

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.

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 (Fig. 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.

Table 1

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

Family	Genus	Species	Feeding habit	Local	Year	Sex	N
Phyllostomidae	Anoura	<i>Anoura geoffroyi</i>	Nectarivorous	Parque Estadual da Lapa Grande	2014	F	1
Phyllostomidae	Artibeus	<i>Artibeus lituratus</i>	Frugivorous	Nossa Senhora das Graças	2014	F	1
Phyllostomidae	Artibeus	<i>Artibeus lituratus</i>	Frugivorous	Parque Municipal do Sapucaia	2014	M	2
Phyllostomidae	Artibeus	<i>Artibeus lituratus</i>	Frugivorous	Parque Municipal do Sapucaia	2014	F	1
Phyllostomidae	Artibeus	<i>Artibeus lituratus</i>	Frugivorous	Vila Luiza	2014	M	2
Phyllostomidae	Artibeus	<i>Artibeus lituratus</i>	Frugivorous	Vila Luiza	2014	F	3
Phyllostomidae	Artibeus	<i>Artibeus lituratus</i>	Frugivorous	Vila Luiza	2014	NI	1
Phyllostomidae	Artibeus	<i>Artibeus planirostris</i>	Frugivorous	Nossa Senhora das Graças	2014	F	3
Phyllostomidae	Artibeus	<i>Artibeus planirostris</i>	Frugivorous	Nossa Senhora das Graças	2014	M	1
Phyllostomidae	Artibeus	<i>Artibeus planirostris</i>	Frugivorous	Parque Estadual da Lapa Grande	2014	F	2
Phyllostomidae	Artibeus	<i>Artibeus planirostris</i>	Frugivorous	Parque Estadual da Lapa Grande	2014	M	1
Phyllostomidae	Artibeus	<i>Artibeus planirostris</i>	Frugivorous	Parque Estadual	2015	M	2
N: Number of specimens; NI: not identified; F: female; M: male							

Family	Genus	Species	Feeding habit	Local	Year	Sex	N
				da Lapa Grande			
Phyllostomidae	Artibeus	<i>Artibeus planirostris</i>	Frugivorous	Parque Estadual da Lapa Grande	2015	F	1
Phyllostomidae	Artibeus	<i>Artibeus planirostris</i>	Frugivorous	Parque Municipal do Sapucaia	2014	M	1
Phyllostomidae	Artibeus	<i>Artibeus planirostris</i>	Frugivorous	Parque Municipal do Sapucaia	2014	F	2
Phyllostomidae	Artibeus	<i>Artibeus planirostris</i>	Frugivorous	Parque Municipal do Sapucaia	2015	F	1
Phyllostomidae	Artibeus	<i>Artibeus planirostris</i>	Frugivorous	Vila Luiza	2014	M	1
Phyllostomidae	Artibeus	<i>Artibeus planirostris</i>	Frugivorous	Vila Luiza	2015	F	2
Phyllostomidae	Carollia	<i>Carollia perspicillata</i>	Frugivorous	Parque Municipal do Sapucaia	2014	M	2
Phyllostomidae	Carollia	<i>Carollia</i> sp.	Frugivorous	Parque Estadual da Lapa Grande	2015	F	1
Phyllostomidae	Carollia	<i>Carollia</i> sp.	Frugivorous	Parque Estadual da Lapa Grande	2015	M	3
Phyllostomidae	Glossophaga	<i>Glossophaga soricina</i>	Nectarivorous	Nossa Senhora das Graças	2014	M	1
Phyllostomidae	Platyrrhinus	<i>Platyrrhinus lineatus</i>	Frugivorous	Nossa Senhora das Graças	2014	F	1
N: Number of specimens; NI: not identified; F: female; M: male							

