Borneo Journal of Resource Science and Technology (2024), 14(2): 188-200
DOI: https:doi.org/10.33736/bjrst.7517.2024

The Gut Microbiomes of Wild Rodents within Forested Environments in Sarawak, Borneo

MUHAMMAD AMIN IMAN AZMI[*1], JULIUS WILLIAM-DEE[1], MUHD AMSYARI MORNI[1,2], NUR AFIQAH AQILAH AZHAR[1], NOR AL-SHUHADAH SABARUDIN[1], EMY RITTA JINGGONG[1], SYAMZURAINI ZOLKAPLEY[1], CHENG-SIANG TAN[3], FAISAL ALI ANWARALI KHAN[*1]

[1]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300, Kota Samarahan, Sarawak, Malaysia; [2]Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, 94300, Kota Samarahan, Sarawak, Malaysia; [3]Centre for Tropical and Emerging Diseases, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, 94300, Kota Samarahan, Sarawak, Malaysia
*Corresponding authors: muhdaminiman@gmail.com; akfali@unimas.my
Received: 1 August 2024 Accepted: 18 December 2024 Published: 31 December 2024

ABSTRACT

The gut microbiota of rodents is shaped by highly diverse bacterial communities. Within the gut environment, there are core gut bacteria that are responsible for facilitating essential bodily processes while maintaining the health of the host rodents. Currently, research on the gut microbiota of wild rodents in Borneo remains limited, especially those encompassing the potential influence of environmental factors. Through the Next-Generation Sequencing (NGS) performed using Oxford Nanopore Technologies, a total of 1052 bacterial genera were detected from 16 rodent individuals of six rodent species. These bacteria were found to be prevalent in the gut microbiota of wild rodents in forested regions. Several bacterial families of importance belonging to the phylum Bacillota were identified, including Lachnospiraceae (18%), Lactobacillaceae (20%) and Oscillospiraceae (19%). They were found to have a high relative abundance when compared with other bacterial families. The diversity of gut microbes among individual rodents showed no significant differences. However, the gut microbiome composition of wild rodents appears to have been influenced by the host species and their life stages. The outcome of this study allows for a better understanding of the prevailing core microbiome members shared across multiple wild rodent individuals within forested areas.

Keywords: Gut microbiota, Malaysia Borneo, Next-generation sequencing, rodent

INTRODUCTION

The gut microbiota of rodents is a complex ecosystem of microorganisms inhabiting their gastrointestinal tracts. It comprises high bacterial diversity, collectively forming a dynamic and intricate community (Coyte & Rakoff-Nahoum, 2019). The gut bacterial community plays a crucial role in maintaining the health of the host by facilitating essential processes such as digestion, nutrient absorption, colonisation resistance and immune system regulation (Kinross *et al.*, 2011; Valdes *et al.*, 2018; Hou *et al.*, 2022). The gut microbiome may potentially influence wildlife behaviour through chemical signalling and impacts on the host nervous system (Archie & Tung, 2015). Hence, the symbiotic relationship between these microorganisms and their host allows the formation of a stable bacterial community, comprising of gut microbiota that contribute to the various physiological processes of wild rodents.

The core gut microbiota refers to the bacteria that would define the overall microbiome composition of the host (Risely, 2020; Perlman *et al.*, 2022; Sharon *et al.*, 2022). These microbes could be essential in sustaining the overall gut microbial community while ensuring host survival. However, identifying keystone taxa that help shape the ecological structure of the gut microbiome is difficult, as highlighted by Perlman *et al.* (2022). Instead, a more suitable approach would be to determine recurring microbiome members in multiple individual hosts to gain an understanding of their significance. The concurrent bacterial taxa found across different individuals, even in an otherwise variable microbial community, could

presumably be considered members of the core gut microbiome (Turnbaugh *et al.*, 2007).

The gut microbiome diversity of rodents could exhibit variation among different host individuals (Viney, 2019). This diversity is shaped by a combination of host genetics and environmental factors (Campbell *et al.*, 2012), and studies have demonstrated that seasonal changes in rodents' diet can lead to short-term shifts in their gut microbial communities (Maurice *et al.*, 2015). Furthermore, the ageing process of rodents was found to significantly alter the composition and diversity of their gut microbiome in the long-term (Fenn *et al.*, 2023). Despite these insights, the specific environmental factors directly influencing gut microbiome diversity in rodents are still not well understood. Environmental factors that are distinct to the rodents' natural habitat could play a role in shaping their gut microbiome composition (Teng *et al.*, 2022).

The characterisation of the gut microbiome within wild rodents would contribute to a better understanding on the intricate relationships between the bacterial community and their rodent hosts. Currently, research on the gut microbiota of wild rodents in Malaysia remains limited, especially those encompassing the potential influence of environmental factors. Recent studies have instead focused on gut microbiome studies of other endangered mammals such as flying foxes (Mohd-Yusof *et al*., 2022), tigers (Khairulmunir *et al*., 2023; Gani *et al*., 2024) and primates (Jose, *et al*., 2024; Sariyati *et al*., 2024). Thus, the main objectives of this study are to taxonomically identify the gut microbiome of wild rodents and to determine the potential environmental factors that could shape their gut microbial diversity.

MATERIALS AND METHODS

Study Sites

The study sites consist of multiple mixed dipterocarp forested areas situated in both protected and non-protected regions of Sarawak, Borneo (Figure 1). The chosen locations host a variety of native forest rodent species, adapted to their respective habitats within the study sites. Among the protected areas are Gunung Gading National Park (N1°41'27.0" E109°50'45.0") and Lambir Hills National Park (N4°11'57.5" E114°02'34.3"), while Marup Atas Engkilili (N1°07'08.3" E111°38'15.8"), Ulu Poi Kanowit (N1°57'09.9" E112°13'23.0"), and Sungai Sibau Kapit (N2°00'02.7" E112°56'16.4") represent the non-protected areas (Supplementary Table 2). Rodent trapping was carried out from October 2021 to March 2023.

