae	Insectivorous	F	Adult	RPPN Porto Cajueiro	Rainy	liver	Negative	1
Histiotus diaphanopterus	Histiotus	Vespertilionidae	Insectivorous	F	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	Negative	2
Histiotus diaphanopterus	Histiotus	Vespertilionidae	Insectivorous	F	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	*Listeria monocitogenes* (spleen)	1
Molossus molossus	Molossus	Molossidae	Insectivorous	F	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	Negative	3

Molossus molossus	Molossus	Molossidae	Insectivorous	M	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	Negative	3
Molossus molossus	Molossus	Molossidae	Insectivorous	F	Youn g	RPPN Porto Cajueiro	Rainy	spleen, liver	Negative	1
Molossus molossus	Molossus	Molossidae	Insectivorous	F	Adult	RPPN Porto Cajueiro	Rainy	spleen, liver	*Brucella* spp. (spleen)	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	F	Adult	PE Vereda Peruaçu	Dry	blood, spleen	Negative	4
Carollia perspicillata	Carollia	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Dry	blood, spleen	Negative	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Dry	blood, spleen	Negative	4
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Dry	blood, spleen	*Salmonella* spp. (blood)	1
Molossops temminckii	Molossops	Molossidae	Insectivorous	M	Adult	PE Vereda Peruaçu	Dry	blood, spleen	*Brucella* spp. (spleen)	2

Artibeus lituratus	Artibeus	Phyllostomidae	Frugivorous	F	Adult	PE Vereda Peruaçu	Dry	blood, spleen	*Salmonella* spp. (blood)	1
Molossops temminckii	Molossops	Molossidae	Insectivorous	M	Adult	PE Vereda Peruaçu	Dry	blood, spleen	Negative	1
Artibeus lituratus	Artibeus	Phyllostomidae	Frugivorous	M	Adult	PE Vereda Peruaçu	Dry	blood, spleen	Negative	3
Molossops temminckii	Molossops	Molossidae	Insectivorous	F	Adult	PE Vereda Peruaçu	Dry	spleen	Negative	1
Molossops temminckii	Molossops	Molossidae	Insectivorous	M	Adult	PE Vereda Peruaçu	Dry	blood, spleen	*Listeria monocitogenes* (spleen)	1
Molossops temminckii	Molossops	Molossidae	Insectivorous	F	Adult	PE Vereda Peruaçu	Dry	blood, spleen	Negative	1
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	F	Adult	PE Vereda Peruaçu	Dry	blood, spleen	*Salmonella* spp. (blood)	1
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	F	Adult	PE Vereda Peruaçu	Dry	blood, spleen	Negative	1

Neoeptesicus furinalis	Neoeptesicus	Vespertilionidae	Insectivorous	F	Adult	PE Vereda Peruaçu	Dry	blood, spleen	Negative	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	RPPN Porto Cajueiro	Dry	spleen	Negative	3
Molossops temminckii	Molossops	Molossidae	Insectivorous	M	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	Negative	4
Artibeus lituratus	Artibeus	Phyllostomidae	Frugivorous	M	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	Negative	2
Molossops temminckii	Molossops	Molossidae	Insectivorous	M	Adult	RPPN Porto Cajueiro	Dry	spleen	Negative	2
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	F	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	Negative	4
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	Negative	22
Artibeus lituratus	Artibeus	Phyllostomidae	Frugivorous	F	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	*Salmonella* spp. (blood)	1

Lophostoma brasiliense	Lophostoma	Phyllostomidae	Insectivorous	F	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	Negative	1
Gardnerycteris crenulatum	Gardnerycteris	Phyllostomidae	Insectivorous	M	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	Negative	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	*Brucella* spp. (spleen)	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Young	RPPN Porto Cajueiro	Dry	blood, spleen	Negative	1
Myotis lavali	Myotis	Vespertilionidae	Insectivorous	M	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	Negative	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	RPPN Porto Cajueiro	Dry	blood	Negative	1
Carollia perspicillata	Carollia	Phyllostomidae	Frugivorous	M	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	Negative	1
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	M	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	Negative	1

Neoeptesicus furinalis	Neoeptesicus	Vespertilionidae	Insectivorous	F	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	Negative	1
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	M	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	*Listeria monocitogenes* (spleen)	2
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	M	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	Negative	5
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	F	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	*Brucella* spp. (blood), *Listeria* monocitogenes (spleen)	1
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	F	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	*Yersinia enterocolitica* (blood, spleen)	1
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	M	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	*Yersinia enterocolitica* (blood, spleen)	1
Glossophaga soricina	Glossophaga	Phyllostomidae	Nectarivorous	F	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	Negative	1

Artibeus fimbriatus	Artibeus	Phyllostomidae	Frugivorous	F	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	Negative	1
Gardnerycteris crenulatum	Gardnerycteris	Phyllostomidae	Insectivorous	M	Adult	RPPN Porto Cajueiro	Dry	blood, spleen	*Salmonella* spp. (spleen)	1
Artibeus lituratus	Artibeus	Phyllostomidae	Frugivorous	M	Adult	RPPN Porto Cajueiro	Dry	spleen	Negative	2
Platyrrhinus lineatus	Platyrrhinus	Phyllostomidae	Frugivorous	F	Adult	RPPN Porto Cajueiro	Dry	spleen	*Listeria monocitogenes* (spleen)	1
Histiotus diaphanopterus	Histiotus	Vespertilionidae	Insectivorous	F	Adult	RPPN Porto Cajueiro	Dry	spleen	Negative	1
Molossus molossus	Molossus	Molossidae	Insectivorous	M	Adult	RPPN Porto Cajueiro	Dry	spleen	Negative	1

ARTICLE 3

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Metataxonomic analysis of rectal samples of synanthropic bats from Montes Claros, Brazil

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ABSTRACT

This study aims to characterize the gut microbiomes of eight species of fruit, insectivorous and nectarivorous bats from Montes Claros, Minas Gerais, Brazil, with an emphasis on identifying potentially pathogenic genera and their implications for human and bat health. Rectal swabs from 44 samples were analysed via high-throughput *16S rRNA* gene sequencing targeting the V3/V4 regions. The results identified Firmicutes, Proteobacteria and Actinobacteria as the predominant phyla. Three hundred nineteen genera were identified, 34 of which were potentially pathogenic, such as *Staphylococcus*, *Enterococcus*, *Bacillus*, *Enterobacter*, *Clostridium sensu stricto 1*, *Salmonella*, *Mycoplasma*, *Bartonella*, *Helicobacter* and *Brucella*. Alpha diversity estimated via the Chao1 index revealed high variability in bacterial richness among the bat samples. This study is the first to report the gut microbiota of *S. lilium*, *A. geoffroyi*, *G. soricina*, and *M. nigricans*. The results contribute to understanding the bat-associated microbiota and reinforce the relevance of integrating wildlife microbiome research from a One Health perspective.

KEY WORDS: *16S rRNA* sequencing, Chiroptera, One Health, pathogen

INTRODUCTION

There are currently approximately 1,460 recognized species of bats (order: Chiroptera) worldwide (Upham, 2022). These mammals have gained prominence not only because of their large number of species but also because of their ability to fly, which enables them to be widely distributed across continents, and their ability to adapt to various habitats (wild, rural and urban) (Teeling et al. 2009). Over the years, several species have adapted to live in urban environments, where they find shelter and food, exhibiting synanthropic behavior. Owing to this adaptation, these animals have been more

frequently involved in the transmission cycle of recent outbreaks of zoonotic diseases (Calisher et al. 2006, Smith and Wang 2013, Wacharapluesadee et al. 2021), underscoring the need for a closer look at the ability of bats to harbor different zoonotic pathogens.

In this context, recent studies have highlighted the diversity of potentially zoonotic bacterial pathogens carried by bats, with more than 100 genera identified in a recent scoping review (Ferreira et al. 2024) and 11 bacterial pathogens of great zoonotic importance identified in a meta-analysis (Szentivanyi et al. 2023). Therefore, the study of the microbiome has been suggested by several authors as an alternative for identifying pathogens (Dimkić et al. 2021, Castelo-Branco et al. 2023), and for this purpose, genomic techniques using next-generation sequencing (NGS) have been implemented (Newman et al. 2018, Selvin et al. 2019, André et al. 2023) to identify nonculturable microorganisms. Among these approaches, short-read sequencing targeting the hypervariable V3–V4 regions of the *16S rRNA* gene stands out because of its low cost, evolutionary conservation, and high taxonomic resolution for profiling prokaryotic communities (Selvin et al. 2019, Kim et al. 2020).

Studies aimed at identifying and understanding the microbiome of these species are rare. In Brazil, there are only a few published studies, particularly those that consider the nation's bat diversity and the ecological and public health importance of these mammals. Research analysing rectal and oral samples of bats revealed the presence of *Escherichia coli*, *Klebsiella oxytoca*, *Serratia marcescens*, *Pseudomonas* spp. and *Staphylococcus* spp., which are highly important pathogens, via culture and MALDI-TOF techniques on samples collected between 2016 and 2017 (Cláudio et al. 2018). In another study conducted in Brazil, the bacterial microbiomes of rectal and oral swabs from bats were also investigated through metagenomic sequencing, which revealed the presence of

clinically significant pathogens, including *Streptococcus*, *Anaplasma*, *Enterobacter*, *Klebsiella*, *Escherichia* and *Enterococcus* (André et al. 2023).

Moreover, in addition to their potential role in transmitting pathogens to other animal species and humans, knowledge of the bat microbiome and the factors that influence its composition are essential for assessing the health of these animals and supporting conservation strategies. The identification of emerging threats to guide management actions aimed at preserving bat diversity is particularly important since bats play important roles in the functioning of the ecosystem, such as seed dispersal, pollination and control of agricultural pests (Teeling et al. 2009).

Therefore, this study aimed to describe the rectal microbiome of samples from eight bat species from Montes Claros, Minas Gerais, Brazil, via high-throughput *16S rRNA* amplicon metagenomic sequencing, with a particular focus on potentially pathogenic bacterial genera. This study also discusses the implications of these findings for the health of bats, humans, and other animal species.

METHODS

Sample collection and study population

The present study included 44 rectal swabs previously collected from bats in Montes Claros, a municipality in northern Minas Gerais, Brazil (Vieira 2016). The collection of samples followed the ethical principles of animal experimentation and was approved by the Animal Experimentation Ethics Committee (CETEA/UFMG) under protocol no. 333/2013. Collections were performed in four regions of the municipality of Montes Claros (Figure 1) for 13 consecutive months, from April 2014 to April 2015. At each collection point, a mist net was installed to assist with capture. The captured bats were placed in individual cotton bags, and the animals were later identified via taxonomic

keys; sex, eating habits and habitat (urban or wild) were also recorded according to the capture location (Gregorin and Taddei 2002). In total, 247 animals were captured, 44 of which were randomly selected for this study (18.81%). In addition to rectal swabs, which were used in the present study, samples from different tissues were collected. The descriptions of the samples are shown in Table 1.