Family	Genus	Species	Feeding habit	Local	Year	Sex	N
Phyllostomidae	Platyrrhinus	<i>Platyrrhinus lineatus</i>	Frugivorous	Vila Luiza	2014	M	2
Phyllostomidae	Sturnira	<i>Sturnira lilium</i>	Frugivorous	Parque Estadual da Lapa Grande	2015	M	1
Vespertilionidae	Myotis	<i>Myotis nigricans</i>	Aerial insectivorous	Parque Estadual da Lapa Grande	2014	F	2
Vespertilionidae	Myotis	<i>Myotis nigricans</i>	Aerial insectivorous	Parque Estadual da Lapa Grande	2014	M	1
Vespertilionidae	Myotis	<i>Myotis nigricans</i>	Aerial insectivorous	Parque Municipal do Sapucaia	2014	F	2
N: Number of specimens; NI: not identified; F: female; M: male							

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 $14,000 \times g$, and the pellets were separated and suspended in 300 μ L 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 (Illumina Inc., USA). Library preparation followed the in-house Neoprosecta 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 Archaea [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 Clostridia [2.79% (86,715/3,106,291)], which was absent in at least one individual of each species except *A. geoffroyi* (Supplementary Material Figure S3).

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

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

At the genus level, 319 groups were classified, with the five most abundant genera being *Staphylococcus*, *Pantoea*, *Paenibacillus*, *Oceanobacillus*, and *Enterococcus*. *Staphylococcus* [10.22% (317,332/3,106,291)] was present in at least one individual of each species; *Pantoea* [9.82% (305,039/3,106,291)] was present only in fruit bats (*A. planirostris* (5), *A. lituratus* (2) and *Carollia* sp. (3)); *Paenibacillus* [9.82% (300,581/3,106,291)] was present in at least one individual of each species; *Oceanobacillus* [8.52% (264,818/3,106,291)] was present in only twelve bats, albeit present in at least one species of each feeding habit; and *Enterococcus* [6.69% (207,734/3,106,291)] was present in only twenty-two bats but was present in at least one species of each feeding habit (Fig. 2 and Supplementary Material Figure S6).

Among the classified genera, 35 potentially pathogenic genera representing one third of the total classified ASVs were identified [33.67% (1,045,971/3,106,291)]. The five pathogenic genera most abundant were *Staphylococcus* [10.22% (317,332/3,106,291)], *Enterococcus* [6.69% (207,734/3,106,291)], *Bacillus* [3.59% (111,408/3,106,291)], *Enterobacter* [2.92% (90,608/3,106,291)] and *Clostridium sensu stricto* 1 [1.95% (60,492/3,106,291)] (Supplementary Material Table S1). A lower relative abundance of other genera with potential clinical relevance, such as *Salmonella* (0.8244%), *Mycoplasma* (0.0334%), *Bartonella* (0.0020%), *Helicobacter* (0.0006%) and *Brucella* (0.0002%), was also identified. The distribution of bats according to their species and feeding habits is shown in Fig. 3 and in Supplementary Material Table S2. Most of these genera were detected in frugivorous bats, particularly in

A. planirostris, except for *Bartonella* and *Rickettsia* [5.71% (2/35)], which were identified exclusively in insectivorous bats (*M. nigricans*). The genera *Staphylococcus* and *Escherichia–Shigella* were detected in all eight bat species. In contrast, eleven genera [31.43% (11/35)], *Bartonella*, *Bordetella*, *Brucella*, *Citrobacter*, *Clostridioides*, *Helicobacter*, *Porphyromonas*, *Proteus*, *Rickettsiella*, *Salmonella* and *Vibrio*, were identified 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 (Fig. 3).

The results of the alpha diversity analysis are shown in Figs. 4 and 5 and were generated on the basis of the Chao1 index used to estimate bacterial richness across the bat samples. In Fig. 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 (Fig. 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 (Figs. 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. liliun*, 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. liliun*, *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. liliun*, *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.

Declarations

COMPETING INTERESTS

The authors declare that they have no conflicts of interest.

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.

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Author Contribution

ACRF: Conceived of or designed study, analyzed data, and wrote the paper. VJMN, BBS, MDD and NCG: performed research, and analyzed 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 authors read and approved the final manuscript.