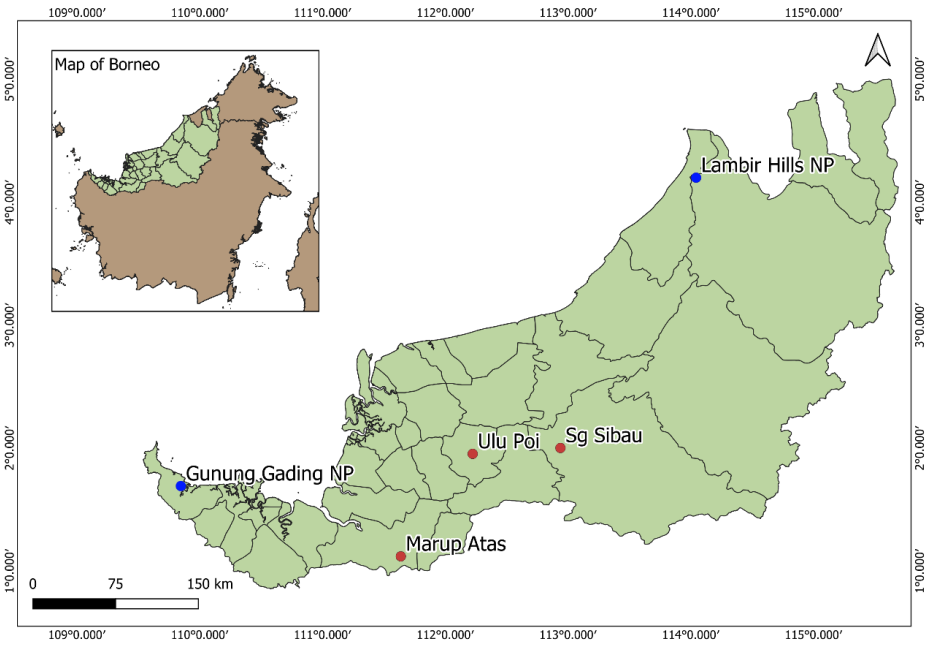

Figure 1. Map of sampling sites in Sarawak, highlighted in green (QGIS v3.21). Non-protected areas are marked with red dots while protected areas are marked as blue

Sampling Methods

Each sampling site involved five nights of trapping, with a total of 50 cage traps and 120 Sherman traps being used for the rodent trapping. The same traps were deployed at each site and sanitised beforehand using 70% ethanol to kill microorganisms and bleach to degrade the DNA of previously trapped rodents. The traps were baited with banana covered with peanut butter. The traps were checked from 0900 hours to 1200 hours, as well as from 1400 hours to 1700 hours, with bait replacement occurring every two days. Morphological measurements of the captured rodents, including head body, tail ventral, ear, hindfoot, sex, weight and life stages, were also recorded. Then, the rodents were transferred to separate sterilised breathable containers to avoid cross contamination during faecal collection. The rodents were identified in the field using descriptions and keys from Payne *et al.* (1985) and Phillipps and Phillipps (2018). Molecular methods were later used to confirm the species identification due to the presence of several cryptic species. Fresh faecal samples were collected using sterile forceps as soon as the rodents defecate in the sterilised containers. The faecal samples of each captured rodent were transferred to sterilised 1.5 ml collection tube containing RNAlater (Ambion, USA) using sterilised forceps. The collection tubes containing faecal samples were immediately stored in a cooler box. Upon arrival at the laboratory, the sample was promptly transferred to a -20 °C freezer for DNA preservation and stability.

DNA Extraction and Molecular Species Identification

A QIAmp PowerFecal Pro DNA Kit (Qiagen, Germany) was used according to the manufacturer's protocol to extract the DNA from collected rodent faecal samples. The extracted DNA was amplified through polymerase chain reaction (PCR), targeting the cytochrome *b* region of the mtDNA. The primer set used were LGL 765 (5'-GAA AAA CCA YCG TTG TWA TTC AAC T-3') and LGL 766 (5'-GTT TAA TTA GAA TYT YAG CTT TGG G-3') (Bickham *et al.*, 1995). The PCR conditions were: pre-denaturation at 94 ºC for 7 minutes, 34 cycles of: denaturation at 92 ºC for 1 minute, annealing at 50 ºC for 1 minute and extension at 72 ºC for 1 minute, followed by 72 ºC final extension for 7 minutes. The PCR products were sent to a commercial sequencing company for purification and sequencing (Apical Scientific, Malaysia). Sequencher 4.1.4 (Gene Code, USA) was then used to clean the obtained DNA sequences and the NCBI Basic Local Alignment Search Tool (BLAST) was used for species identification based on the lowest E-values of the uploaded sequences. The lower E-values indicate high degree of similarity between sequences defined by many identical residues and few substitutions or gaps (Kerfeld & Scott, 2011). Finally, the DNA sequences, along with sequences from GenBank were analysed in MEGA-X 10.2.4 software to generate a Maximum-Likelihood phylogenetic tree and calculate the pairwise genetic distance between rodent individuals subjected to Kimura two-parameter model (Kimura, 1980). The sequences of the sampled rodents were submitted to the GenBank and Sequence Read Archive databases (Supplementary Table 3).

Next-Generation Sequencing

A DeNovix DS-11 Spectrophotometer (Denovix, Delaware, USA) was used for quantification of the extracted faecal DNA as preparation for nanopore sequencing. DNA with a concentration above 20 µg/ml and a purity ratio between 1.8 and 2.0 at 260 nm and 280 nm absorbance are considered to have passed the quality check. The PCR amplification of 16S rRNA gene (27F and 1482R) were done using 16S Barcoding Kit (SQK-RAB204) from Oxford Nanopore Technologies, United Kingdom (ONT). The quality of PCR products were checked and only passed products (concentration above 20 µg/ml; absorbance ratio between 1.8 and 2.0 at 260/280 ratio) proceeded to sample pooling and library preparation following the manufacturer's protocol (ONT). The Flow Cell Priming Kit (EXP-FLP002) was then used to prime the flow cell (R9.4.1) and load the samples. Lastly, a MinION Mk1C sequencer (ONT) was used to perform nanopore sequencing for around 17 hours for each sequencing run.

Data Analysis

FASTQ files of the 16S rRNA sequences were obtained using Guppy 5.1.12 (ONT), which is the basecaller integrated in the MinKNOW 21.11.6 (ONT) operating system. The EPI2ME

(ONT) application was used to perform taxonomic classification of bacterial species according to the National Center for Biotechnology Information (NCBI) taxonomic records at 95% confidence threshold. Reads below the threshold of the default quality score of 7 were excluded. The range of the library sizes was between ~25,000 and ~1,300,000 reads. The R package phyloseq (McMurdie & Holmes, 2013) was used to normalise the dataset to the library with the lowest number of reads for further analysis using the rarefying method. The gut microbiome of rodents was visualised using the MiscMetabar package (Taudière, 2023) to produce a Sankey chart. Also, the ggplot2 package (Wickham, 2011) was used to produce an abundance graph of bacterial genera for each rodent individual. Alpha diversity was estimated using the Shannon index and plots of diversity indices were done using the phyloseq package (McMurdie & Holmes, 2013).

RESULTS

A total of 16 rodent individuals (15 adult; 1 juvenile) belonging to six different species from various study sites were used in this study (Table 1). Among the sites, Sungai Sibau Kapit had the highest number of rodent captures, with a total of five individuals. For the lowest number of sampled rodents, Gunung Gading National Park and Lambir Hills National Park recorded only two individuals each. Furthermore, Sungai Sibau Kapit had the highest diversity as four species were caught during rodent sampling. In contrast, Gunung Gading National Park recorded just one species. The phylogenetic tree and genetic distances between rodent's species (K2P) confirm the species identification of the wild rodents (Table 2; Figure 2).