DNA extraction

To increase the yield of DNA from the samples, additional processing was included as a first step. One millilitre of phosphate-buffered saline (PBS) (0.01 M, pH 7.4) was added to each tube containing the swab, and the tubes were homogenized for 30 minutes. The swabs were then removed and transferred to a new tube. The tubes containing the samples were centrifuged again for 30 minutes at 14,000 × g, and the pellets were separated and suspended in 300 μL of PBS. The tubes were subsequently homogenized for 30 minutes, after which the samples were stored at -80°C until processing. DNA extraction was performed automatically via the MagMAX™CORE nucleic acid purification kit on the KingFisher Flex Robot (Thermo Fisher Scientific). The samples were purified via OneStep PCR Inhibitor Removal Kits (ZymoResearch©) according to the manufacturer's instructions. In this step, a tube with an aliquot of PBS (negative control), another with an aliquot of the MagMAX™CORE nucleic acid purification kit (extraction kit control) and another with an aliquot of the ZymoBIOMICS Microbial Community Standards (ZymoResearch©) (positive control) were included. The DNA concentration was measured with a spectrophotometer (Nanodrop, Thermo Fisher Scientific Inc., Waltham, MA, United States).

Genomic sequencing

The extracted DNA was sequenced via MiSeq Sequencing System equipment (lllumina Inc., USA). Library preparation followed the in-house Neoprospecta Microbiome Technologies (Florianópolis, SC, Brazil) protocol. High-throughput sequencing of the V3/V4 regions of the *16S rRNA* gene was performed after amplification via the primers 341F (CCTACGGGRSGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) (Caporaso, et al. 2012). The libraries were sequenced via paired-end sequencing on the Illumina platform (MiSeq), employing the V3 (600 cycles; 2 × 300 bp) and V2 (500 cycles; 2 × 250 bp) kits.

Analysis

Quality checks of the sequence reads were performed via FASTQC (v.0.12.1), and Quantitative Insights Into Microbial Ecology 2 (QIIME2, v. 2024.2) was used. Trimming and denoising (DADA2) of the amplicon variant sequences (ASVs) were performed via QIIME2. An average Phred score of 30 or higher (Q30) was implemented to filter out low-quality reads. The Silva database (v. 138 - available online: https://www.arb-silva.de/documentation/release-138/, accessed on March 22, 2024) was used as the *16S rRNA* reference database for the classification of amplicon sequence variants (ASVs) via the classify-consensus-blast. Differential diversity and abundance analyses were conducted in R (v. 4.3.3) (R Core Team, 2024) via the phyloseq package (McMurdie and Holmes 2013, Love, Huber et al. 2014). Alpha diversity was assessed via the Chao1 estimator, a nonparametric estimator of species richness that assigns greater weight to rare ASVs. Control samples (Mock, PBS and Kit) were removed from this analysis; therefore, alpha diversity was calculated for only the 44 bat samples.

RESULTS

A total of 47 samples (3 controls and 44 bat samples) were analysed, from which a total of 3,106,291 ASVs were generated. As expected, the negative control (PBS) did not yield any sequences, and all bacterial genera from the commercial community ZymoBIOMICS, which was used as the positive control, were identified after analysis. Furthermore, when the extraction kit was amplified, 2,327 ASVs were generated. These sequences were not removed from the samples, as they represent a very low percentage of the total ASVs [0.07% (2,327/3,106,291)].

The 3,106,291 ASVs were distributed between the kingdom Bacteria [99.99% (3,106,209/3,106,291)] and Archea [0.01% (82/3,106,291)], with the latter present only in two bats of the genus *Carollia* (Supplementary Material Figure S1). Nineteen groups were classified at the phylum level. The three most abundant bacteria were Firmicutes [60.18% (1,869,407/3,106,291)] present in all the samples; Proteobacteria [25.84% (802,745/3,106.291)] were absent in only one *C. perspicillata* individual; and Actinobacteriota [13.06% (405,874/3,106,291)], which was absent in some individuals of three frugivorous species (*A. planirostris (3)*, *A. lituratus (3)*, and *C. perspicillata (1)*) (Supplementary Material Figure S2).

Thirty-nine groups were classified according to class. The most abundant bacteria were Bacilli [57.23% (1,777,691/3,106,291)], which were absent only in one *C. perspicillata* individual; Gammaproteobacteria [19.41% (602,804/3,106,291)], which were absent only in one *A. perspicillata* individual; Actinobacteria [12.76% (395,303/3,106,291)], which was absent in two *A. planirostris individuals and two A. lituratus individuals*; Alphaproteobacteria [6.43% (199,874/3,106,291)], which were absent in one C. *perspicillata* individual and two *A. planirostris individuals*; and

171 Clostridia [2.79% (86,715/3,106,291)], which was absent in at least one individual of
172 each species except *A. geoffroyi* (Supplementary Material Figure S3).

173 At the order level, Bacillales [27.03% (839,568/,106,291)] were the most
174 abundant among the one hundred classified groups; however, it was absent in at least one
175 individual of each species except *A. geoffroyi*; followed by Enterobacterales [17.43%
176 (541,328/3,106,291)], absent in only one *A. planirostris individual*; Staphylococcales
177 [10.22% (317,352/3,106,291)], present in at least one individual of each species;
178 Lactobacillales [10.17% (315,995/3,106,291)], absent in one bat of each species, *G.*
179 *soricina*, *C. perspicillata and A. planirostris;* and Paenibacillales [9.68%
180 (300,662/3106,291)] present in at least one individual of each studied bat species
181 (Supplementary Material Figure S4).

182 One hundred and seventy-two groups were classified at the family level. The
183 Bacillaceae [15.97% (496,089/3,106,291)] family was the most abundant and was present
184 in at least one individual of each species, except for *S. lilium*, *G. soricina* and *P. lineatus*.
185 Next, the family Planococcaceae [11.06% (343,476/3,106,291)] was observed in only
186 twelve bats, although it was present in at least one species associated with each feeding
187 habit. Similarly, Staphylococcaceae [10.22% (317,349/3,106,291)] was present for at
188 least one individual of each species; Erwiniaceae [9.82% (305,039/3,106,291)] was
189 present only in fruit bats *A. planirostris* (5), *A. lituratus* (1) and *Carollia* sp. (2);
190 Paenibacillaceae [9.68% (300,662/3,106,291)] was present in at least one individual of
191 each species; and Enterobacteriaceae [6.12% (190,217/3,106,291)] was present in all
192 individuals, except for one individual of *A. planirostris* (Supplementary Material Figure
193 S5).

194 At the genus level, 319 groups were classified, with the five most abundant genera
195 being *Staphylococcus*, *Pantoea*, *Paenibacillus*, *Oceanobacillus*, and *Enterococcus*.

196 *Staphylococcus* [10.22% (317,332/3,106,291)] was present in at least one individual of
197 each species; *Pantoea* [9.82% (305,039/3,106,291)] was present only in fruit bats (*A.*
198 *planirostris* (5), *A. lituratus* (2) and *Carollia* sp. (3)); *Paenibacillus* [9.82%
199 (300,581/3,106,291)] was present in at least one individual of each species;
200 *Oceanobacillus* [8.52% (264.818/3.106.291)] was present in only twelve bats, albeit
201 present in at least one species of each feeding habit; and *Enterococcus* [6.69%
202 (207,734/3,106,291)] was present in only twenty-two bats but was present in at least one
203 species of each feeding habit (Figure 2 and Supplementary Material Figure S6).

204 Among the classified genera, 35 potentially pathogenic genera representing one
205 third of the total classified ASVs were identified [33.67% (1.045.971/310.629)]. The five
206 pathogenic genera most abundant were *Staphylococcus* [10.22% (317,332/3,106,291)],
207 *Enterococcus* [6.69% (207,734/3,106,291)], *Bacillus* [3.59% (111,408/3,106,291)],
208 *Enterobacter* [2.92% (90,608/3,106,291)] and *Clostridium sensu stricto 1* [1.95%
209 (60,492/3,106,291)] (Supplementary Material Table S1). A lower relative abundance of
210 other genera with potential clinical relevance, such as *Salmonella* (0.8244%),
211 *Mycoplasma* (0.0334%), *Bartonella* (0.0020%), *Helicobacter* (0.0006%) and *Brucella*
212 (0.0002%), was also identified. The distribution of bats according to their species and
213 feeding habits is shown in Figure 3 and in Supplementary Material Table S2. Most of
214 these genera were detected in frugivorous bats, particularly in *A. planirostris*, except for
215 *Bartonella* and *Rickettsia [5.71% (2/35)]*, which were identified exclusively in
216 insectivorous bats (*M. nigricans*). The genera *Staphylococcus* and *Escherichia–Shigella*
217 were detected in all eight bat species. In contrast, eleven genera *[31.43% (11/35)]*,
218 *Bartonella, Bordetella, Brucella, Citrobacter, Clostridioides, Helicobacter,*
219 *Porphyromonas, Proteus, Rickettsiella, Salmonela and Vibrio,* were identified
220 exclusively in a single bat species. *The genera Rhodococcus, Escherichia–Shigella*,

Enterococcus, *Enterobacter*, *Bacillus*, and *Acinetobacter* were present in at least one individual from each feeding group (Figure 3).

The results of the alpha diversity analysis are shown in Figures 4 and 5 and were generated on the basis of the Chao1 index used to estimate bacterial richness across the bat samples. In Figure 4, marked heterogeneity was observed, both among bat species and within individuals of the same species (e.g., of two *A. planirostris* bats, one exhibited more than 600 taxa and the other close to 0). In general, frugivorous species presented a wide range of Chao1 values, with the highest richness estimates, such as an *A. lituratus* bat with more than 800 taxa. Insectivorous species presented relatively low richness values, but there was still variation in the number of ASVs observed among individuals of the same species (*M. nigricans*). Nectarivorous species, represented by *G. soricina* and *A. geoffroy*, presented intermediate Chao1 values compared with those of the other feeding groups. Figure 5 shows that the majority of samples had Chao1 values of approximately 200 ASVs, while variability among samples was evident.

DISCUSSION

Bats are among the most abundant animal groups in the world (Simmons 2005, Teeling, Hedges et al. 2009). In Brazil, 186 species distributed across 68 genera and 9 families have been identified to date, representing a significant portion of the global bat diversity (Garbino, Cláudio et al. 2024). These animals play important ecological roles in ecosystems, acting as seed dispersers, pollinators, and regulators of insect populations, making them essential for maintaining biodiversity and ecosystem stability (RAMÍREZ-FRÁNCEL, GARCÍA-HERRERA et al. 2022). However, the synanthropic behavior of several species and their high mobility, owing to their flight ability, also position them as potential reservoirs and dispersers of pathogens across large geographic areas, which

raises concerns about the transmission of zoonotic diseases to humans and other wildlife (Nunes, Rocha et al. 2017). In contrast to their abundance and diversity in Brazil, knowledge of the intestinal microbial composition of these animals is limited, precluding the development of both conservation strategies and surveillance measures focused on potential zoonotic risks. In this context, this study provides, for the first time, a characterization of the rectal microbiome of eight different bat species from Montes Claros, Minas Gerais, Brazil, with more than 3 million ASVs classified among 44 bat samples, representing a considerable level of intestinal bacterial richness. Our results contribute substantially to the growing knowledge of the microbiota associated with bats, particularly in synanthropic species in Brazil, where such studies are still scarce.

