

Bacterial Communities in Fecal Samples of *Myotis chiloensis* from Southern, Chile

Comunidades Bacterianas Presentes en Muestras Fecales
de *Myotis chiloensis* en el Sur de Chile

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SUMMARY: The insectivorous bat *Myotis chiloensis* is endemic of South America. Even though potentially pathogenic bacterial species of *Mycoplasma* have been reported from this species, there are no further studies regarding the bacterial communities they harbor. This may provide important insights for the better understanding of its ecology, diet and implications in cross-species pathogens transmission. Here we report a first survey on bacterial communities of *M. chiloensis* based on metagenomic analysis of fecal samples. We found that taxonomic profile is dominated by Proteobacteria (23.7 to 57.7 %) and Firmicutes (11.8 to 61.6 %), which main families are represented by *Burkholderiaceae-Enterobacteriaceae* and *Veillonellaceae-Bacillaceae*, respectively. Phyla Bacteroidetes, Actinobacteria, Cyanobacteria, Planctomycetes and Acidobacteria were also present with abundance above 1 % of the total reads. Variations among individuals could be observed at genus level and no significant differences were found between sex groups regarding taxonomic profiles and diversity. Potentially pathogenic species were also detected in all the samples, including *Staphylococcus aureus* and *Clostridium perfringens*. Our results highlight the significance *M. chiloensis* as a reservoir of pathogenic bacteria and its microbiota as an interesting ecological model due to its wide distribution. Further metagenomic studies are necessary for a better understanding of *M. chiloensis* diet and its host-symbiont relationships.

KEY WORDS: Chiroptera; Guano; Metagenomics; Microbiota; Pathogenic bacteria.

INTRODUCTION

Bats comprises 25 % of living mammalian species—the most abundant group of mammal— exhibiting an extraordinary ecological value as prey and predator, arthropod suppression, seed dispersal, pollination, material and nutrient distribution, and recycle (Banskar *et al.*, 2016b; De Leon *et al.*, 2018). *Myotis chiloensis* (Waterhouse, 1838) is endemic of South America, along with *Histiotus magellanicus*, present the southernmost known distribution for any bat in the world (Ossa & Rodríguez-San Pedro, 2015). *M. chiloensis* is only found in Chile (from Coquimbo to Navarino Island) and south Argentina (from Neuquén to Tierra del Fuego) (Ossa & Rodríguez-San Pedro). In south Chile, this bat inhabits native temperate forests and exotic tree plantations —Pinus and Eucalyptus— (Meynard *et al.*, 2014) and is found also in buildings, particularly walls, attics and under the roof (Ossa & Rodríguez-San Pedro).

Bats can harbor multiple pathogens, viruses have been well-studied (e.g. Rabies, Coronaviruses, Ebolavirus and Paramyxoviruses), however, the role of bats as reservoirs of pathogenic bacteria has been under-explored (Banskar *et al.*, 2016a; Mühlendorfer, 2013). Some studies have confirmed the presence of multiple bacterial pathogens in different bat species: by culture-dependent techniques it has been reported putative pathogens belonging to Enterobacteriaceae on *Rousettus leschenaultia* samples (Banskar *et al.*, 2016a); Bartonella species were studied and isolated from the Finland bat *M. daubentonii* (Veikkolainen *et al.*, 2014); the presence of *Staphylococcus* pathogens with high frequency of antimicrobial resistance was reported in guano of *Myotis* bats (Vandzurová *et al.*, 2012); and Mycoplasmas — including potentially novel species— were reported for multiple Chilean bats (Millán *et al.*, 2019).

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Comprehensive studies on the bacterial flora of bats are necessary for a better understanding of its zoonotic threat to humans (Veikkolainen *et al.*). In fact, this feature has been proposed as an indicator of environmental stress and climate change, since pathogen spreading may be related to habitat alteration and physiological stress (Jones *et al.*, 2009). Recently, changes such as rapid land-use were reported to shift gut microbial communities in bats —because the exposure to novel bacteria or by changing of food resources—, which consequences to host health remain unknown and may have important implications for cross-species pathogen transmission (Ingala *et al.*, 2019).