The combined gut microbiome of all rodent individuals was distinguished at different taxonomic levels (Figure 3). After filtering, the average read length of all sequences of bacteria were around 1,500 bp. Overall, the most abundant phylum across all rodent species is Bacillota (formerly Firmicutes) which represents around 78% of the total abundance. The three most dominant bacterial families belong to this phylum, which includes Lachnospiraceae (18%), Lactobacillaceae (20%) and Oscillospiraceae (19%). At the genus level, a total of 1052 bacterial genera were detected, with the most abundant consisting of *Ruminococcus* (12.2%), *Ligilactobacillus* (8.9%), and *Lactobacillus* (7.2%) (Table 3). Comparing each rodent individual, their gut microbiome composition did not adhere to a strict pattern (Figure 4). The relative abundance of these predominant genera differs from each rodent individual. In particular, one juvenile rodent individual from Lambir Hills National Park (L1) had genus *Ligilactobacillus* (77.8%) as the most dominant genus.

The estimated alpha diversity, based on the Shannon diversity index values, displayed variation among the gut microbiomes of all rodent individuals, ranging from 2.2 in Lambir Hills National Park to 5.0 in Sungai Sibau Kapit (Figure 5). Non-parametric Kruskal-Wallis test was used to assess the differences between alpha diversity according to each study site and revealed these differences were not significant ($p < 0.05$).

Table 1. Number of individuals of rodent species from Gunung Gading National Park (GGNP), Lambir Hills National Park (LHNP), Marup Atas Engkilili (MAE), Sungai Sibau Kapit (SSK) and Ulu Poi Kanowit (UPK)

Rodent Species	GGNP	LHNP	MAE	SSK	UPK
Maxomys surifer	0	0	0	1	0
Maxomys tajuddini	0	0	2	1	1
Maxomys whiteheadi	2	0	1	0	0
Niviventer cremoriventer	0	1	1	2	1
Rattus tanezumi	0	0	0	0	1
Sundamys muelleri	0	1	0	1	0
Total	**2**	**2**	**4**	**5**	**3**

Table 2. Average percentage of K2P genetic distance values among rodent species based on cytochrome *b* gene

No.	Rodent Species	1	2	3	4	5	6
1	*Maxomys surifer*	-					
2	*Maxomys tajuddini*	14.5	-				
3	*Maxomys whiteheadi*	12.6	9.7	-			
4	*Niviventer cremoriventer*	16.6	16.3	15.9	-		
5	*Rattus tanezumi*	16.5	17.3	16.5	16.2	-	
6	*Sundamys muelleri*	17.8	18.0	17.0	17.2	15.9	-

Table 3. Relative abundance table in percentages of the top 23 most abundant bacterial genera (>1% overall relative abundance) according to rodent species

Bacterial Genera	*Maxomys surifer* (n=1)	*Maxomys tajuddini* (n=4)	*Maxomys whiteheadi* (n=3)	*Niviventer cremoriventer* (n=5)	*Rattus tanezumi* (n=1)	*Sundamys muelleri* (n=2)	Overall
Ruminococcus	9.69	5.68-26.8	2.59-12.5	0.76-14.2	5.54	22.5-40.4	12.2
Ligilactobacillus	0.26	0.03-0.12	0.06-0.10	0.03-77.8	0.38	0.03-0.09	8.93
Lactobacillus	11.61	3.64-9.17	11.6-25.5	0.03-1.49	4.66	1.00-8.11	7.19
Blautia	5.37	1.91-8.80	0.97-5.49	0.45-11.38	3.62	1.93-3.59	4.07
Lachnoclostridium	5.87	2.91-12.1	1.27-3.97	0.41-7.80	2.96	1.87-2.59	3.68
Flintibacter	3.12	1.82-5.28	0.66-2.76	0.24-2.80	6.22	3.24-4.80	2.80
Limosilactobacillus	1.59	0.75-5.46	2.10-4.04	0.03-4.33	6.12	1.11-3.42	2.62
Flavonifractor	2.18	2.40-3.48	1.31-3.00	0.22-4.00	4.82	2.43-3.79	2.52
Clostridium	3.57	1.09-3.05	1.09-6.40	0.34-3.03	1.90	1.11-2.11	2.19
Duncaniella	1.90	0.64-2.13	2.45-3.81	0.39-5.74	4.77	0.56-0.91	2.04
Klebsiella	0.06	0.38-3.35	1.00-7.38	0.07-11.9	0.09	0.06	1.89
Anaerostipes	0.96	1.41-4.21	0.55-1.34	0.26-6.39	1.55	1.37-2.31	1.72
Muribaculum	1.61	0.52-1.89	2.17-3.32	0.34-4.53	3.30	0.33-0.54	1.70
Prevotella	0.68	0.37-2.80	0.83-4.52	0.06-2.80	3.38	1.54-2.72	1.59
Romboutsia	4.85	0.02-4.06	0.02-11.1	0.01-1.65	0.13	0.03-0.14	1.49
Lacrimispora	1.03	0.92-3.13	0.20-1.45	0.40-2.82	1.53	1.10-3.40	1.48
Escherichia	0.31	0.29-3.13	0.84-1.71	0.13-3.73	0.18	0.16-0.27	1.43
Intestinimonas	2.26	1.01-2.23	0.52-2.35	0.10-1.44	1.85	1.82-1.86	1.35
Lachnospira	0.82	0.21-3.42	0.07-0.29	0.06-3.98	0.77	0.63-1.39	1.16
Phascolarctobacterium	0.74	0.00-3.59	0.00-5.61	0.00-0.17	0.00	0.69-2.25	1.08
Bacillus	1.06	0.79-1.28	0.82-1.08	0.98-1.55	1.11	0.61-1.03	1.07
Bacteroides	1.92	0.25-2.63	0.87-3.62	0.28-0.94	1.02	0.38-0.61	1.06
Anaerobutyricum	0.52	0.53-3.47	0.14-0.87	0.12-2.69	1.18	0.64-1.26	1.00