In fact, microbiome studies are fundamental for characterizing the composition and dynamics of microbial communities associated with an organism (Berg, Rybakova et al. 2020). The taxonomic composition observed in this study represents another step toward understanding the gut microbiome of bats. In addition to being a definitive study, this work contributes to others by providing evidence of the predominance of Firmicutes, Proteobacteria, and Actinobacteria in the gut microbiome of bats. This taxonomic pattern is consistent with findings reported in bat species from Brazil, including *A. lituratus*, *A. planirostris*, *Eptesicus furinalis*, *C. perspicillata*, and *P. lineatus*, as well as in *Rhinolophus monoceros* in India and *Vespertilio sinensis* in China (Selvin, Lanong et al. 2019, Li, Chu et al. 2021, André, Ikeda et al. 2023). Furthermore, studies in bat communities in Brazil and elsewhere suggest that diet influences the gut bacterial structure, with frugivorous, nectarivorous, and insectivorous species exhibiting distinct microbial signatures, which was also observed in the descriptive analysis in the present study (Figure 4) (Phillips, Phelan et al. 2012, Carrillo-Araujo, Taş et al. 2015, André, Ikeda et al. 2023, Dai, Leng et al. 2024).

In addition to describing the overall microbial diversity, this study also identified several potentially pathogenic bacterial genera present in the gut microbiota of all the assessed bat species, including bacterial species known to be associated with opportunistic or zoonotic infections, such as *Staphylococcus*, *Escherichia-Shigella*, *Brucella*, *Mycoplasma*, *Klebsiella*, and *Burkholderia*. The presence of genera that harbor clinically relevant bacterial species, also identified by others (André, Dumler et al. 2016, Selvin, Lanong et al. 2019, Popov, Popov et al. 2023), emphasizes the importance of continuous surveillance. However, it is important to emphasize that the detection of these genera through *16S rRNA* sequencing does not necessarily imply the presence of pathogenic bacterial species since many species within these groups are commensal members of the gut microbiota. Indeed, identifying bacteria at the genus level limits the ability to assess pathogenic potential, virulence factors, or antimicrobial resistance profiles, highlighting the need for further studies using different approaches, such as shotgun sequencing, to identify bacterial species and the resistome. This limitation reinforces the need for complementary approaches, such as shotgun sequencing, which allows for species-level identification and resistome characterization.

In this context, the identification of potentially pathogenic genera in this study should be interpreted as an initial screening step that can guide future targeted investigations. Given the wide microbial diversity present in bats, directing efforts towards specific bacterial groups is essential to deepen the understanding of their role in the maintenance and transmission of pathogens. The detection of genera such as *Brucella*, *Mycoplasma* and *Escherichia-Shigella* highlights the importance of complementary approaches, including PCR, bacterial isolation, or whole-genome sequencing, to allow the identification of these microorganisms at the species level, as well as the characterization of virulence profiles and antimicrobial resistance. These approaches are

relevant both for the discovery of new bacterial species, such as *Brucella nosferati* (Hernández-Mora, Chacón-Díaz et al. 2023), and for improving our understanding of the pathogenic potential of known microorganisms, including *Salmonella* spp. Therefore, microbiome-based surveys represent a valuable tool for prioritizing pathogens of interest and supporting more focused epidemiological and molecular investigations.

With respect to the median value of the alpha diversity index Chao1, a moderate level of bacterial richness was observed throughout the dataset, along with marked interindividual variability across bat species and dietary groups (Figures 4 and 5). These results reinforce what has been detected by other authors: gut bacterial richness varies widely among bat species and individuals, with specific host factors, such as food group, host species phylogeny, and life stage, being suggested to influence the structure of the gut microbiota (Phillips, Phelan et al. 2012, Carrillo-Araujo, Taş et al. 2015, Li, Chu et al. 2021, Dai, Leng et al. 2024).

Unfortunately, the low representation of some species, particularly *A. geoffroyi*, *G. soricina*, and *S. lilium*, limits the availability of taxonomic keys and robust interspecific comparisons of sex, feeding habits, and habitat (urban or wild), preventing a full characterization of their gut microbiomes. Consequently, conclusions about differences between food groups should be interpreted with caution. Nevertheless, despite the small sample sizes for some species, this study represents the first report of the gut microbiota of *S. lilium*, *A. geoffroyi*, *G. soricina*, and *M. nigricans*, thereby contributing novel data to the literature. Furthermore, the rigorous methodological approach adopted reinforces the reliability of the results. The use of high-throughput, culture-independent *16S rRNA* amplicon sequencing, which targets the hypervariable V3–V4 regions, allowed for a comprehensive characterization of the bacterial communities, including genera that are fastidious and difficult to identify by culture-dependent methods, such as *Leptospira*

spp., *Mycoplasma* spp., *Rickettsia* spp., and others. Additionally, the inclusion of negative, positive, and extraction controls further ensured analytical robustness, minimized contamination risk and validated the bioinformatics pipeline. Finally, the absence of amplification in the negative control and the successful recovery of all expected genera in the ZymoBIOMICS™ microbial standard reinforce the validity and accuracy of the generated data. From the One Health perspective, understanding the pathogenic microbial community associated with synanthropic bats contributes to the assessment of the risks and benefits of the interaction between wildlife, domestic animals, and human populations, particularly to date, where interactions between humans and bats have increased owing to their adaptation to urban environments and the growing interest in wildlife-based activities such as ecotourism (Russo and Ancillotto 2015, Fornazari 2021).

CONCLUSION

Our results revealed a diverse bacterial community dominated by Firmicutes, Proteobacteria, and Actinobacteria in the analysed bats, with marked interspecific and interindividual variability, suggesting the influence of ecological and host-related factors. The detection of several bacterial genera, including those potentially pathogenic, further highlights the importance of continuous surveillance and deeper genomic investigations to assess their functional and epidemiological significance. Notably, this study is the first to report the gut microbiota of specimens of the species *S. lilium*, *A. geoffroyi*, *G. soricina*, and *M. nigricans*. Together, our results facilitate understanding of the bat-associated microbiota and reinforce the relevance of integrating wildlife microbiome research from a One Health perspective.

ADDITIONAL REQUEST TEXT

Figure S1: Relative abundance of bacterial Kindom of bats captured in Montes Claros, Minas Gerais Brazil. Stacked bar plots depict the percentage (%) composition of the most abundant Kindom in each sample.

Figure S2: Relative abundance of bacterial Phylum of bats captured in Montes Claros, Minas Gerais Brazil. Stacked bar plots depict the percentage (%) composition of the most abundant Phylum in each sample.

Figure S3: Relative abundance of bacterial Class of bats captured in Montes Claros, Minas Gerais Brazil. Stacked bar plots depict the percentage (%) composition of the most abundant Class in each sample.

Figure S4: Relative abundance of bacterial Order of bats captured in Montes Claros, Minas Gerais Brazil. Stacked bar plots depict the percentage (%) composition of the most abundant Order in each sample.

Figure S5: Relative abundance of bacterial Family of bats captured in Montes Claros, Minas Gerais Brazil. Stacked bar plots depict the percentage (%) composition of the most abundant Family in each sample.

Figure S6: Relative abundance of bacterial Genus of bats captured in Montes Claros, Minas Gerais Brazil. Stacked bar plots depict the percentage (%) composition of the most abundant Genus in each sample.

Table S1: Absolute (ASVs) and relative abundance of potentially pathogenic bacterial genera identified in bat samples from Montes Claros, Minas Gerais.

Table S2: Absolute abundance (ASVs) of potentially pathogenic bacterial genera identified by bat specie, of the eigth bats species from Montes Claros, Minas Gerais.

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472

473 **STATEMENTS & DECLARATIONS**

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COMPETING INTERESTS

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

ACRF: Conceived of or designed study, analysed data, and wrote the paper. VJMN, BBS, MDD and NCG: performed research, and analysed data. MBH, CRP, TMV and EMSD: designed research and supervised the writing of the manuscript. CMFG and GSS: supervised the writing of the manuscript. All the authors have read and approved the final manuscript.

DATA AVAILABILITY

Raw sequence data generated in this study will be deposited in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA1450673 and will be made publicly available upon acceptance of the manuscript. Associated metadata and processed data will also be made available in a public repository.

ETHICS APPROVAL

The collection of samples followed the ethical principles of animal experimentation and was approved by the Animal Experimentation Ethics Committee (CETEA/UFMG), under protocol no. 333/2013.

CONSENT TO PARTICIPATE

Not applicable.

CONSENT TO PUBLISH

Not applicable.

505 **Tables**

506

507 **Table 1:** Family distribution, genus, species, feeding habits (diet), collection location and
508 sex of the 44 bats studied, captured between 2014 and 2015 in the municipality of Montes
509 Claros, Minas Gerais.

Family	Genus	Species	Feeding habit	Local	Year	Sex	N
Phyllostomidae	Anoura	*Anoura geoffroyi*	Nectarivorous	Parque Estadual da Lapa Grande	2014	F	1
Phyllostomidae	Artibeus	*Artibeus lituratus*	Frugivorous	Nossa Senhora das Graças	2014	F	1
Phyllostomidae	Artibeus	*Artibeus lituratus*	Frugivorous	Parque Municipal do Sapucaia	2014	M	2
Phyllostomidae	Artibeus	*Artibeus lituratus*	Frugivorous	Parque Municipal do Sapucaia	2014	F	1
Phyllostomidae	Artibeus	*Artibeus lituratus*	Frugivorous	Vila Luiza	2014	M	2
Phyllostomidae	Artibeus	*Artibeus lituratus*	Frugivorous	Vila Luiza	2014	F	3
Phyllostomidae	Artibeus	*Artibeus lituratus*	Frugivorous	Vila Luiza	2014	NI	1
Phyllostomidae	Artibeus	*Artibeus planirostris*	Frugivorous	Nossa Senhora das Graças	2014	F	3
Phyllostomidae	Artibeus	*Artibeus planirostris*	Frugivorous	Nossa Senhora das Graças	2014	M	1
Phyllostomidae	Artibeus	*Artibeus planirostris*	Frugivorous	Parque Estadual da Lapa Grande	2014	F	2
Phyllostomidae	Artibeus	*Artibeus planirostris*	Frugivorous	Parque Estadual da Lapa Grande	2014	M	1