Bats have also been recently proposed as a good model for microbiome evolutionary studies in mammals, due to its wide species richness and multiple dietary niches, its important ecological role on seed spreading, and pest control (Lutz *et al.*, 2019). First bacterial community from guano by next-generation sequencing was reported in 2015 (De Mandal *et al.*, 2015). Since then, more reports on microbial diversity from different samples and bats species have been published (Banskar *et al.*, 2016b; De Leon *et al.*; Dietrich & Markotter, 2019; Ingala *et al.*; Selvin *et al.*, 2019). However, studies on bacterial communities associated with bats are still limited, and one of the reasons is the time and cost of sample collection for large-scale studies (Fofanov *et al.*, 2018). To date, there have been no reports on the bacterial symbionts of *Myotis chiloensis*. Thus, here we described the first survey on metagenomic profiling for bacterial species composition and diversity found on fecal material of the bat *Myotis chiloensis*.

MATERIAL AND METHOD

Fieldwork was conducted in November 2017 at Laguna del Laja National Park (WGS84; 19H 286739 E - 5858648 S; Bio-Bio, Chile). The wildlife capture and investigation activities of this work were conducted with the ethical approval of the Agricultural and Livestock Service (SAG), Government Administration of Chile, resolution 2770-2017, following the Chilean National laws 4.601 and 19.473. We captured individuals of *M. chiloensis* and directly collected fecal samples from the bats in sterile microtubes under the same conditions. This method (direct fecal sampling from each individual) is more complex limiting the access to suitable number of samples but guarantees a higher and more intact diversity of the sample to be described. All captures were made with the permission of SAG and SNAPE of the Chilean Government. DNA extraction was performed using the PowerSoil DNA Extraction Kit (Qiagen) from fecal samples of two adult

males —BC04, BC06— and two adult females —BC02, BC08—. DNA was amplified using Illustra Genomiphi V2 DNA Amplification kit (GE LifeSciences) to increase the genomic DNA concentration. DNA quality and concentration were verified using Qubit with the Quantifluor ONE dsDNA System kit (Promega), and absorbance ratios at 260/280 and 260/230, respectively. Sequencing of 16S rRNA genes was performed with Oxford Nanopore MinION platform using the Rapid 16S Amplicon Barcoding Kit (SQK-RAB201) for library preparation. Sequences were obtained by the MinKNOW suite and basecalling with Guppy 3.0. Reads were filtered by length (>1500 bp) and quality (>10) using NanoFilt 1.1.0 (De Coster *et al.*, 2018), adapters and barcodes were trimmed with qcat-1.1.0. Taxonomic assignment at genus level was carried out with Centrifuge 10.3-beta (Kim *et al.*, 2016), using Silva 132 database (Quast *et al.*, 2013) based on a 95 % of identity threshold, while species level was assigned using Greengenes database (McDonald *et al.*, 2012). Plots and analysis of taxonomic abundance were made with Pavian-0.3 (Breitwieser & Salzberg, 2020) and MicrobiomeAnalyst (Dhariwal *et al.*, 2017).

RESULTS AND DISCUSSION

The Chilean myotis (*Myotis chiloensis*) had a forearm length of 37.8 ± 0.81 , third finger length of 57.4 ± 1.5 , fifth finger length of 46.0 ± 1.0 and a weight of 6.4 ± 0.53 . It is a medium size bat with preference for water courses and riparian vegetation. Its roosts are tree holes and houses. Regarding the sequencing results, more than 93 % of those read were successfully classified for the samples (Table I). Common phyla were identified among the samples, including Proteobacteria (from 23.7 to 57.7 %) and Firmicutes (from 11.8 to 61.6 %) as more abundant groups, followed by Bacteroidetes, Actinobacteria, Cyanobacteria, Planctomycetes and Acidobacteria with counts above 1 % of the total reads (Fig. 1a). Very similar phyla composition was previously found for bat guano samples in the Cabalorisa Caves, Philippines (De Leon *et al.*), with the dominance of Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Chloroflexi, Saccharibacteria and Planctomycetes.

Main families of Proteobacteria were found to be *Burkholderiaceae* (2.7 % to 16 %) and *Enterobacteriaceae* (5.8 % to 12.3 %), while Firmicutes were mainly represented by *Veillonellaceae* (2 % to 22.2 %) and *Bacillaceae* (1.8 % to 5.2 %). Similar results were reported for afrotrropical bats, whose gut microbiota was dominated by Proteobacteria (60.4 %) and Firmicutes (27.4 %), where *Bacillaceae* and *Enterobacteriaceae* were reported to be significantly

Table I. Collected data for bats during fecal sampling.