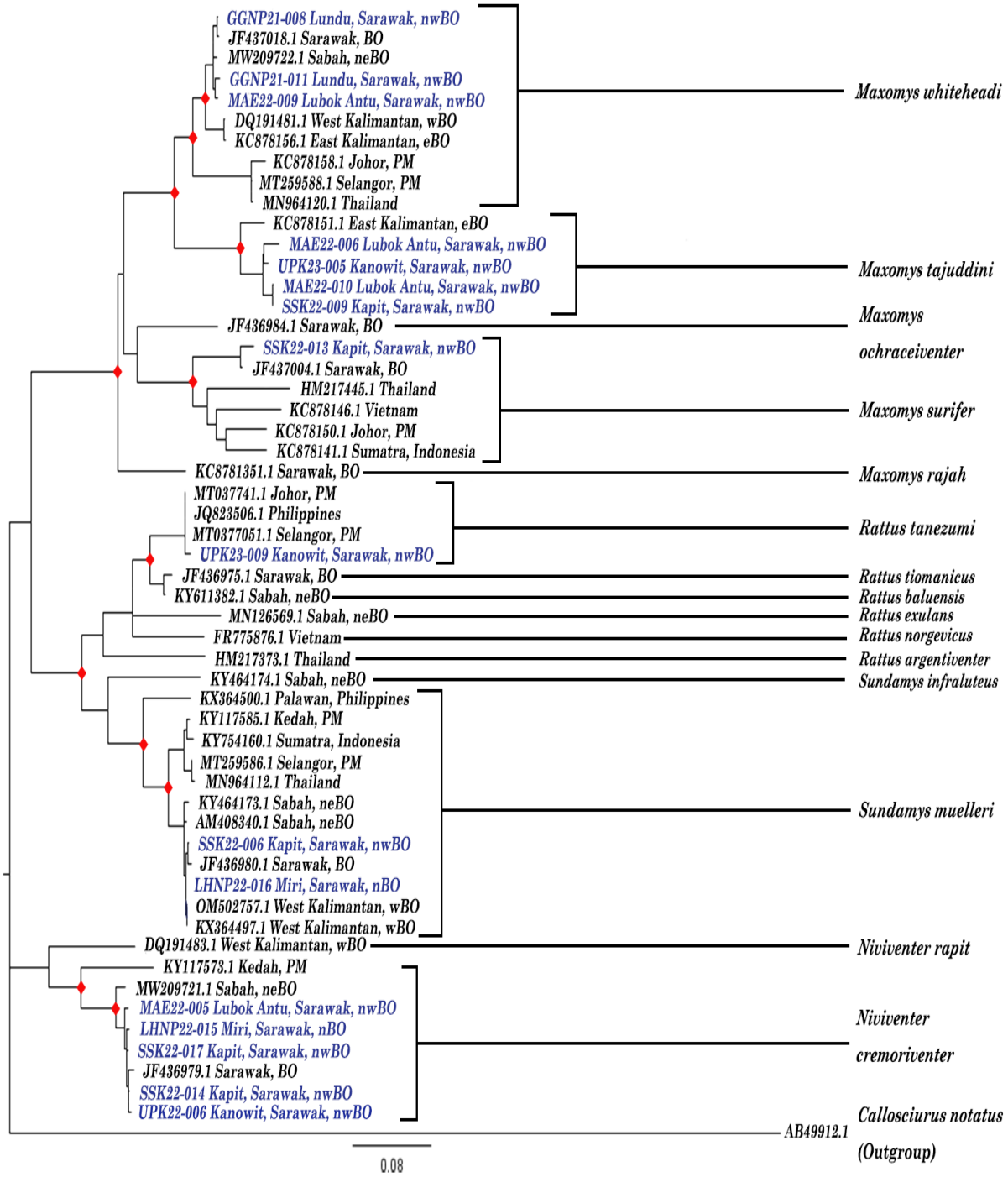

Figure 2. Maximum-Likelihood phylogenetic tree of sequenced rodent individuals highlighted in blue font. Red diamonds indicate nodes with bootstrap values >90. (PM = Peninsular Malaysia, BO = Borneo, nBO = North Borneo, nwBO = Northwest Borneo, neBO = Northeast Borneo, wBO = West Borneo, eBO = East Borneo)