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Data Availability

The BioProject for this study has been registered in the NCBI database under accession number PRJNA1450673. Raw sequencing data are currently being submitted to the NCBI Sequence Read Archive (SRA) and will be made publicly available upon acceptance of the manuscript.

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31. STATEMENTS & DECLARATIONS

Figures

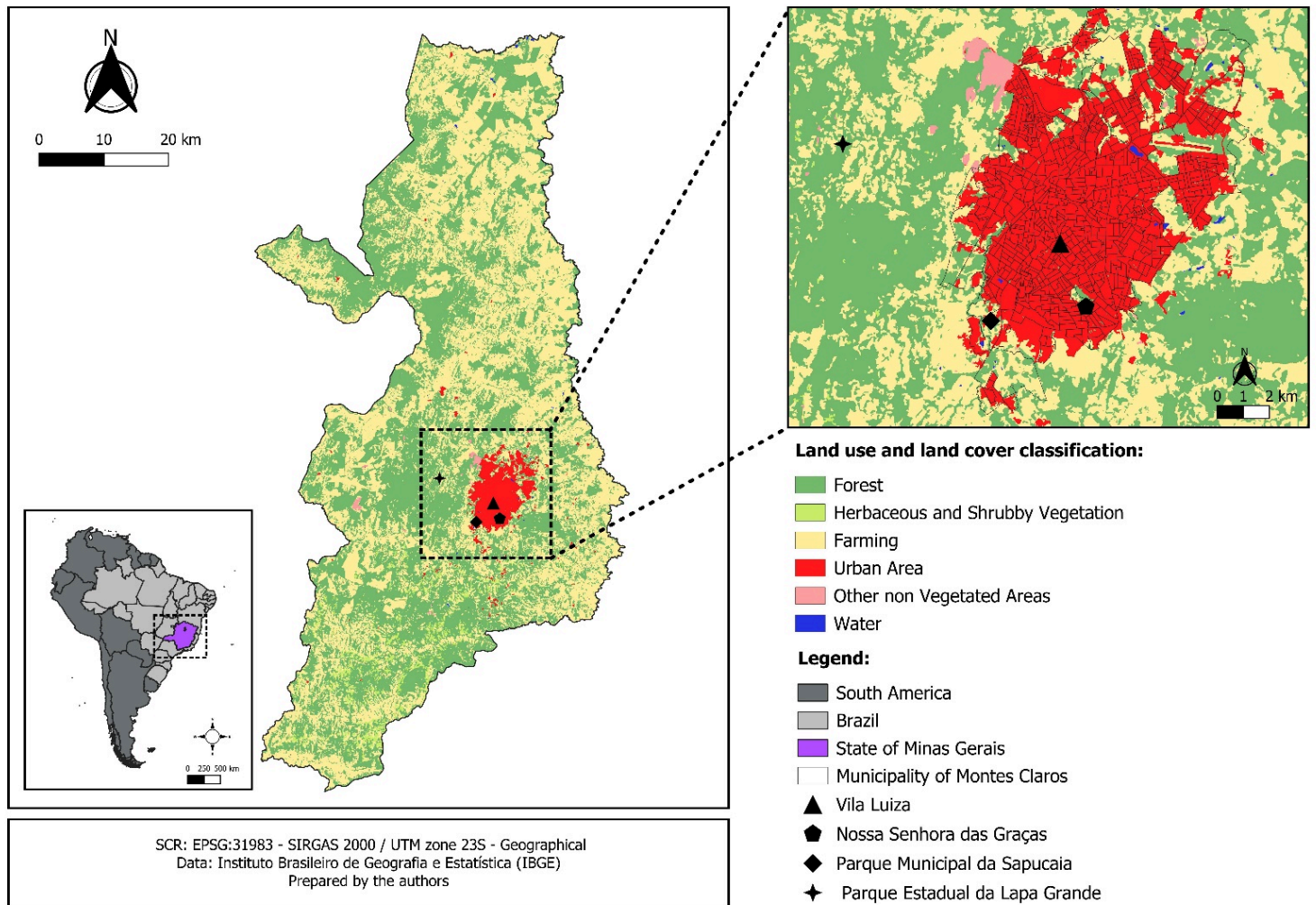


Figure 1