ID	Specie	Sex	Age	FA	Gross Weight	Size/Weight	Net Weight	Sequencing data	
								Total Raw	Classified reads
BC08	<i>Myotis chiloensis</i>	Female	Adult	37.5	15.6	8.5	7.1	416 217	95.3/94.3 %
BC06	<i>Myotis chiloensis</i>	Male	Adult	36.7	14.3	8.5	5.8	131 886	95.4/94.0 %
BC04	<i>Myotis chiloensis</i>	Male	Adult	38.3	15.3	8.5	6.8	51 091	93.8/94.1 %
BC02	<i>Myotis chiloensis</i>	Female	Adult	36.8	14.2	8.5	5.7	20 359	93.6/93.1 %

associated with insectivorous bats (Lutz *et al.*), coincidently with *M. chiloensis* diet. Proteobacteria has also been found as the most abundant phyla independently of the feeding strategy for insectivorous, hematophagous, nectarivores and frugivorous bats (Banskar *et al.*, 2016b; Carrillo-Araujo *et al.*, 2015). However, our results showed the major colonization of Firmicutes occurs in some individuals (Fig. 1a). A similar result was only reported in gut of *Rhinolophus*

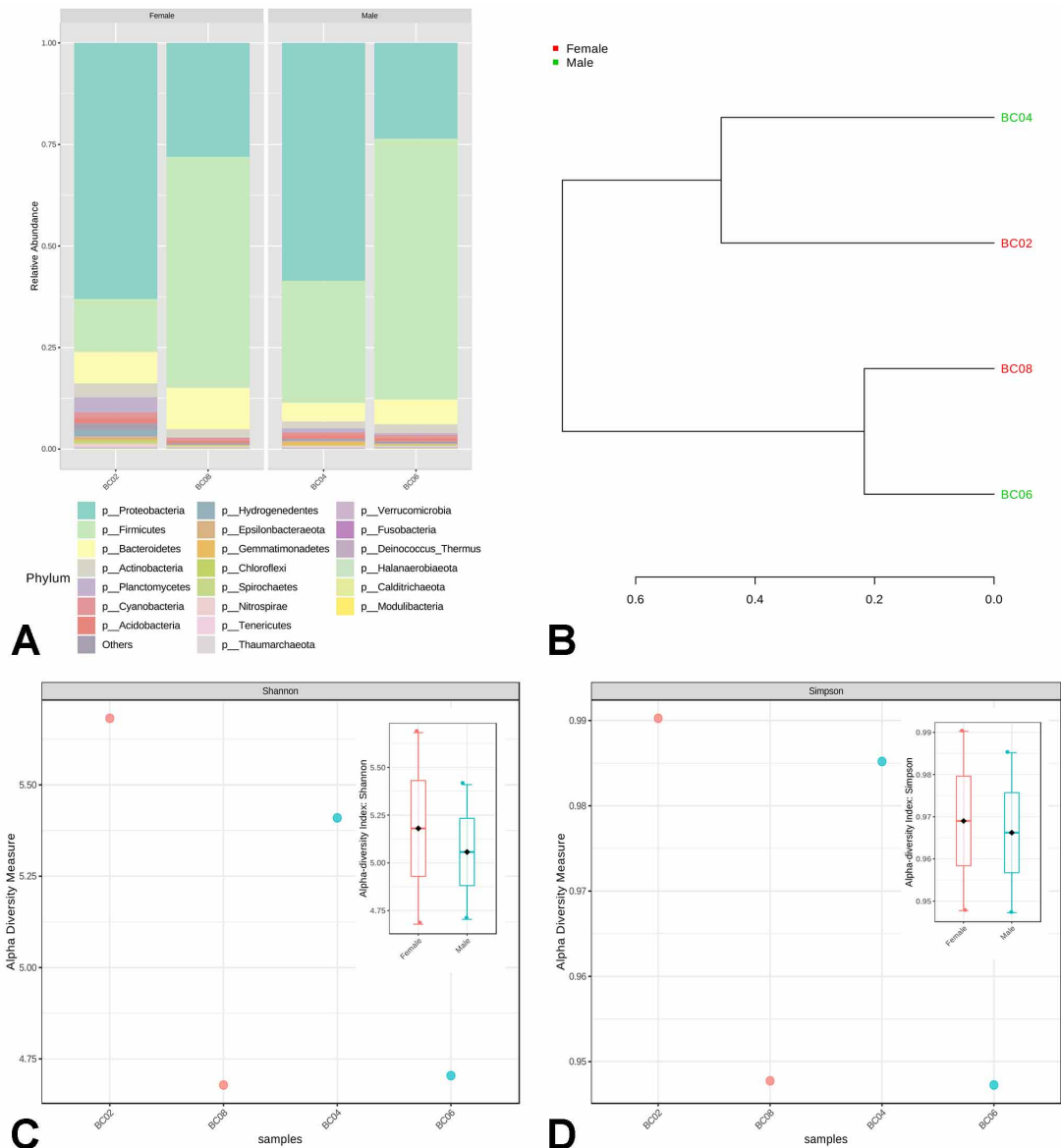


Fig. 1. Microbiome analysis of four fecal samples of *Myotis chiloensis*: (a) Phylum profiles based on relative abundance of reads; (b) Dendrogram analysis based on Bray-Curtis Index; (c) Alpha-diversity based on Shannon, p-value: 0.86191, [T-test] statistic: 0.19985; (d) Alpha-diversity based on Simpson, p-value: 0.93133, [T-test] statistic: 0.097491.

monoceros from Meghalaya, India (Selvin *et al.*), where abundance of Firmicutes (50.6 %) dominates in some samples. Completely different profiles have also been reported for guano samples from caves in India, where most dominant bacteria belongs to Chloroflexi (29.97 %) and Actinobacteria (22.55 %) (De Mandal *et al.*). Even though, dominance of Proteobacteria —particularly *Enterobacteriaceae* family— have been commonly reported in different bat guano/gut samples (Vengust *et al.*, 2018), this data suggests that due to the vast diversity of bat species and diets, a generalization of bat bacterial communities composition remains impossible to be defined.