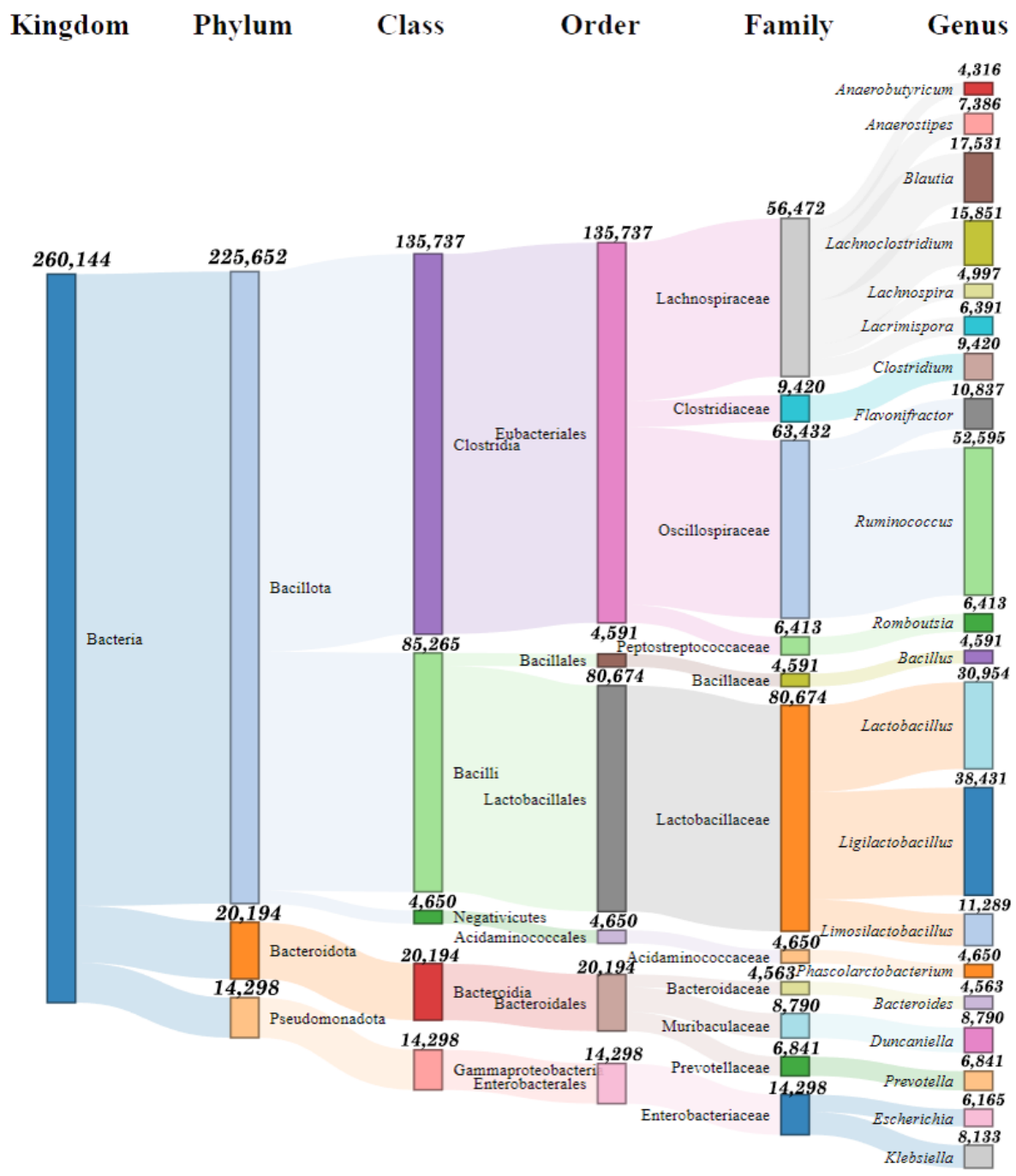

Figure 3. Sankey chart of combined gut microbiome composition of all rodent individuals (n = 16) according to different taxa levels. Only bacterial genera with relative abundance of >1% were shown. Number above nodes indicate the number of reads assigned for each taxon

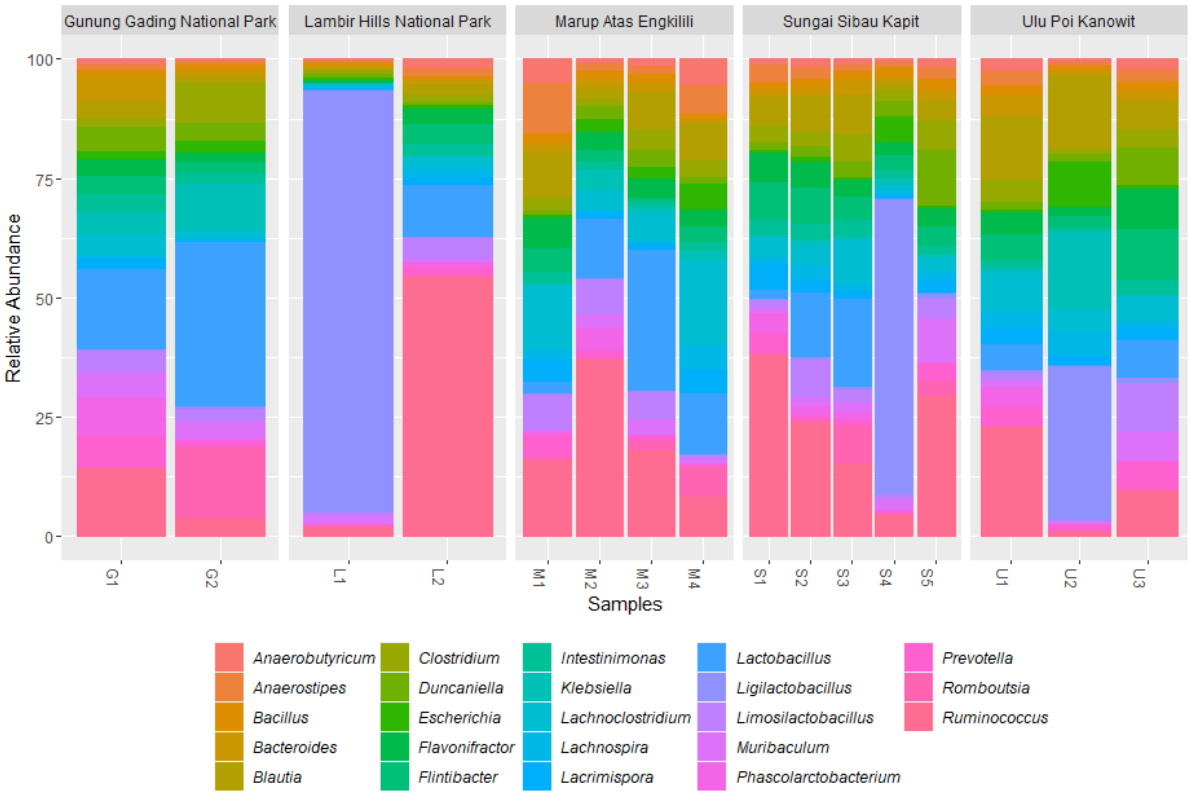

Figure 4. Abundance bar plots of the top 23 most abundant bacterial genera from rodent individuals across different localities. The top 23 genera were selected as they represent >1% of the relative abundance of the total bacterial abundance. Abbreviations of samples follow Supplementary Table 1

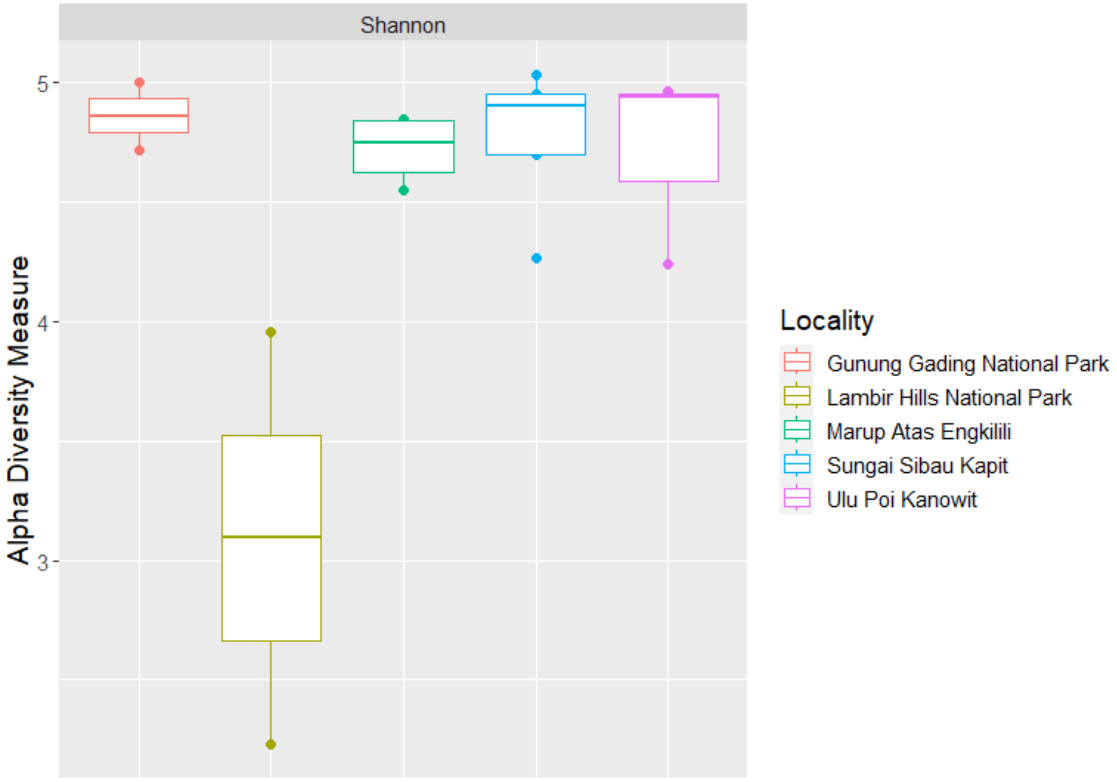

Figure 5. Box plot of Shannon diversity index of the gut microbiome of rodent individuals across different study sites