Phyllostomidae	Artibeus	*Artibeus planirostris*	Frugivorous	Parque Estadual da Lapa Grande	2015	M	2
Phyllostomidae	Artibeus	*Artibeus planirostris*	Frugivorous	Parque Estadual da Lapa Grande	2015	F	1
Phyllostomidae	Artibeus	*Artibeus planirostris*	Frugivorous	Parque Municipal do Sapucaia	2014	M	1
Phyllostomidae	Artibeus	*Artibeus planirostris*	Frugivorous	Parque Municipal do Sapucaia	2014	F	2
Phyllostomidae	Artibeus	*Artibeus planirostris*	Frugivorous	Parque Municipal do Sapucaia	2015	F	1
Phyllostomidae	Artibeus	*Artibeus planirostris*	Frugivorous	Vila Luiza	2014	M	1
Phyllostomidae	Artibeus	*Artibeus planirostris*	Frugivorous	Vila Luiza	2015	F	2
Phyllostomidae	Carollia	*Carollia perspicillata*	Frugivorous	Parque Municipal do Sapucaia	2014	M	2
Phyllostomidae	Carollia	*Carollia* sp.	Frugivorous	Parque Estadual da Lapa Grande	2015	F	1
Phyllostomidae	Carollia	*Carollia* sp.	Frugivorous	Parque Estadual da Lapa Grande	2015	M	3
Phyllostomidae	Glossophaga	*Glossophaga soricina*	Nectarivorous	Nossa Senhora das Graças	2014	M	1
Phyllostomidae	Platyrrhinus	*Platyrrhinus lineatus*	Frugivorous	Nossa Senhora das Graças	2014	F	1
Phyllostomidae	Platyrrhinus	*Platyrrhinus lineatus*	Frugivorous	Vila Luiza	2014	M	2

	Phyllostomidae	Sturnira	*Sturnira lilium*	Frugivorous	Parque Estadual da Lapa Grande	2015	M	1
	Vespertilionidae	Myotis	*Myotis nigricans*	Aerial insectivorous	Parque Estadual da Lapa Grande	2014	F	2
	Vespertilionidae	Myotis	Myotis *nigricans*	Aerial insectivorous	Parque Estadual da Lapa Grande	2014	M	1
	Vespertilionidae	Myotis	*Myotis nigricans*	Aerial insectivorous	Parque Municipal do Sapucaia	2014	F	2

510 N: Number of specimens; NI: not identified; F: female; M: male

Figures

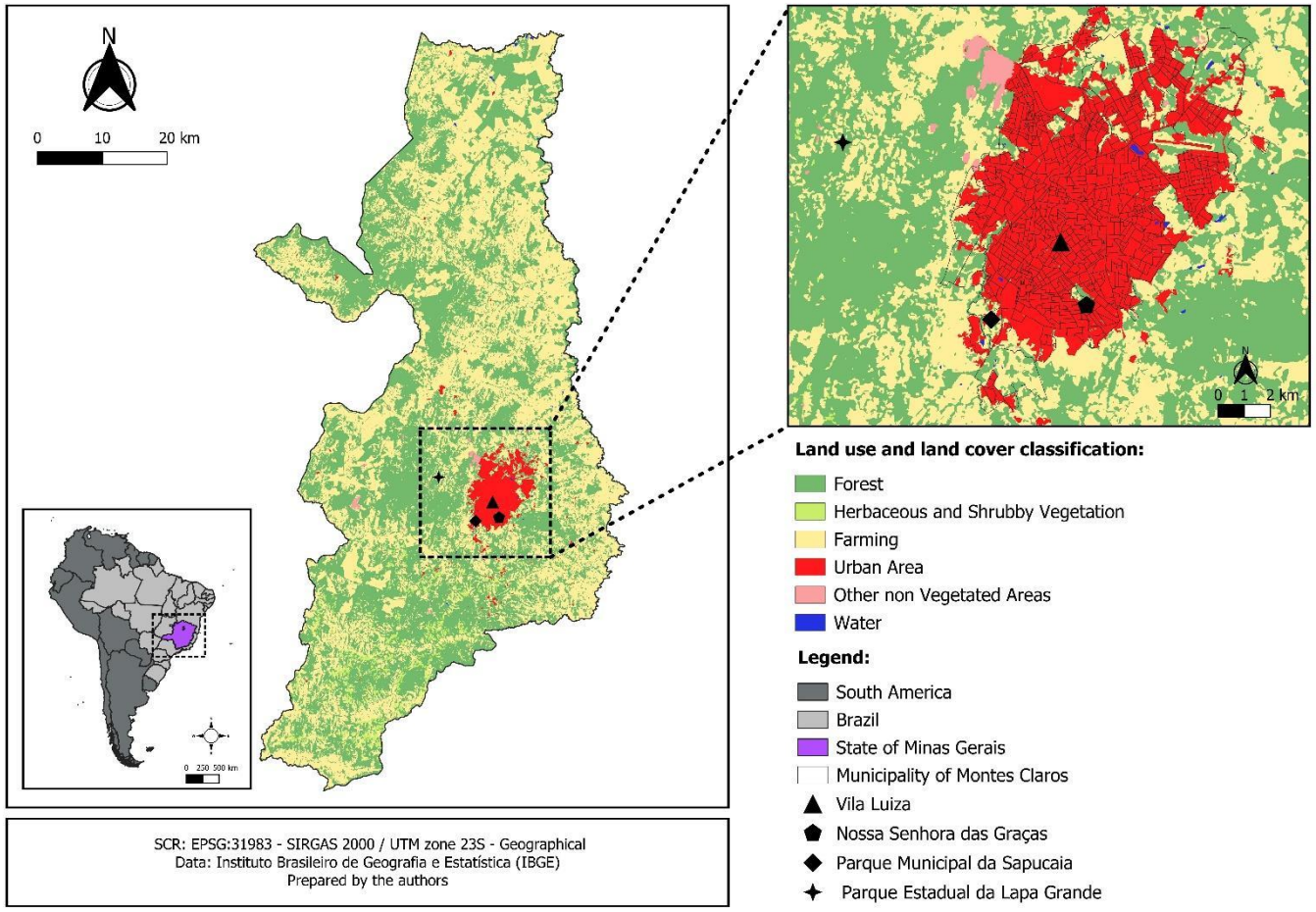
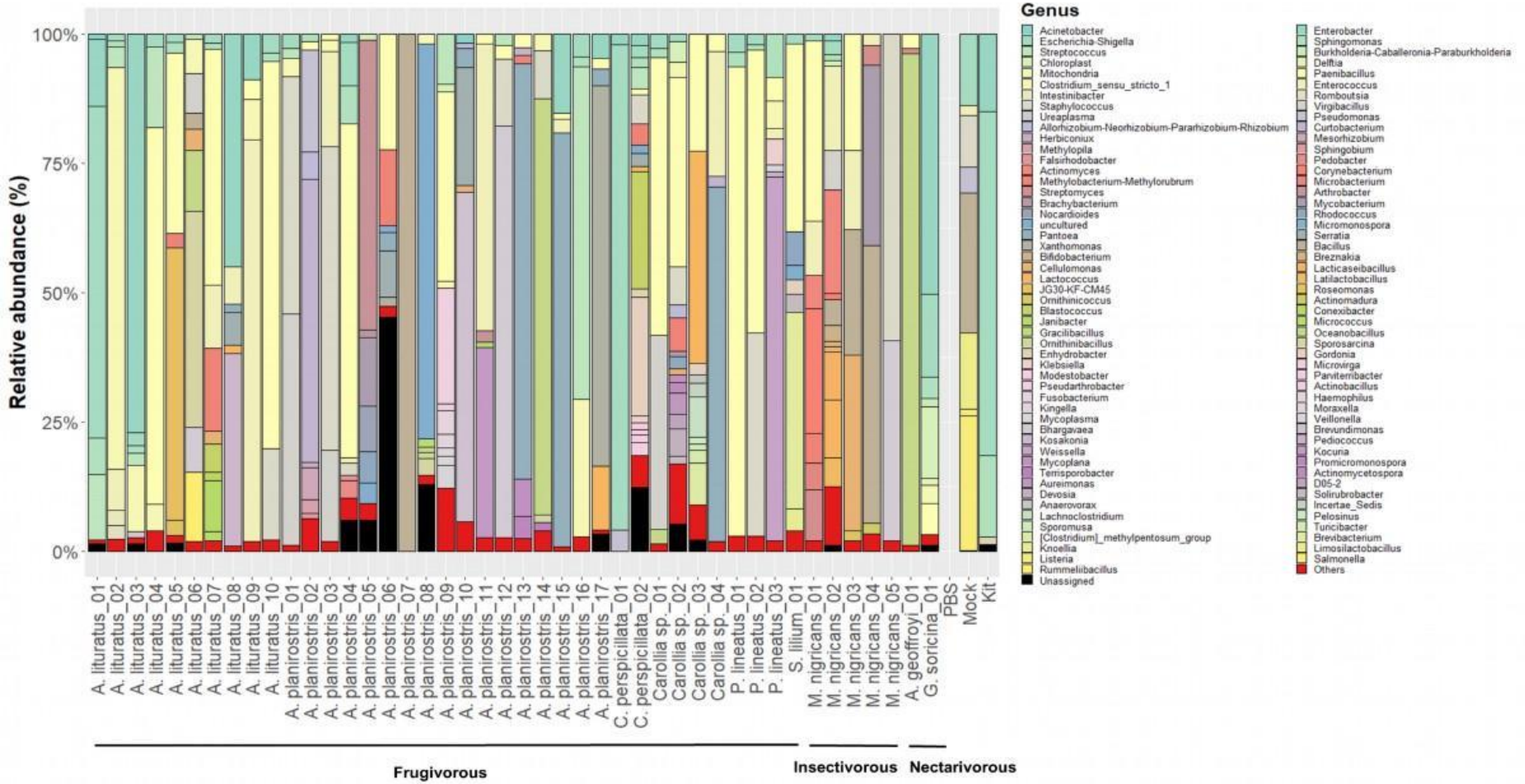
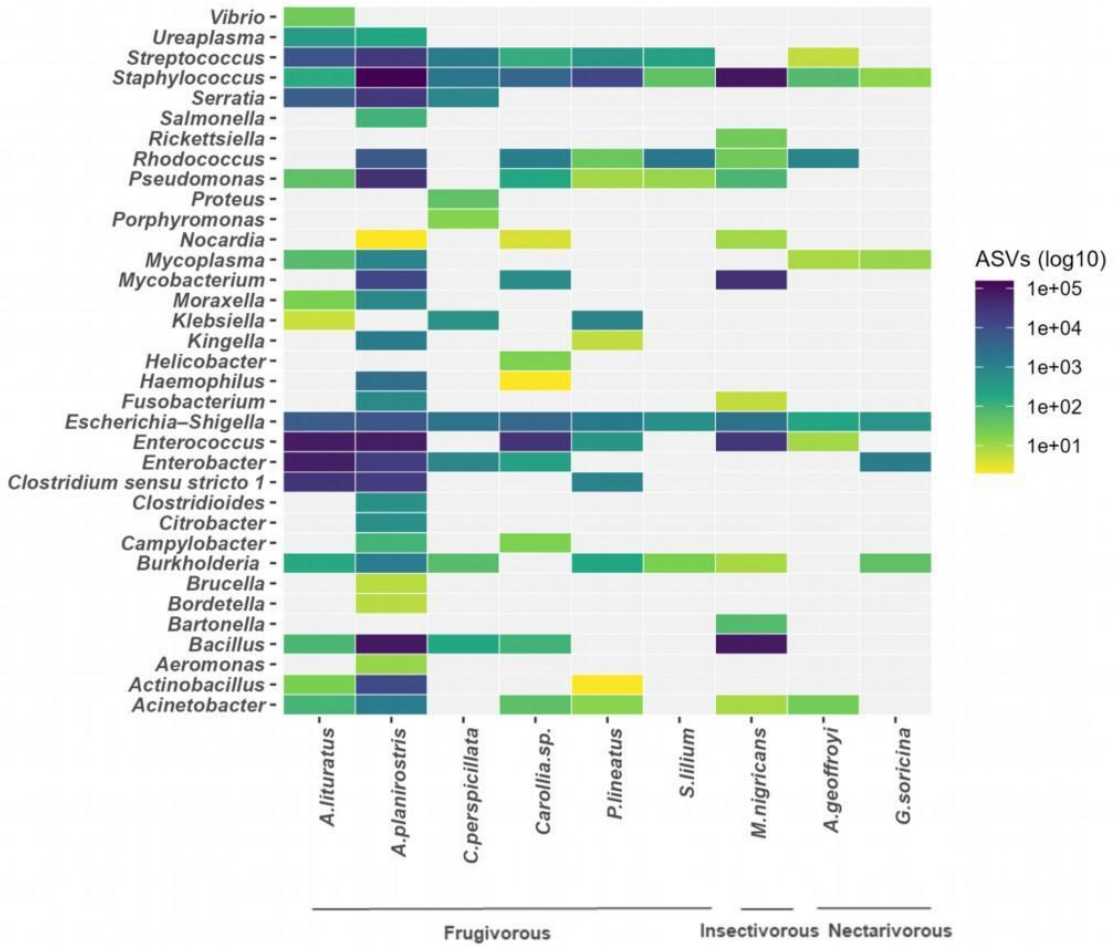