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

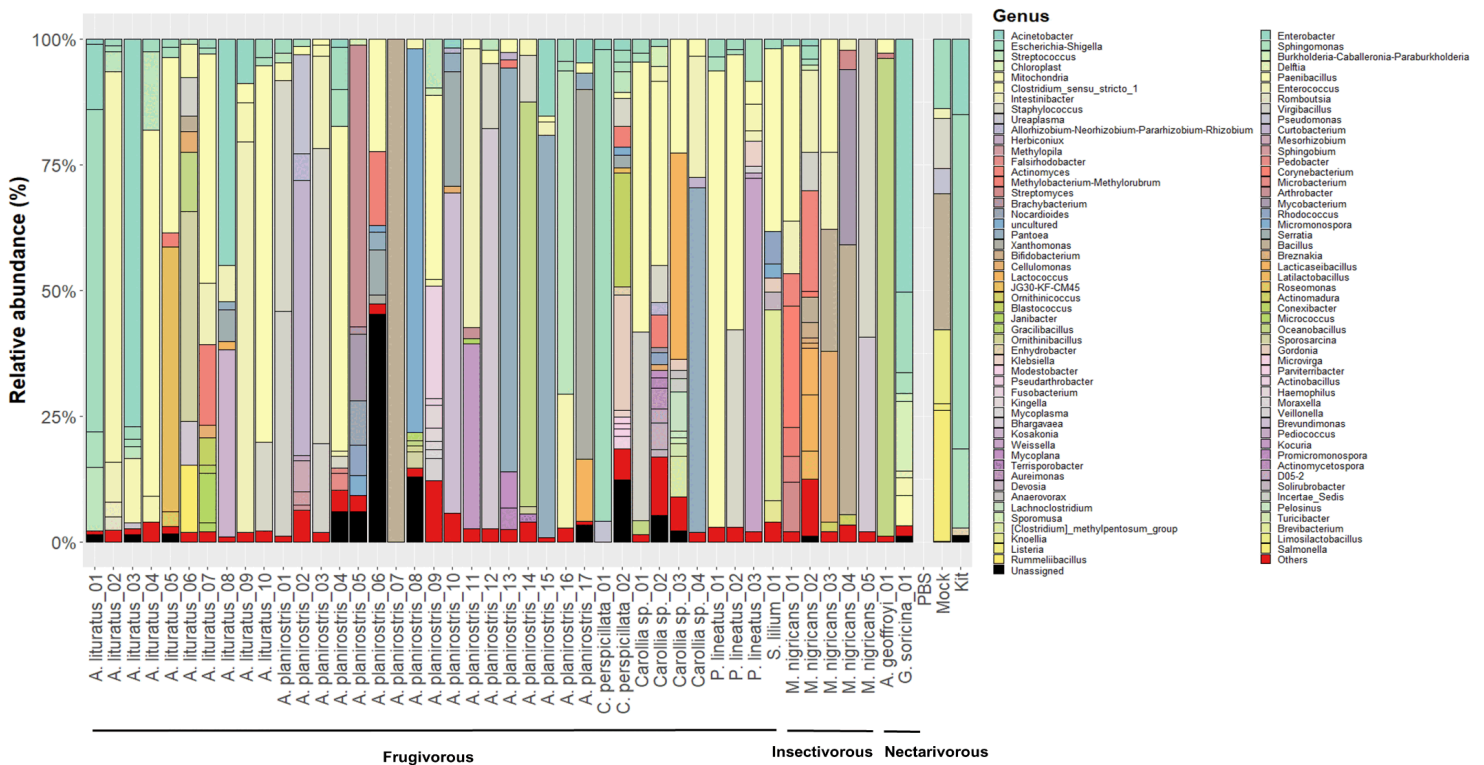


Figure 2

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

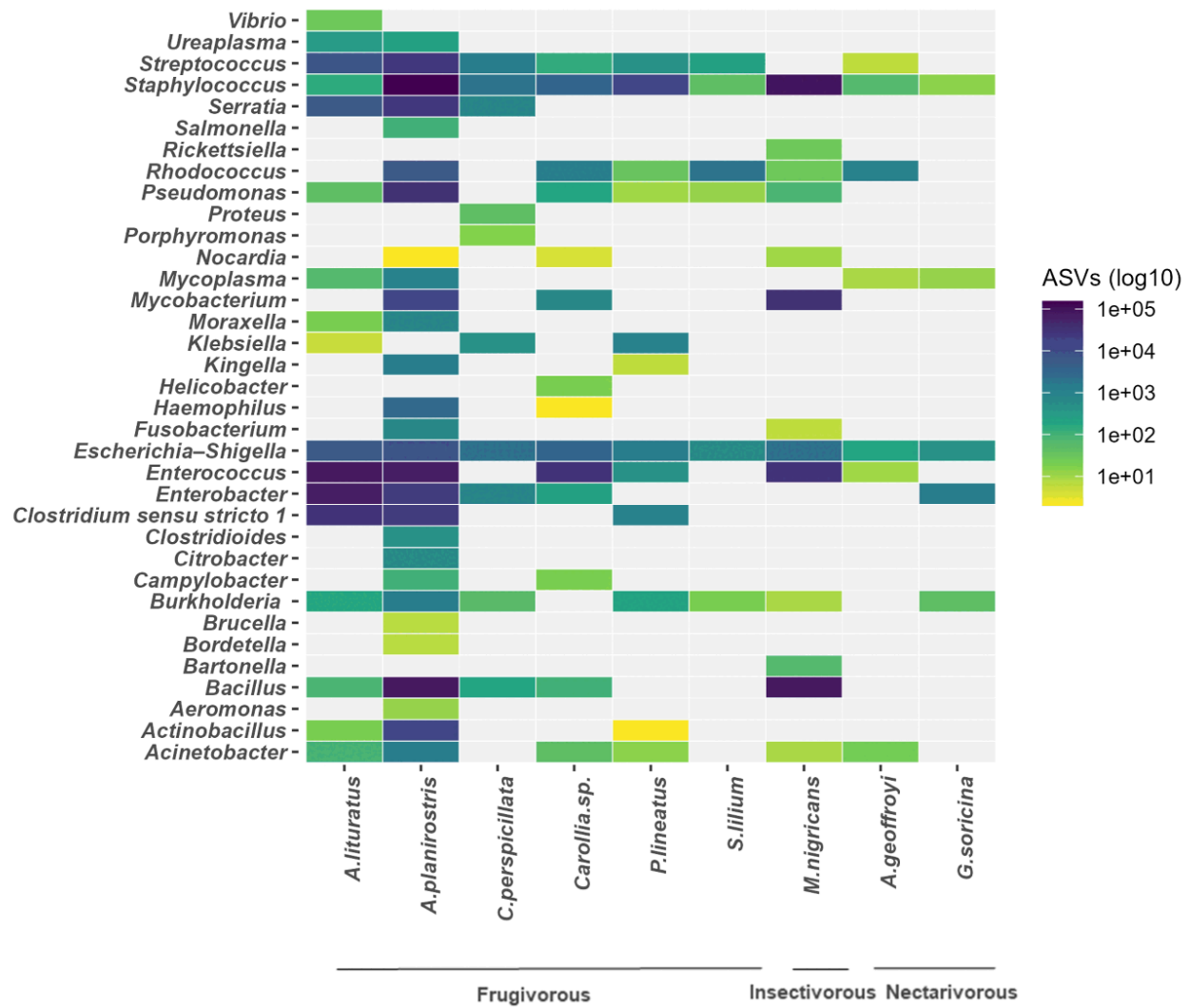


Figure 3

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.