DISCUSSION

The gut microbiomes across different rodent individuals have shown patterns of variation, indicating the presence of distinct bacterial communities. Various factors, including dietary preferences, host genetics, and environmental exposures, have been reported to contribute to the observed heterogeneity (Campbell *et al.*, 2012; Maurice *et al.*, 2015). However, a consistent finding throughout all these microbiomes is the dominance of the Bacillota phylum. Similarly, recent studies have identified Bacillota, previously recognised as Firmicutes, as one of the most abundant bacterial phyla within the gut microbiota of wild rodents (Weldon *et al.*, 2015; Jahan *et al.*, 2021). These microbes are commonly found within the mammalian gut microbiota, contributing to the regulation of the host's immune system and the development of the gut epithelium (Leser & Mølbak, 2009).

One of the most abundant bacterial family observed in the gut microbiota of rodents was Oscillospiraceae. This family is also recognised as Ruminococcaceae, which is considered as a heterotypic synonym to Oscillospiraceae (Euzéby, 2010). Members of this bacterial family, alongside Lachnospiraceae, are common in the gut environment and plays a vital role in digestion and nutrient absorption. They were found to be specialised for degrading complex plant material through enzymatic activities (Biddle *et al.*, 2013). The genus *Ruminococcus*, belonging to the family Oscillospiraceae, is the most abundant genus observed across all rodent individuals. Studies have shown that *Ruminococcus* is a constituent of the rodent core gut bacteria based on their significant abundance (Wang *et al.*, 2019). The high prevalence of this bacteria observed in wild rodents could have potential importance to their feeding behaviours (e.g., Kohl *et al.*, 2022). Forest rodents in Borneo have very specialised diets and lifestyles (Phillipps & Phillipps, 2018). Since most of the rodent species found in this study typically inhabit forested areas, the presence of *Ruminococcus* may suggest that the readily abundant vegetation leads them to consume a substantial amount of plant material.

Lachnospiraceae is also a prominent bacterial family and is represented by multiple genera at varying abundances. Among these, the genus *Blautia* was observed as the most highly present genus. Liu *et al.* (2021) have highlighted the nutritional benefits of some *Blautia* species for their probiotic properties in mammals. However, they have also stated that further research is required due to the lack of comprehensive review concerning this genus. Although it was observed at relatively low abundance, another noteworthy bacterial genus within the same Lachnospiraceae family is *Anaerobutyricum*. Similar to *Blautia*, certain strains of *Anaerobutyricum* could potentially serve as probiotics, and recent studies have undergone preclinical testing in mice models for medical use (Wortelboer *et al.*, 2022). The presence of various Lachnospiraceae members within the gut microbiota suggests their pivotal role as a component of the bacterial community, and further research on the significance of this family could contribute to advancements in wildlife and humans in general.

Another dominant bacterial family is Lactobacillaceae which recorded the highest abundance for the overall combined gut microbiome of wild rodents. This family represents the lactic acid bacteria that are responsible for metabolising lactose within the gut environment (König & Fröhlich, 2017). This includes the genus *Lactobacillus* which were found to be significantly higher in reproductively active wild female rodents (Maurice *et al.*, 2015). Notably, the gut microbiota of a juvenile *Niviventer cremoriventer* individual from Lambir Hills National Park was observed to be predominantly *Ligilactobacillus*, another genus from the family Lactobacillaceae that was formerly known as the *Lactobacillus salivarius* group (Zheng *et al.*, 2020). It is possible that *Ligilactobacillus* could share similar properties with *Lactobacillus* as lactic acid bacteria. The relative abundance of *Ligilactobacillus* in the other adult *N. cremoriventer* individuals were noticeably lower (0% to 39.8%) than the juvenile individual (77.8%). The prevalence of this genus in the gut of the young rodent could imply its significance during infancy, potentially contributing to the digestion of maternal milk.

Several potentially pathogenic bacterial genera were detected at low abundance in multiple rodent individuals from various localities. This includes *Bacillus*, *Bacteroides*, *Clostridium*, and *Escherichia*. Certain species

from these genera are considered pathogenic to humans as they can cause illnesses and can be transmitted to both humans and animals through multiple pathways (e.g., direct contact with infected animals or ingestion of contaminated food or water) (Wexler, 2007; Erickson, 2016; Carlson *et al*., 2019; Fagre *et al*., 2022). While the combined abundance of these bacteria is lower than that of the other bacterial taxa, their prevalence in all wild rodent individuals indicates the potential existence of a commensal relationship between them. These bacteria may possess the capability to coexist within the established bacterial community in the rodent gut microbiota, capitalising on the nutrient-rich environment derived from the gut microbiota (Bäumler & Sperandio, 2016). Nevertheless, rodents are recognised as reservoir hosts for pathogenic bacteria, and conducting specific analyses that emphasise the prevalence of pathogenic bacteria occurring at low abundances could lead to a better understanding of their transmission dynamics.

The interactions between specific environmental factors and the composition of the wild rodent gut microbiome remains largely unknown. Shifting the focus towards the dietary behaviours and the vegetation composition of the natural habitat would help determine the factors that contribute to the variations in the gut microbial communities within mammals (e.g., Fan *et al.*, 2022). However, it is apparent that the host rodent species plays a substantial role in shaping their gut microbiota. For instance, in *Maxomys* species, *Lactobacillus* is one of the most dominant taxa with a relative abundance ranging from 3.64% to 25.50%. In contrast, for other rodent genera (*Niviventer*, *Sundamys* and *Rattus*), the relative abundance of *Lactobacillus* falls between 0.03% to 8.11%. Anders *et al*. (2021) reported similar findings, noting clear differences in the gut microbiome compositions among three wild rodent species (*Apodemus speciosus*, *A. argenteus* and *Myodes rufocanus*). The monophyletic relationship among *Maxomys* individuals implies that the phylogenetic similarity of rodent species may reflect their gut microbiome composition. According to Wang *et al.* (2022), gut microbiota among individuals of the same species from two distant locations are more similar, compared to individuals of closely related species within the same geographical area. However, the habitats are similar overall, with the main differences being in annual temperature and precipitation. Nevertheless, due to the higher number of rodent individuals within genus *Maxomys*, this assumption needs to be treated with caution. On the other hand, the life stage of the rodents seems to affect their gut microbiota based on the observed low alpha diversity exhibited by the juvenile *N. cremoriventer* individual from Lambir Hills National Park. As highlighted by Fenn *et al.* (2023), microbiome alpha diversity increases with age for wild rodents as there is a positive shift in species richness. This pattern might arise due to the incomplete development of the bacterial community within the gut environment of young rodents.