Main genera found in *Myotis chiloensis* are summarized in Table II. All bats share the presence of *Bacillus*, *Escherichia-Shigella* and *Serratia* with similar abundance. Differences in the percentage of the sequences for some groups were found for each individual sample, independent of sex. Sample BC06 showed an increased abundance of *Carnobacterium*, as well as *Dysgonomonas* in sample BC08. Sample BC02 showed a reduced percentage of *Enterococcus*, *Staphylococcus* and *Vagococcus* when compared with the other bats and BC04 was the only individual with significant

presence of *Nevskia* genus; those might be individual differences related to diet. Studying of individual significant variants on *M. chiloensis* microbiota could be valuable for a better comprehension of each community diet and ecological implications. Further identification of species was conducted with Greengenes taxonomy. Results showed that most abundant species correspond to *Janthinobacterium lividum* (53.9 % - 1.2 %), *Serratia marcescens* (4.2 % - 1.8 %), *Propionibacterium acnes* (2.2 % - 0.8 %), *Staphylococcus aureus* (7.5 % - 1.9 %), *Veillonella dispar* (8.2 % - 0.6 %), *Haemophilus parainfluenzae* (2.2 % - 0.9 %) and *Staphylococcus epidermidis* (5.8 % -1.2 %) (Fig. 2). Other potential pathogens were found in less proportion (<1 %) such as *Salmonella enterica*, *Neisseria cinerea* and *Clostridium perfringens*. Most of these species are usual members of intestinal microbiota on mammals, however, some others can be potentially pathogenic for humans, highlighting the necessity of further studies of *M. chiloensis* as a reservoir of pathogenic bacteria. Potentially pathogenic genera such as *Burkholderia*, *Corynebacterium*, *Francisella*, *Legionella*, *Mycobacterium*, *Pseudomonas*, and *Rickettsia* were also found on metagenomic data from guano collected in caves (De Leon *et al.*).

Table II. Comparison of most abundant genera o four *Myotis chiloensis* fecal samples.