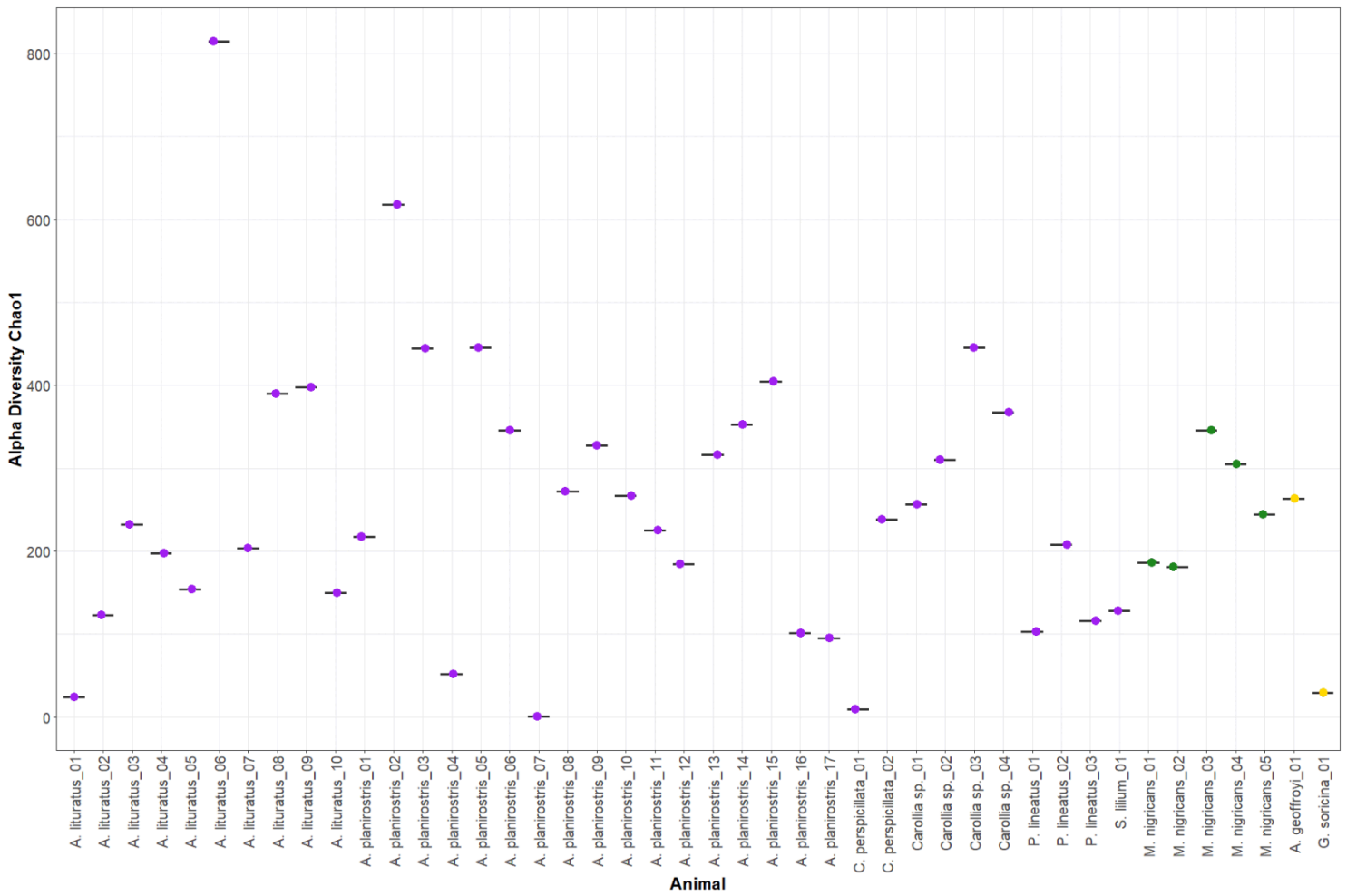


Figure 4

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.