Figure 1: Location of bat capture and sampling points in four regions of Montes Claros, Minas Gerais, Brazil.


Relative abundance (%)
100%
75%
50%
25%
0%
A. lituratus_01
A. lituratus_02
A. lituratus_03
A. lituratus_04
A. lituratus_05
A. lituratus_06
A. lituratus_07
A. lituratus_08
A. lituratus_09
A. lituratus_10
A. planirostris_01
A. planirostris_02
A. planirostris_03
A. planirostris_04
A. planirostris_05
A. planirostris_06
A. planirostris_07
A. planirostris_08
A. planirostris_09
A. planirostris_10
A. planirostris_11
A. planirostris_12
A. planirostris_13
A. planirostris_14
A. planirostris_15
A. planirostris_16
A. planirostris_17
C. perspicillata_01
C. perspicillata_02
Carollia sp._01
Carollia sp._02
Carollia sp._03
Carollia sp._04
P. lineatus_01
P. lineatus_02
P. lineatus_03
S. lilium_01
M. nigricans_01
M. nigricans_02
M. nigricans_03
M. nigricans_04
M. nigricans_05
A. geoffroyi_01
G. soricina_01
PBS
Mock
Kit
Frugivorous
Insectivorous
Nectarivorous
Genus
Acinetobacter
Escherichia-Shigella
Streptococcus
Chloroplast
Mitochondria
Clostridium_sensu_stricto_1
Intestinibacter
Staphylococcus
Ureaplasma
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
Herbiconiux
Methylopila
Falsirhodobacter
Actinomyces
Methylobacterium-Methylorubrum
Streptomyces
Brachybacterium
Nocardioides
uncultured
Pantoea
Xanthomonas
Bifidobacterium
Cellulomonas
Lactococcus
JG30-KF-CM45
Ornithinicoccus
Blastococcus
Janibacter
Gracilibacillus
Ornithinibacillus
Enhydrobacter
Klebsiella
Modestobacter
Pseudarthrobacter
Fusobacterium
Kingella
Mycoplasma
Bhargavaea
Kosakonia
Weissella
Mycoplana
Terrisporobacter
Aureimonas
Devosia
Anaerovorax
Lachnoclostridium
Sporomusa
[Clostridium]_methylpentosum_group
Knoellia
Listeria
Rummeliibacillus
Unassigned
Enterobacter
Sphingomonas
Burkholderia-Caballeronia-Paraburkholderia
Delftia
Paenibacillus
Enterococcus
Romboutsia
Virgibacillus
Pseudomonas
Curtobacterium
Mesorhizobium
Sphingobium
Pedobacter
Corynebacterium
Microbacterium
Arthrobacter
Mycobacterium
Rhodococcus
Micromonospora
Serratia
Bacillus
Breznakia
Lacticaseibacillus
Latilactobacillus
Roseomonas
Actinomadura
Conexibacter
Micrococcus
Oceanobacillus
Sporosarcina
Gordonia
Microvirga
Parviterribacter
Actinobacillus
Haemophilus
Moraxella
Veillonella
Brevundimonas
Pediococcus
Kocuria
Promicromonospora
Actinomycetospora
DB5-2
Solirubrobacter
Incertae_Sedis
Pelosinus
Tunicibacter
Brevibacterium
Limosilactobacillus
Salmonella
Others

514

515 **Figure 2:** Relative abundance of bacterial genera in bats captured in Montes Claros, Minas Gerais, Brazil. The stacked bar graphs represent the
516 percentage composition (%) of genera with a composition greater than 1% in each sample. Genera less than 1% were grouped into others,
517 represented in red.


Vibrio
Ureaplasma
Streptococcus
Staphylococcus
Serratia
Salmonella
Rickettsiella
Rhodococcus
Pseudomonas
Proteus
Porphyromonas
Nocardia
Mycoplasma
Mycobacterium
Moraxella
Klebsiella
Kingella
Helicobacter
Haemophilus
Fusobacterium
Escherichia–Shigella
Enterococcus
Enterobacter
Clostridium sensu stricto 1
Clostridioides
Citrobacter
Campylobacter
Burkholderia
Brucella
Bordetella
Bartonella
Bacillus
Aeromonas
Actinobacillus
Acinetobacter
A.lituratus
A.planirostris
C.perspicillata
Carollia.sp.
P.lineatus
S.lilium
M.nigricans
A.geoffroyi
G.soricina
Frugivorous
Insectivorous
Nectarivorous
ASVs (log10)
1e+05
1e+04
1e+03
1e+02
1e+01

518

Figure 3: Heatmap illustrating the distribution of potentially pathogenic bacterial genera among bat species from Montes Claros, Minas Gerais, Brazil. The color gradient indicates the absolute abundance of amplicon sequence variants (ASVs) on a logarithmic scale (log10), highlighting differences in genus composition across hosts. The gray cells represent zero abundance.

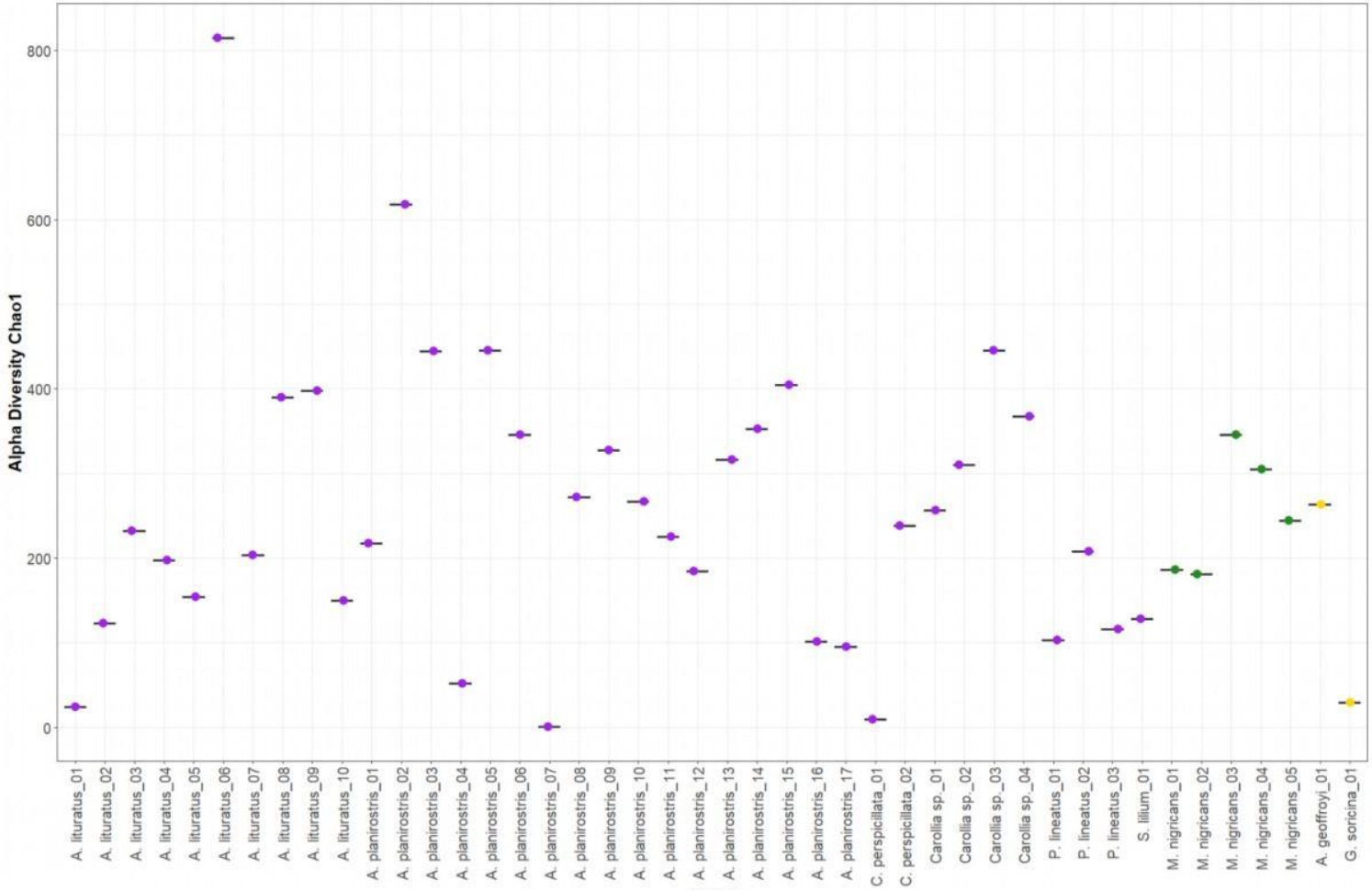


Figure 4: Bacterial alpha diversity estimated by the Chao1 index in bat samples of different species from Montes Claros, Minas Gerais, Brazil. Each point represents an individual sample, colored by feeding habit (frugivores: purple, insectivores: green, and nectarivores: yellow). The y-axis indicates the estimated richness of bacterial taxa, whereas the x-axis shows the samples organized by species.