Genera	Female-BC02	Female-BC08	Male-BC04	Male-BC06	mic lineage
<i>Bacillus</i>	1.58 %	3.01 %	3.08 %	4.70 %	Firmicutes>Bacilli>Bacillales>Bacillaceae
<i>Budvicia</i>	1.27 %	1.29 %	1.37 %	0.56 %	ProteoGammaproteoEnterobacteriales>Enterobacteriaceae
<i>Carnobacteriummm</i>		0.30 %	0.66 %	3.34 %	Firmicutes>Bacilli>Lactobacillales>Carnobacteriaceae
<i>Dysgonomonas</i>	0.36 %	8.88 %	1.76 %	4.21 %	Bacteroidetes>Bacteroidia>Bacteroidales>Dysgonomonadaceae
<i>Enterobacter</i>	1.16 %	1.15 %	1.13 %	0.68 %	ProteoGammaproteoEnterobacteriales>Enterobacteriaceae
<i>Enterococcus</i>	0.71 %	3.92 %	3.94 %	8.65 %	Firmicutes>Bacilli>Lactobacillales>Enterococcaceae
<i>Escherichia-Shigella</i>	2.51 %	2.79 %	2.42 %	1.20 %	ProteoGammaproteoEnterobacteriales>Enterobacteriaceae
<i>Haliangium</i>	2.32 %	0.03 %	0.34 %	0.22 %	ProteoDeltaproteoMyxococcales>Haliangiaceae
<i>Janthinobacteriummm</i>	3.81 %	0.07 %	2.86 %	0.71 %	ProteoGammaproteoBetaproteobacteriales>Burkholderiaceae
<i>Lactobacillus</i>	0.21 %	0.89 %	0.95 %	1.56 %	Firmicutes>Bacilli>Lactobacillales>Lactobacillaceae
<i>Listeria</i>		0.11 %	0.12 %	1.19 %	Firmicutes>Bacilli>Bacillales>Listeriaceae
<i>Massilia</i>	3.98 %	0.20 %	3.25 %	0.82 %	ProteoGammaproteoBetaproteobacteriales>Burkholderiaceae
<i>Nevskia</i>		0.00 %	2.78 %	0.00 %	ProteoGammaproteoSalinisphaerales>Solimonadaceae
<i>Pseudomonas</i>	2.68 %	0.51 %	2.05 %	0.58 %	ProteoGammaproteoPseudomonadales>Pseudomonadaceae
<i>Serratia</i>	1.31 %	1.93 %	1.40 %	0.67 %	ProteoGammaproteoEnterobacteriales>Enterobacteriaceae
<i>Sporomusa</i>	0.14 %	0.92 %	0.10 %	0.60 %	Firmicutes>Negativicutes>Selenomonadales>Veillonellaceae
<i>Staphylococcus</i>	0.82 %	7.82 %	5.05 %	5.97 %	Firmicutes>Bacilli>Bacillales>Staphylococcaceae
<i>Stenotrophomonas</i>	1.95 %	0.14 %	0.75 %	0.17 %	ProteoGammaproteoXanthomonadales>Xanthomonadaceae
<i>Streptococcus</i>	0.27 %	1.27 %	1.38 %	1.70 %	Firmicutes>Bacilli>Lactobacillales>Streptococcaceae
<i>Sulfuritalea</i>	0.08 %	0.03 %	1.39 %	0.04 %	ProteoGammaproteoBetaproteobacteriales>Rhodocyclaceae
uncultured	4.44 %	19.39 %	1.70 %	20.31 %	Firmicutes>Negativicutes>Selenomonadales>Veillonellaceae
uncultured	3.31 %	0.56 %	1.42 %	1.44 %	ProteoGammaproteoBetaproteobacteriales>Burkholderiaceae
uncultured	2.34 %	0.00 %	0.59 %	0.27 %	Planctomycetes>Planctomycetacia>Pirellulales>Pirellulaceae
uncultured	2.04 %	0.02 %	0.07 %	0.01 %	ProteoGammaproteoGammaproteobacteria Incertae Sedis>Unknown Family
uncultured	1.70 %	0.01 %	0.33 %	0.21 %	Bacteroidetes>Bacteroidia>Chitinophagales>Saprospiraceae
uncultured	1.31 %	0.06 %	0.16 %	0.07 %	ProteoAlphaproteoRhodobacterales>Rhodobacteraceae
uncultured	0.24 %	1.32 %	3.36 %	0.90 %	ProteoAlphaproteoRhizobiales>Beijerinckiaceae
<i>Vagococcus</i>	0.01 %	5.79 %	6.65 %	4.25 %	Firmicutes>Bacilli>Lactobacillales>Enterococcaceae
<i>Veillonella</i>	0.21 %	0.95 %	0.15 %	0.96 %	Firmicutes>Negativicutes>Selenomonadales>Veillonellaceae

Color indicates lower(blue) and higher (red) values in the sample.