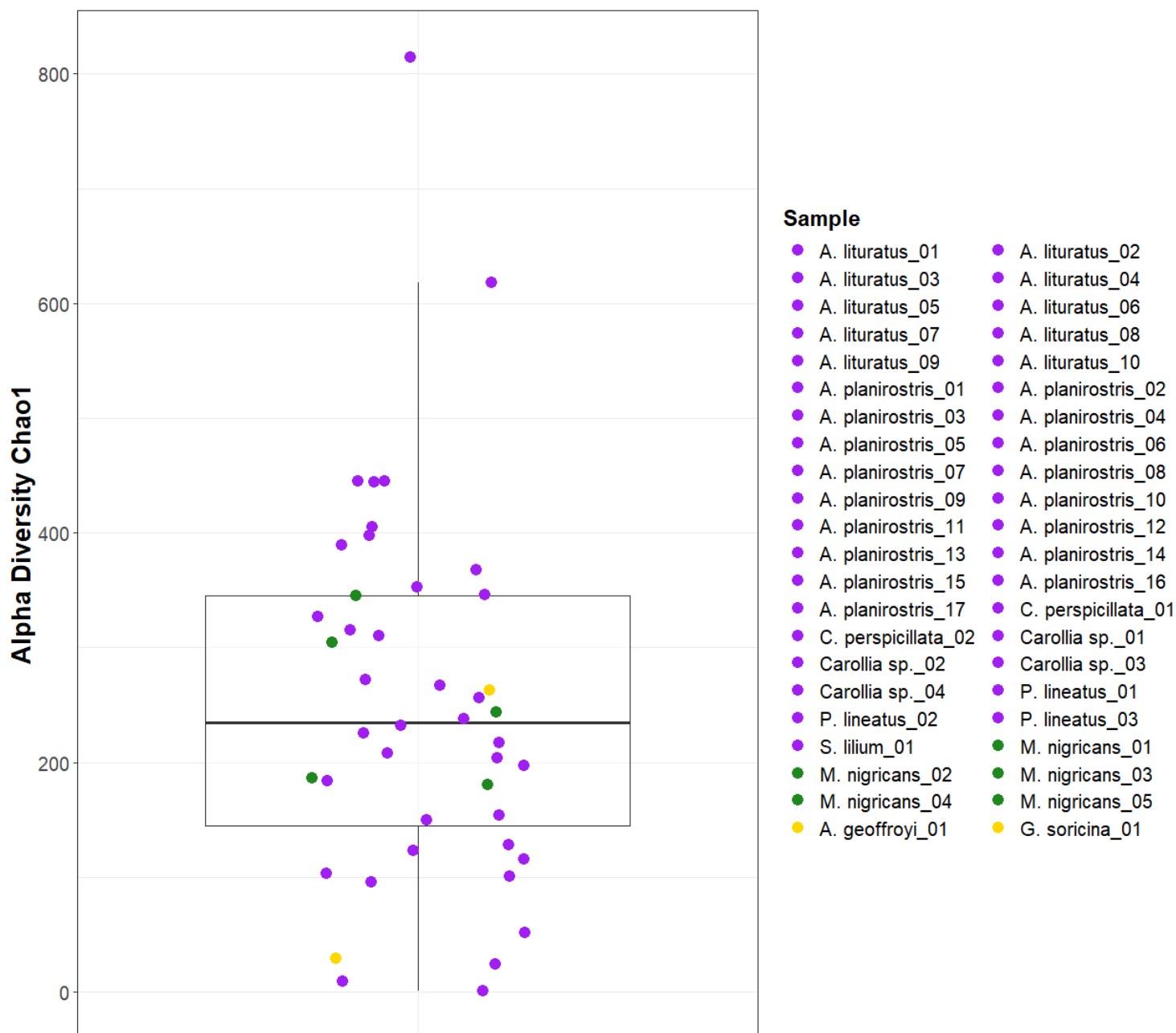


Figure 5

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).

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