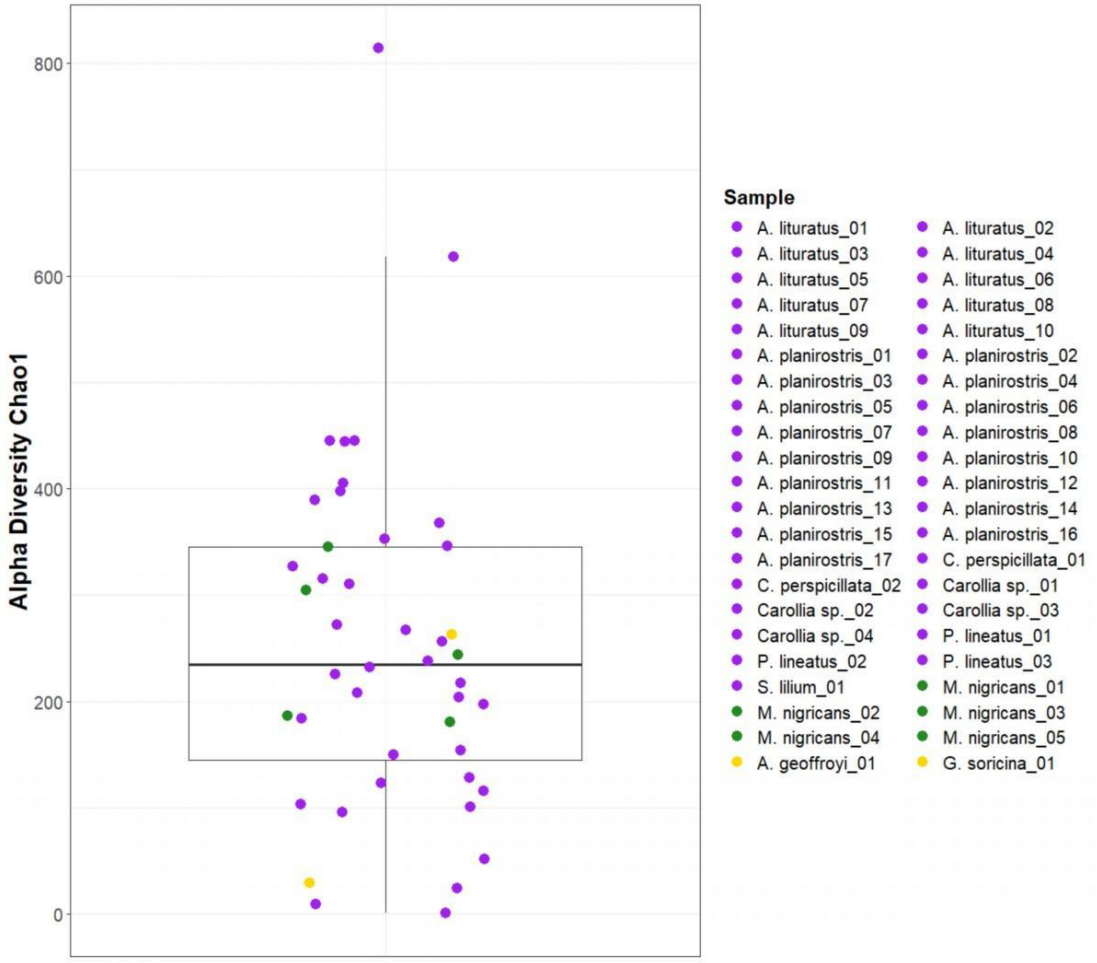


Figure 5: Alpha diversity of the bat microbiota estimated via the Chao1 index across 44 bat samples from Montes Claros, Minas Gerais, Brazil. The boxplot shows the distribution of estimated bacterial richness, with each point corresponding to an individual sample colored according to feeding habits (frugivores purple, insectivores green, and nectarivores yellow).

Supplementary Material

Metataxonomic of the rectal samples of synanthropic bats from Montes Claros, Brazil

Figures

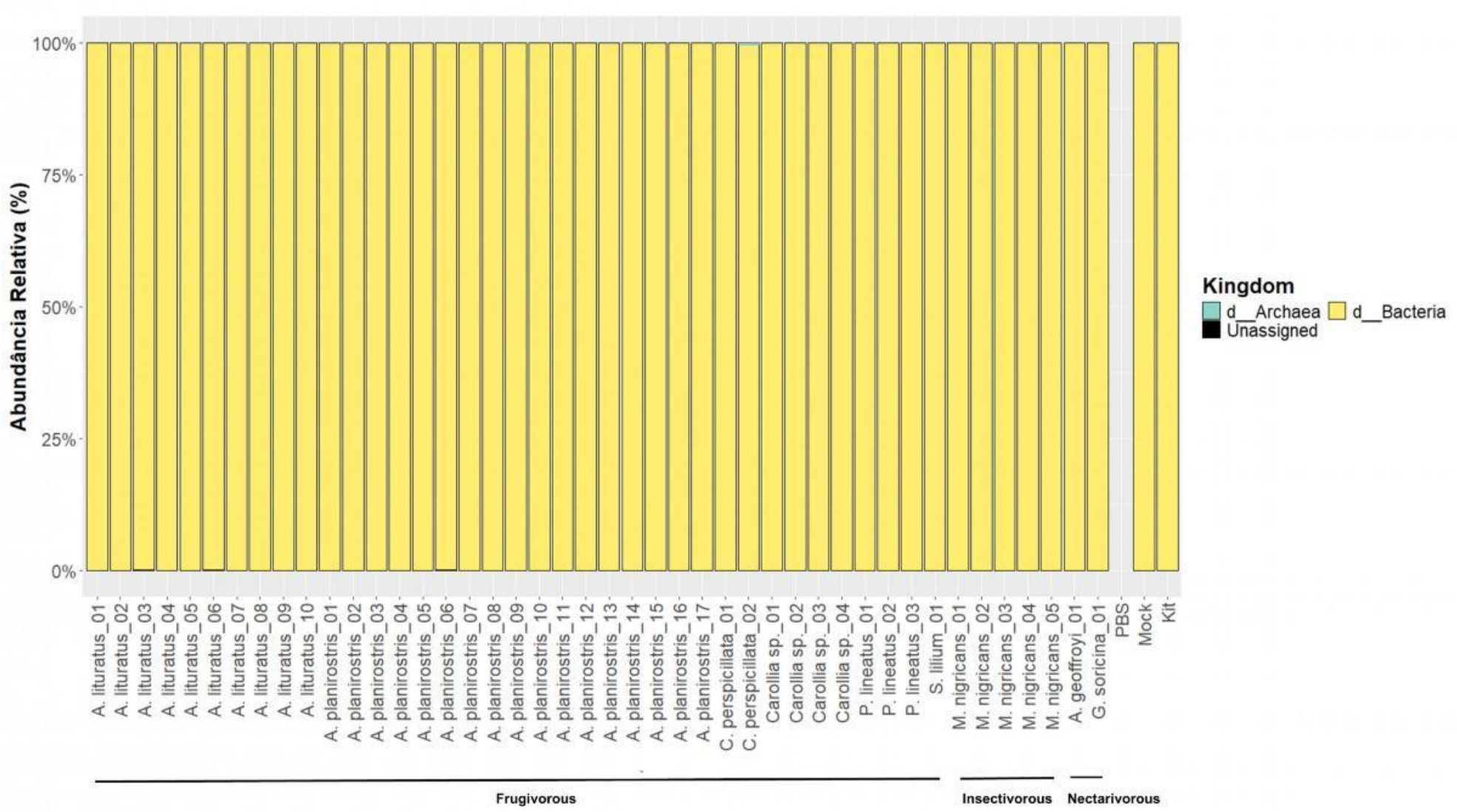


Figure S1: Relative abundance of bacterial Kindom of bats captured in Montes Claros, Minas Gerais Brazil. Stacked bar plots depict the percentage (%) composition of the most abundant Kindom in each sample.

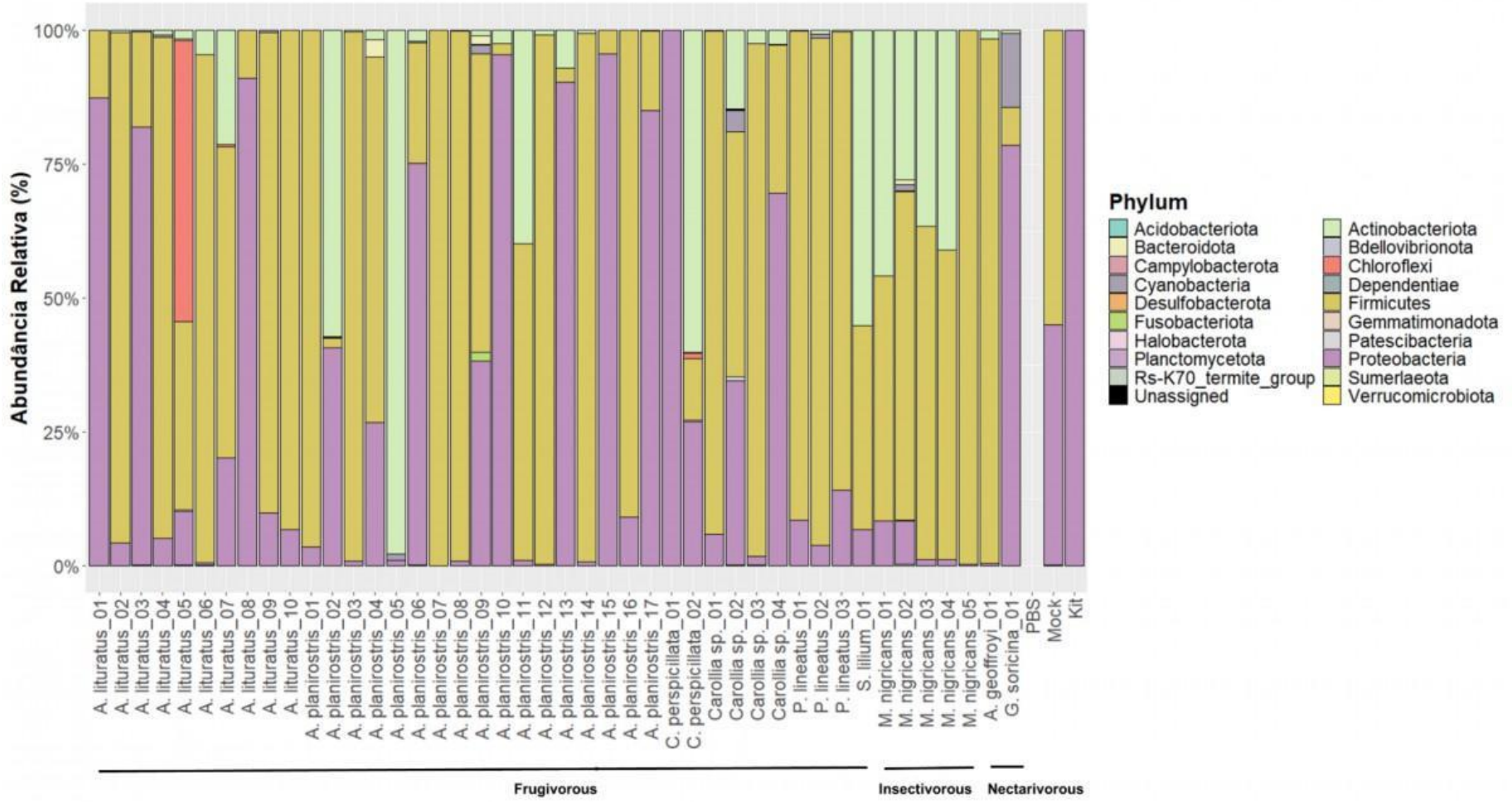


Figure S2: Relative abundance of bacterial Phylum of bats captured in Montes Claros, Minas Gerais Brazil. Stacked bar plots depict the percentage (%) composition of the most abundant Phylum in each sample.