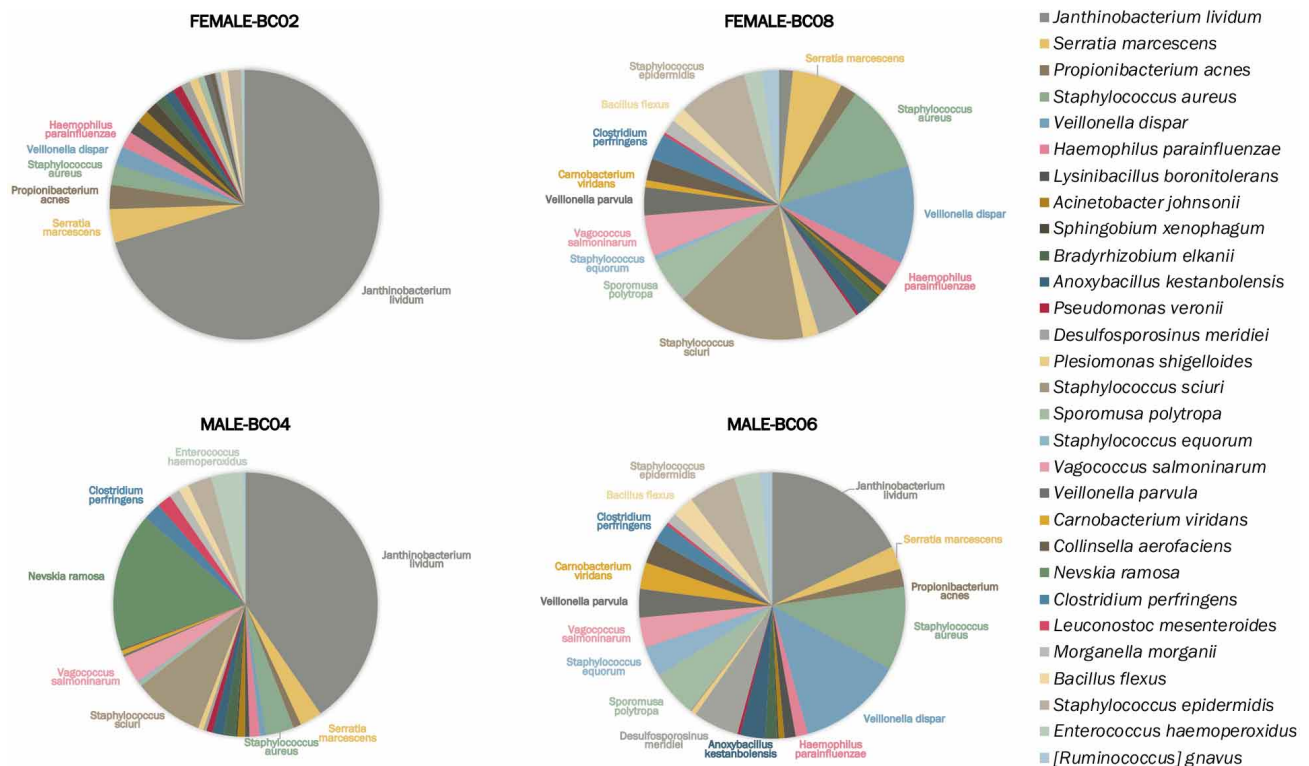


Fig. 2. Comparison of main species found in four *Myotis chiloensis* fecal samples by 16S rARN metagenomics analysis.