The characterisation of the combined core gut microbiota in this study may not accurately reflect the microbiome composition of distinct rodent species. Given the non-invasive nature of faecal sampling, it is recommended to obtain a larger sample size that encompasses a diverse range of wild rodent species and accounts for different life stages. This approach would yield a more comprehensive understanding on the dynamics of the host-specific core gut microbiome, especially with an increased number of representatives for each species. Furthermore, including more localities with distinct habitat types and incorporating additional environmental data into the characterisation of the rodents' gut microbiome would provide insights into the constituents that influence the structure of the bacterial community (e.g., Lobato-Bailón *et al.*, 2023). Determining the effects of distinct habitats on the rodent gut microbiome could have significant implications for zoonotic disease risks, as certain environmental factors may potentially influence pathogenic bacteria transmission.

CONCLUSION

This study effectively characterises the gut microbiome of multiple wild rodents inhabiting forested areas in Sarawak, Borneo as bacteria with high relative abundance hold potential as core constituents of the rodent gut microbiome. Furthermore, it appears that the gut microbiome composition of wild rodents is influenced by the host rodent species and their life stages, as evidenced by the abundance patterns of certain bacterial taxa.

ACKNOWLEDGEMENTS

The authors sincerely thank Sarawak Research and Development Council for their funding and support (GL/F07/SRDC/05/2021; RDCRG02/CAT/2020/_33), and Sarawak Forestry Corporation for the permission to conduct our work in Sarawak (Permit No. SFC.810-4/6/1 (2021) and Park Permit No. WL 21/2021). We also thank Faculty of Resource Science and Technology at Universiti Malaysia Sarawak (UNIMAS) and its staff for assisting in logistics and laboratory support. UNIMAS also supported some of the authors through the Zamalah Graduate Scholarship and Postgraduate Research Grant (F07/PGRG/2046/2020). Lastly, we are thankful for FAAK lab members for their cooperation and commitment during the sampling process for this study. The substance of this manuscript was submitted by MAIA to the Faculty of Resource Science and Technology, Universiti Malaysia Sarawak as partial fulfilment of the M.Sc. degree in Zoology.

REFERENCES

Anders, J.L., Moustafa, M.A.M., Mohamed, W.M.A., Hayakawa, T., Nakao, R., & Koizumi, I. (2021). Comparing the gut microbiome along the gastrointestinal tract of three sympatric species of wild rodents. *Scientific Reports*, 11(1), 19929. DOI:10.1038/s41598-021-99379-6

Archie, E.A., & Tung, J. (2015). Social behavior and the microbiome. *Current Opinion in Behavioral Sciences*, 6: 28-34. DOI: 10.1016/j.cobeha.2015.07.008

Bäumler, A.J., & Sperandio, V. (2016). Interactions between the microbiota and pathogenic bacteria in the gut. *Nature*, 535(7610): 85-93. DOI:10.1038/nature18849

Bickham, J.W., Wood, C.C., & Patton, J.C. (1995). Biogeographic implications of cytochrome b sequences and allozymes in sockeye (*Oncorhynchus nerka*). *Journal of Heredity*, 86(2): 140-144. DOI:10.1093/oxfordjournals.jhered.a111544

Biddle, A., Stewart, L., Blanchard, J., & Leschine, S. (2013). Untangling the genetic basis of fibrolytic specialization by Lachnospiraceae and Ruminococcaceae in diverse gut communities. *Diversity*, 5(3): 627-640. DOI:10.3390/d5030627

Campbell, J.H., Foster, C.M., Vishnivetskaya, T., Campbell, A.G., Yang, Z.K., Wymore, A., Palumbo, A.V., Chesler, E.J., & Podar, M. (2012). Host genetic and environmental effects on mouse intestinal microbiota. *The ISME Journal*, 6(11): 2033-2044. DOI:10.1038/ismej.2012.54

Carlson, CJ., Kracalik, I.T., Ross, N., Alexander, K.A., Hugh-Jones, M.E., Fegan, M., Elkin B.T., Epp, T., Shury, T.K., Zhang, W., Bagirova, M., Getz, W.M., & Blackburn, J.K. (2019). The global distribution of *Bacillus anthracis* and associated anthrax risk to humans, livestock and wildlife. *Nature Microbiology*, 4(8), 1337-1343. DOI:10.1038/s41564-019-0435-4

Coyte, K.Z., & Rakoff-Nahoum, S. (2019). Understanding competition and cooperation within the mammalian gut microbiome. *Current Biology*, 29(11): R538-R544. DOI:10.1016/j.cub.2019.04.017

Erickson, M.C. (2016). Overview: Foodborne pathogens in wildlife populations. In: Jay-Russell, M., & Doyle, M.P. (eds.), *Food Safety Risks from Wildlife: Challenges in Agriculture, Conservation, and Public Health*. Cham: Springer. pp. 1-30. DOI:10.1007/978-3-319-24442-6_1

Euzéby, J. (2010). List of new names and new combinations previously effectively, but not validly, published. *International Journal of Systematic and Evolutionary Microbiology*, 60(3): 469-472. DOI:10.1099/ijs.0.022855-0

Fagre, A.C., Cohen, L.E., Eskew, E.A., Farrell, M., Glennon, E., Joseph, M.B., Frank, H.K., Ryan, S.J., Carlson, C.J., & Albery, G.F. (2022). Assessing the risk of human-to-wildlife pathogen transmission for conservation and public health. *Ecology Letters*, 25(6), 1534-1549. DOI:10.1111/ele.14003

Fan, C., Zhang, L., Jia, S., Tang, X., Fu, H., Li, W., Liu, C., Zhang, H., Cheng, Q., & Zhang, Y. (2022). Seasonal variations in the composition and functional profiles of gut microbiota reflect dietary changes in plateau pikas. *Integrative Zoology*, 17(3): 379-395. DOI:10.1111/1749-4877.12630

Fenn, J., Taylor, C., Goertz, S., Wanelik, K.M., Paterson, S., Begon, M., Jackson, J., & Bradley, J. (2023). Discrete patterns of microbiome variability across timescales in a wild rodent population. *BMC Microbiology*, 23(1): 1-17. DOI:10.1186/s12866-023-02824-x

Gani, M., Mohd-Ridwan, A.R., Sitam, F.T., Kamarudin, Z., Selamat, S.S., Awang, N.M.Z., Karuppannan, K.V., & Md-Zain, B. M. (2024).