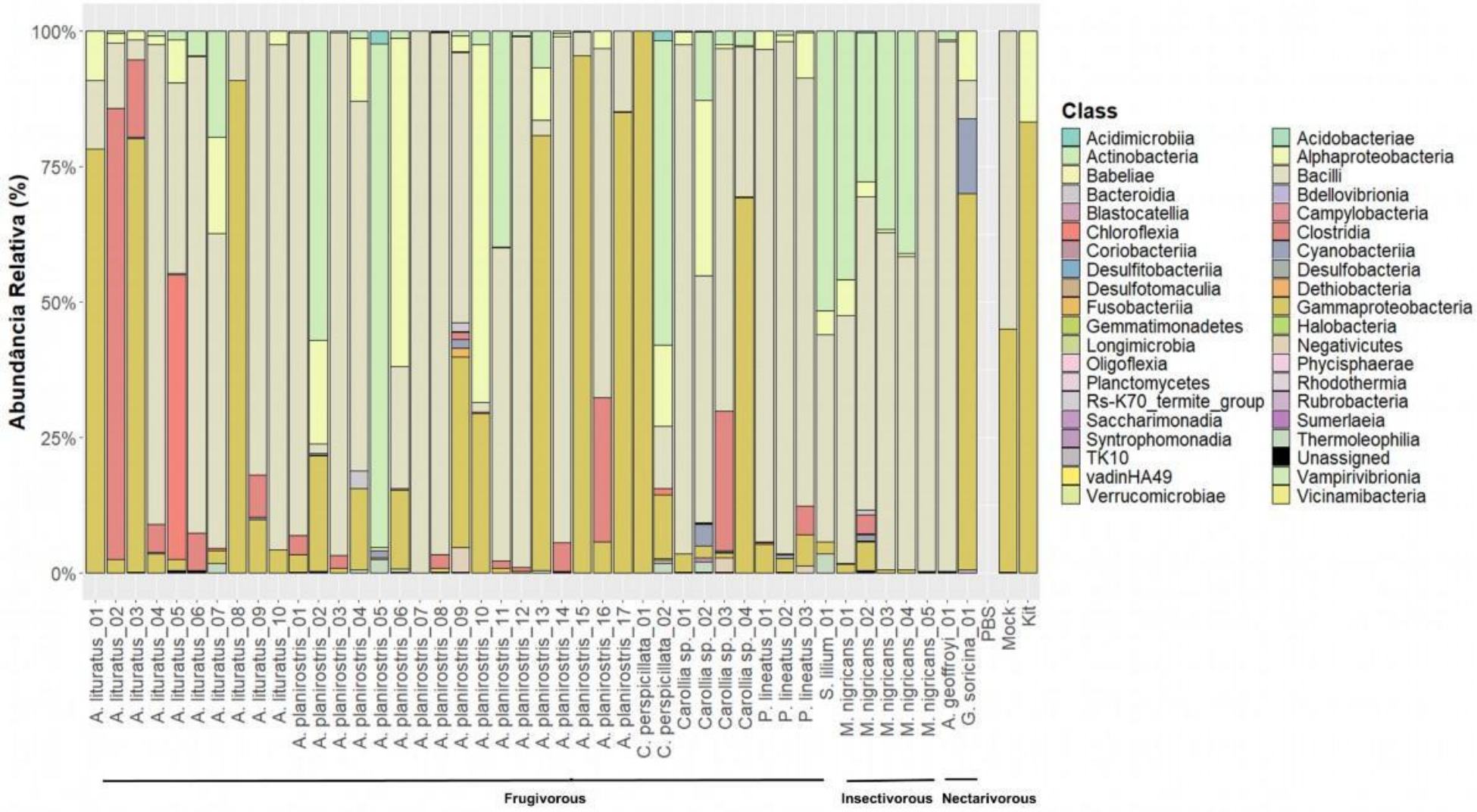


Figure S3: Relative abundance of bacterial Class of bats captured in Montes Claros, Minas Gerais Brazil. Stacked bar plots depict the percentage (%) composition of the most abundant Class in each sample.

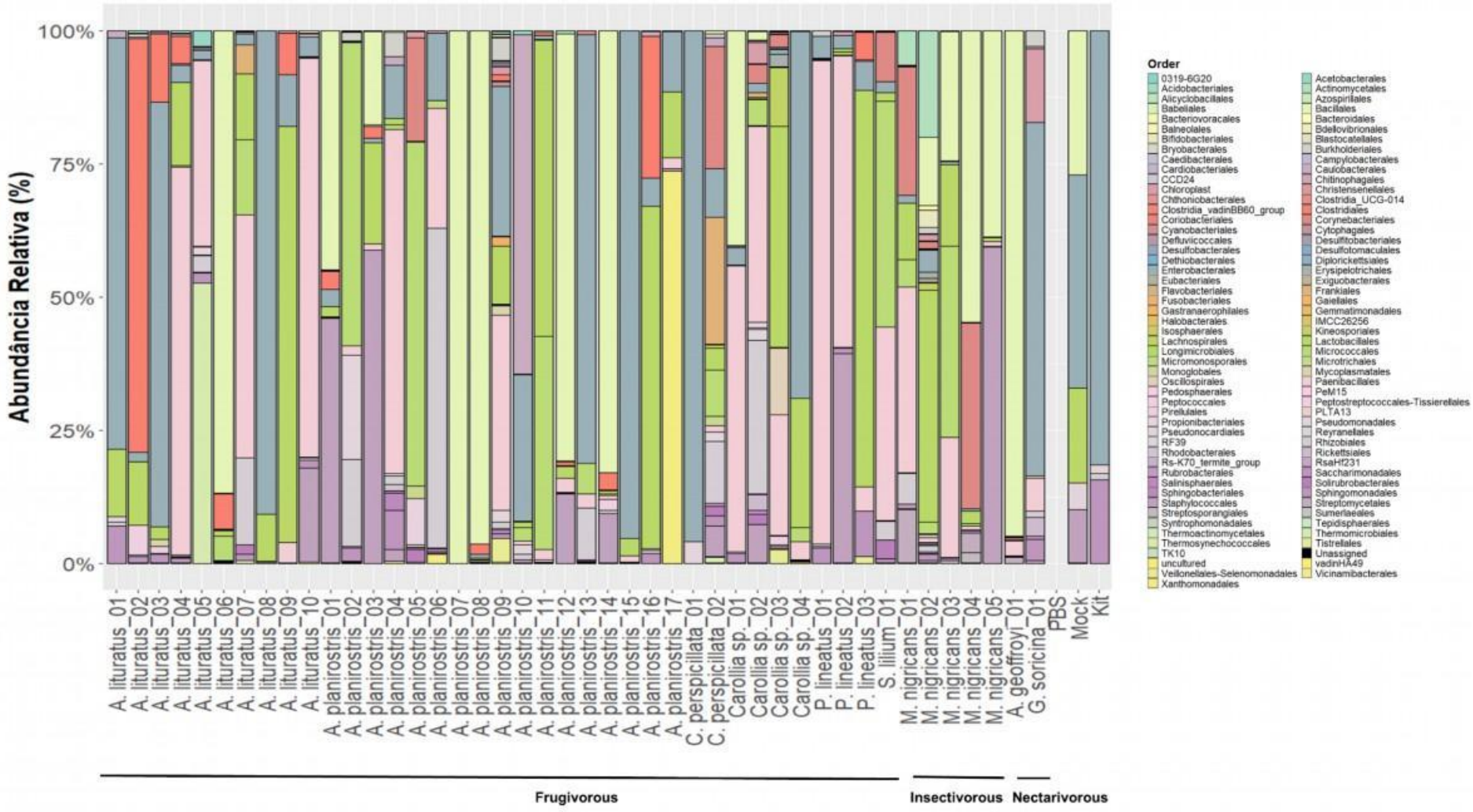


Figure S4: Relative abundance of bacterial Order of bats captured in Montes Claros, Minas Gerais Brazil. Stacked bar plots depict the percentage (%) composition of the most abundant Order in each sample.