This report showed no evidence of deferential microbiomes for males and females of *M. chiloensis* bats. No differences in species richness were found between groups. In fact, more similar metagenomic profiles were found between individual samples from male BC04 and female BC02, as well as for male BC06 and female BC08, which was demonstrated by dendrogram analysis (Fig. 1b) and by alpha-diversity (Fig. 1c-d). Although our data has an optimum percentage of classified reads for the microbiota characterization, it is recommended to compare these results with more replicates, since differences among BC06/BC08 and BC02/CB04 could also be attributed to the total reads per sample (Table I). A result on microbiota based on the sex of bats was reported, showing significant differences only when N<10 data set were used (Lutz *et al.*). Based on these results, sex is not considered as a predictor of the bats microbiome, as other ecological factors such as geographic locality, an important predictor for Afrotropical bats microbiome (Lutz *et al.*). Diet has been proposed to be a primary factor defining the gut microbiome of bats (Carrillo-Araujo *et al.*), since it has been evidenced that feeding strategies contribute to the development of different intestinal microbiomes (Carrillo-Araujo *et al.*; Lutz *et al.*). Due to the wide distribution of *M. chiloensis* in a variety of ecological niches, it may act as a good model to study the microbiome shifting across geographical regions.

Both Simpson and Shannon indexes showed a high diversity in all the samples (>4 based on Shannon's), which is higher than previously reported for gut bacterial communities of afrotropical bat species and agrees with gut microbiome analysis of Phyllostomid bats (Carrillo-Araujo *et al.*). Differences on bacterial diversity of fecal samples can be explained by host bat species, diet and the sampling method. While other studies on guano have been conducted with guano samples collected from the floor (De Mandal *et al.*), our study was conducted after direct sampling from individual bats and higher counts of bacteria can be found in fresh fecal samples (Banskar *et al.*, 2016a). Additionally, no clear differences have been observed for bacterial community patterns between insectivorous and frugivorous bats (Banskar *et al.*, 2016b), but higher diversity was reported for insectivores species (Carrillo-Araujo *et al.*), which is the case of *M. chiloensis*. In this study we provide a first survey on bacterial communities associated to fecal material of *M. chiloensis* bats, which showed some similarities with previously reported microbiomes of insectivorous bats and the presence of potential pathogens. This data along with further studies on *M. chiloensis* microbiome will provide important insights about the ecology of this species, particularly regarding the better understanding of its diet, host-symbiont relationships, and ecological implications.

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NÚÑEZ-MONTERO, K.; SANTOS, A.; QUEZADA-SOLÍS, D.; MARTÍNEZ-URTAZA, J.; LISÓN, F. & BARRIENTOS, L. Comunidades bacterianas presentes en muestras fecales de *Myotis chiloensis* en el Sur de Chile. *Int. J. Morphol.*, 39(1):57-63, 2021.

RESUMEN: El murciélago insectívoro *Myotis chiloensis* es endémico de América del Sur. A pesar de que en esta especie se han reportado bacterias potencialmente patógenas tipo Mycoplasma, no existen estudios sobre sus comunidades bacterianas, lo cual podría proporcionar información importante para una mejor comprensión de su ecología, dieta e implicaciones en la transmisión de patógenos. En el presente trabajo se realiza una descripción de las comunidades bacterianas del murciélago *M. chiloensis* basada en análisis metagenómico de muestras fecales. El perfil taxonómico encontrado fue dominado por Proteobacterias (23,7-57,7 %) y Firmicutes (11,8-61,6 %), cuyas principales familias fueron representadas por *Burkholderiaceae*-*Enterobacteriaceae* y *Veillonellaceae*-*Bacillaceae*, respectivamente. También se encontraron los filos Bacteroidetes, Actinobacteria, Cyanobacteria, Planctomycetes y Acidobacteria con una abundancia superior al 1 %. Se observaron variaciones entre los individuos a nivel de género, sin diferencias significativas de los perfiles taxonómicos y diversidad según sexo. Se detectaron especies potencialmente patógenas en todas las muestras, entre ellos *Staphylococcus aureus* y *Clostridium perfringens*. Nuestros resultados destacan la importancia de *M. chiloensis* como un reservorio de bacterias patógenas y el estudio de su microbiota como un modelo ecológico debido a su amplia distribución. Más estudios metagenómicos son necesarios para comprender la dieta de *M. chiloensis* y sus relaciones huésped-simbionte.

PALABRAS CLAVE: Chiroptera; guano; Metagenómica; Microbiota; Bacterias patógenas.

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