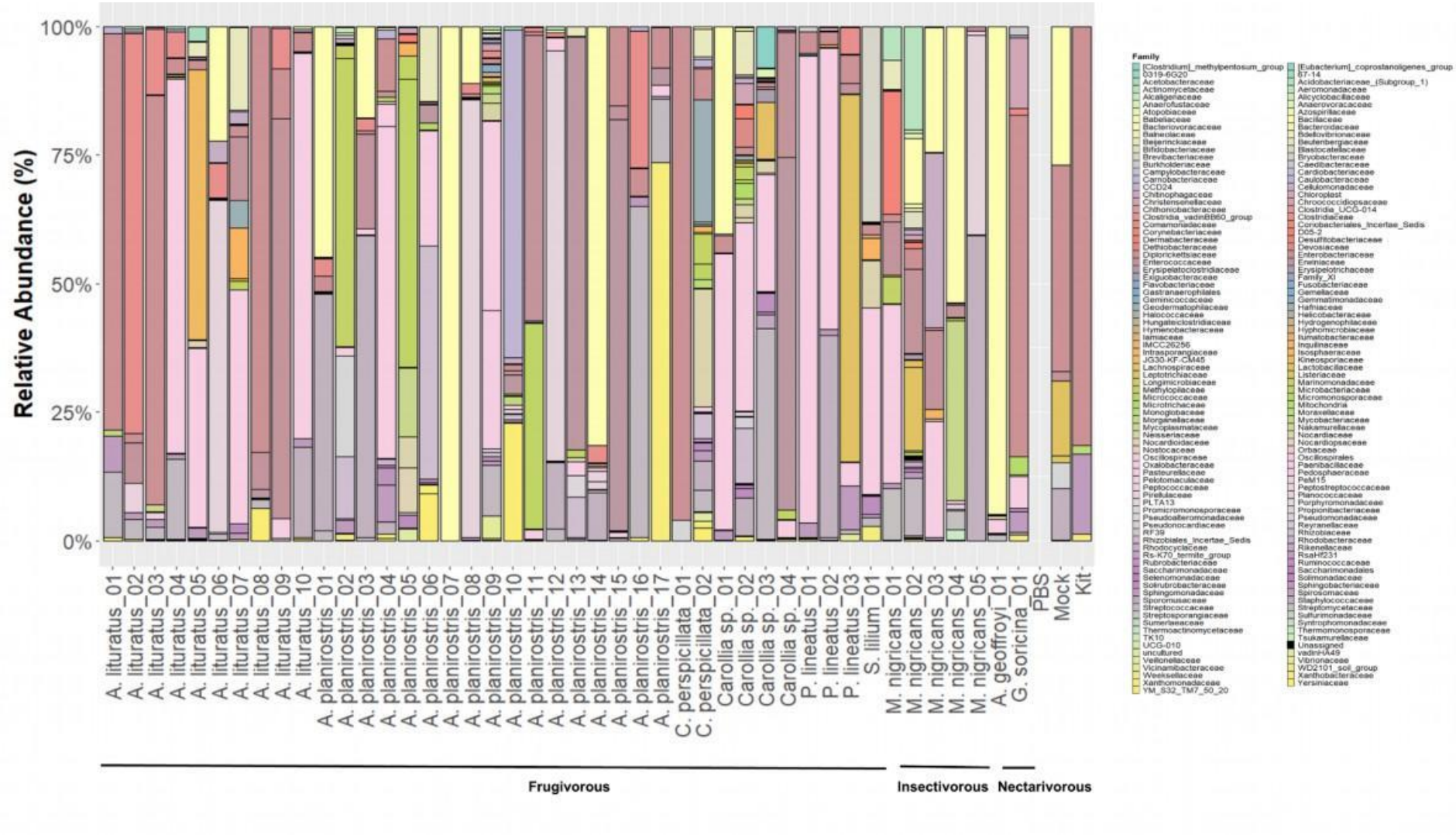


Figure S5: Relative abundance of bacterial Family of bats captured in Montes Claros, Minas Gerais Brazil. Stacked bar plots depict the percentage (%) composition of the most abundant Family in each sample.

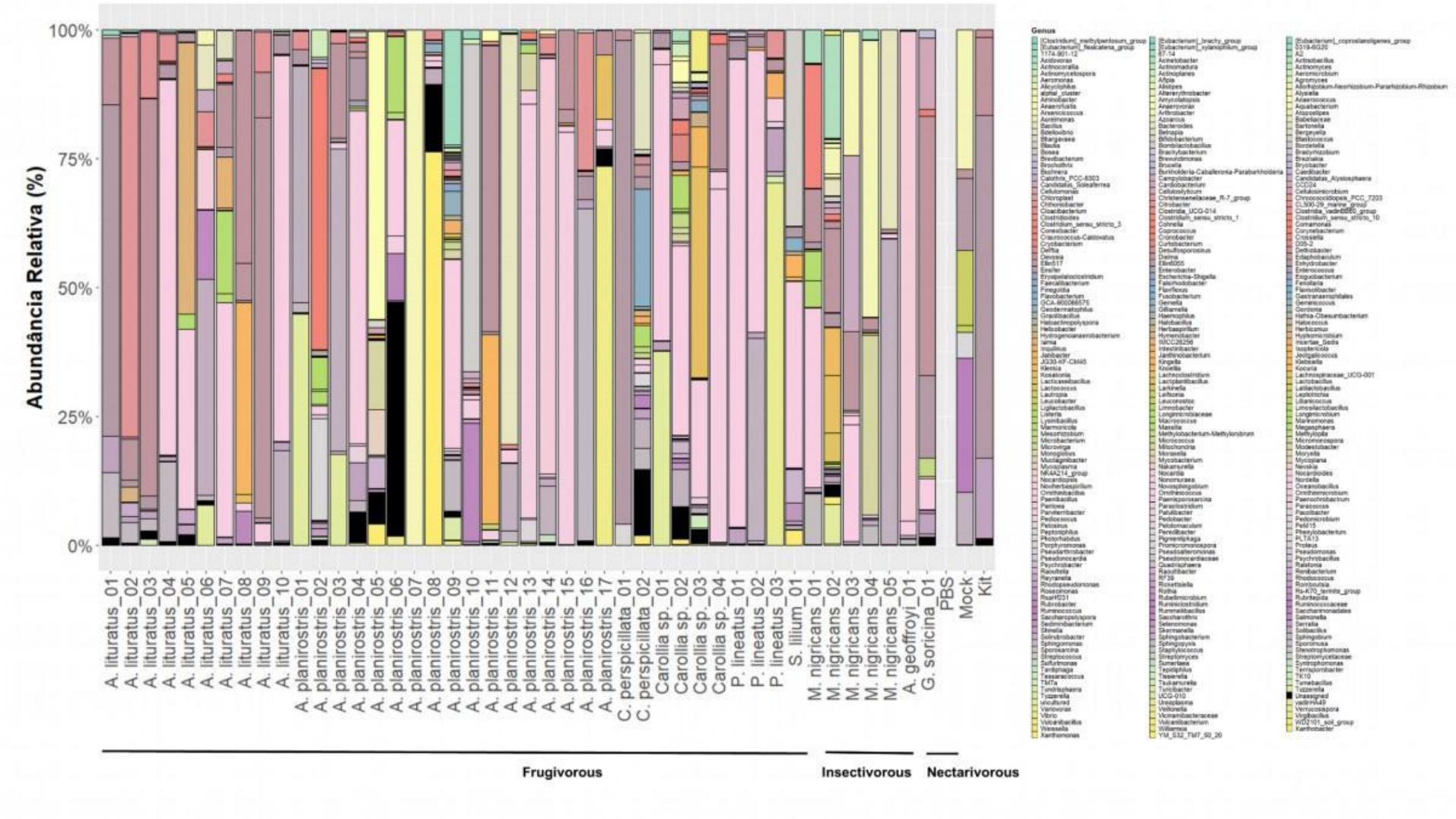


Figure S6: Relative abundance of bacterial Genus of bats captured in Montes Claros, Minas Gerais Brazil. Stacked bar plots depict the percentage (%) composition of the most abundant Genus in each sample.

Tables

Table S1: Absolute (ASVs) and relative abundance of potentially pathogenic bacterial genera identified in bat samples from Montes Claros, Minas Gerais.

Pathogenic Genus	**Absolute abundance (ASVs)**	**Relative abundance (%)***
Staphylococcus	317332	10.2158
Enterococcus	207734	6.6875
Bacillus	111408	3.5865
Enterobacter	90608	2.9169
Clostridium sensu stricto 1	60492	1.9474
Mycobacterium	47736	1.5368
Escherichia–Shigella	40395	1.3004
Pseudomonas	39364	1.2672
Streptococcus	36445	1.1733
Serratia	31283	1.0071
Salmonella	25608	0.8244
Actinobacillus	11859	0.3818
Rhodococcus	10256	0.3302
Haemophilus	2594	0.0835
Burkholderia	1753	0.0564
Acinetobacter	1589	0.0512
Moraxella	1589	0.0512
Kingella	1439	0.0463
Nocardia	1427	0.0459
Klebsiella	1404	0.0452
Mycoplasma	1038	0.0334
Fusobacterium	718	0.0231
Citrobacter	571	0.0184
Ureaplasma	520	0.0167
Clostridioides	489	0.0157
Campylobacter	99	0.0032
Bartonella	63	0.0020
Proteus	44	0.0014
Vibrio	27	0.0009
Rickettsiella	27	0.0009
Helicobacter	18	0.0006
Porphyromonas	16	0.0005
Aeromonas	12	0.0004
Bordetella	7	0.0002
Brucella	7	0.0002
Total	1.045.971	33.6726

*Calculated in relation to the 3.106.291 ASVs generated by all samples.

Table S2: Absolute abundance (ASVs) of potentially pathogenic bacterial genera identified by bat specie, of the eigth bats species from Montes Claros, Minas Gerais.

Patogenic Genus	***A. lituratus***	***A. planirostris***	***C. perspicillata***	***Carollia sp.***	***P. lineatus***	***S. lilium***	***M. nigricans***	***A. geoffroyi***	***G. soricina***
Staphylococcus	157	153844	1866	3644	14315	45	90945	66	14
Enterococcus	77200	70510	0	29773	407	0	27999	10	0
Bacillus	83	80745	177	103	0	0	79475	0	0
Enterobacter	66602	20508	793	234	0	0	0	0	1368
Clostridium sensu stricto 1	29707	21384	0	0	1008	0	0	0	0
Mycobacterium	0	13720	0	646	0	0	33370	0	0
Escherichia–Shigella	5870	7712	2151	3336	1536	573	2205	211	437
Pseudomonas	45	34045	0	177	10	12	82	0	0
Streptococcus	7351	25625	1346	138	410	238	0	6	0
Serratia	5297	25178	808	0	0	0	0	0	0
Salmonella	0	102	0	0	0	0	0	0	0
Actinobacillus	19	11838	0	0	2	0	0	0	0
Rhodococcus	0	6152	0	1224	32	1870	27	946	0
Haemophilus	0	2592	0	2	0	0	0	0	0
Burkholderia	169	1268	56	0	185	19	9	0	47
Acinetobacter	92	1396	0	48	15	0	9	24	0
Moraxella	19	807	0	0	0	0	0	0	0
Kingella	0	1424	0	0	6	0	0	0	0
Nocardia	0	2	0	4	0	0	10	0	0
Klebsiella	5	0	456	0	939	0	0	0	0
Mycoplasma	62	955	0	0	0	0	0	9	12
Fusobacterium	0	712	0	0	0	0	6	0	0

Citrobacter	0	571	0	0	0	0	0	0	0
Ureaplasma	308	196	0	0	0	0	0	0	0
Clostridioides	0	489	0	0	0	0	0	0	0
Campylobacter	0	99	0	18	0	0	0	0	0
Bartonella	0	0	0	0	0	0	63	0	0
Proteus	0	0	44	0	0	0	0	0	0
Vibrio	27	0	0	0	0	0	0	0	0
Rickettsiella	0	0	0	0	0	0	27	0	0
Helicobacter	0	0	0	18	0	0	0	0	0
Porphyromonas	0	0	16	0	0	0	0	0	0
Aeromonas	0	12	0	0	0	0	0	0	0
Bordetella	0	7	0	0	0	0	0	0	0
Brucella	0	7	0	0	0	0	0	0	0

GENERAL CONCLUSION

The main findings of this thesis include the detection of *Salmonella* spp., *Bartonella* spp., *Brucella* spp., *Yersinia enterocolitica*, *Staphylococcus aureus*, *Coxiella burnetii* and *Listeria monocytogenes* in liver samples of Brazilian bats. In addition, 35 potencially pathogenic genus were classified in the metagenomic sequencing of rectal swab samples from bats from Montes Claros, Minas Gerais, Brazil, among them bacterial genera of great zoonotic importance such as *Brucella*, *Staphylococcus*, *Salmonella*, *Bartonella* and others. The association between bats and zoonotic bacterial pathogens is eminent, and an increasing number of studies have demonstrated the capacity of these animals to harbor numerous pathogens. Although there is still no clarity or consensus in the literature about the role of bats in the transmission cycle of zoonotic bacteria, the evidence points to the need for public policies aimed at constant monitoring of these animals. A continuous effort of surveillance and education must be implemented to contain potential risks of disease transmission and also to conserve this species, which plays a fundamental ecological role.