Habitat shapes the gut microbiome diversity of Malayan tigers (*Panthera tigris jacksoni*) as revealed through metabarcoding 16S rRNA profiling. *World Journal of Microbiology and Biotechnology*, 40(4), 111. DOI:10.1007/s11274-023-03868-x

Hou, K., Wu, Z.X., Chen, X.Y., Wang, J.Q., Zhang, D., Xiao, C., Zhu, D., Koya, J.B., Wei, L., Li, J., & Chen, Z.S. (2022). Microbiota in health and diseases. *Signal Transduction and Targeted Therapy*, 7(1): 135. DOI:10.1038/s41392-022-00974-4

Jahan, N.A., Lindsey, L.L., Kipp, E.J., Reinschmidt, A., Heins, B.J., Runck, A.M., & Larsen, P.A. (2021). Nanopore-based surveillance of zoonotic bacterial pathogens in farm dwelling peridomestic rodents. *Pathogens*, 10(9): 1183. DOI:10.3390/pathogens10091183

Jose, L., Lee, W., Hanya, G., Tuuga, A., Goossens, B., Tangah, J., Matsuda, I., & Kumar, V.S. (2024). Gut microbial community in proboscis monkeys (*Nasalis larvatus*): Implications for effects of geographical and social factors. *Royal Society Open Science*, 11(7), 231756. DOI:10.1098/rsos.231756

Kerfeld, C.A., & Scott, K.M. (2011). Using BLAST to teach "E-value-tionary" concepts. *PLoS Biology*, 9(2): e1001014. DOI:10.1371/journal.pbio.1001014

Khairulmunir, M., Gani, M., Karuppannan, K.V., Mohd-Ridwan, A.R., & Md-Zain, B.M. (2023). High-throughput DNA metabarcoding for determining the gut microbiome of captive critically endangered Malayan tiger (*Pantheratigrisjacksoni*) during fasting. *Biodiversity Data Journal*, 11, e104757. DOI:10.3897/BDJ.11.e104757

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16: 111-120. DOI:10.1007/BF01731581

Kinross, J.M., Darzi, A.W., & Nicholson, J.K. (2011). Gut microbiome-host interactions in health and disease. *Genome Medicine*, 3: 1-12. DOI:10.1186/gm228

Kohl, K.D., Dieppa-Colón, E., Goyco-Blas, J., Peralta-Martínez, K., Scafidi, L., Shah, S., Zawacki, E., Barts, N., Ahn, Y., Hedayati, S., Secor, S.M., & Rowe, M.P. (2022). Gut microbial ecology of five species of sympatric desert rodents in relation to herbivorous and insectivorous feeding strategies. *Integrative and Comparative Biology*, 62(2): 237-251. DOI:10.1093/icb/icac045

König, H., & Fröhlich, J. (2017). Lactic acid bacteria. In: Konig, H., Unden, G., & Frohlich, J. (eds.), *Biology of Microorganisms on Grape, in Must and in Wine*. Cham: Springer. pp. 3-41. DOI:10.1007/978-3-319-60021-5

Leser, T.D., & Mølbak, L. (2009). Better living through microbial action: the benefits of the mammalian gastrointestinal microbiota on the host. *Environmental Microbiology*, 11(9): 2194-2206. DOI:10.1111/j.1462-2920.2009.01941.x

Liu, X., Mao, B., Gu, J., Wu, J., Cui, S., Wang, G., Zhao, J., Zhang, J., & Chen, W. (2021). *Blautia*—a new functional genus with potential probiotic properties? *Gut Microbes*, 13(1): 1875796. DOI:10.1080/19490976.2021.1875796

Lobato-Bailón, L., García-Ulloa, M., Santos, A., Guixé, D., Camprodon, J., Florensa-Rius, X., Molleda, R., Manzano, R., Ribas, M.P., Espunyes, J., Dias-Alves, A., Marco, I., Migura-Garcia, L., & Martínez-Urtaza, J. (2023). The fecal bacterial microbiome of the Kuhl's pipistrelle bat (*Pipistrellus kuhlii*) reflects landscape anthropogenic pressure. *Animal Microbiome*, 5(1): 1-14. DOI:10.1186/s42523-023-00229-9

Maurice, C.F., Knowles, S.C., Ladau, J., Pollard, K.S., Fenton, A., Pedersen, A.B., & Turnbaugh, P.J. (2015). Marked seasonal variation in the wild mouse gut microbiota. *The ISME Journal*, 9(11): 2423-2434. DOI:10.1038/ismej.2015.53

McMurdie, P.J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PloS ONE*, 8(4): e61217. DOI:10.1371/journal.pone.0061217

Mohd-Yusof, N.S., Abdul-Latiff, M.A.B., Badrulisham, A.S., Othman, N., Yaakop, S., Md-Nor, S., & Md-Zain, B.M. (2022). First report on metabarcoding analysis of gut microbiome in Island Flying Fox (*Pteropus hypomelanus*) in island populations of Malaysia. *Biodiversity Data Journal*, 10, e69631. DOI:10.3897/BDJ.10.e69631

Payne, J., Francis, C.M., & Phllipps, K. (1985). *A field guide to the mammals of Borneo*. Kota Kinabalu: The Sabah Society.

Perlman, D., Martínez-Álvaro, M., Moraïs, S., Altshuler, I., Hagen, L.H., Jami, E., Roehe, R., Pope, P.B., & Mizrahi, I. (2022). Concepts and consequences of a core gut microbiota for animal growth and development. *Annual Review of*

Animal Biosciences, 10: 177-201. DOI:10.1146/annurev-animal-013020-020412

Phillipps, Q., & Phillipps, K. (2018). *Phillipps field guide to the mammals of Borneo*. 2nd Edition. Oxford, UK: John Beaufoy Publishing.

Risely, A. (2020). Applying the core microbiome to understand host–microbe systems. *Journal of Animal Ecology*, 89(7): 1549-1558. DOI:10.1111/1365-2656.13229

Sariyati, N.H., Othman, N., Abdullah-Fauzi, N.A.F., Chan, E., Md-Zain, B.M., Karuppannan, K.V., & Abdul-Latiff, M.A.B. (2024). Characterizing the gastrointestinal microbiome diversity in endangered Malayan Siamang (*Symphalangus syndactylus*): Insights into group composition, age variability and sex-related patterns. *Journal of Medical Primatology*, 53(5), e12730. DOI:10.1111/jmp.12730

Sharon, I., Quijada, N.M., Pasolli, E., Fabbrini, M., Vitali, F., Agamennone, V., Dötsch, A., Selberherr, E., Grau, J.H., Meixner, M., Liere, K., Ercolini, D., de Filippo, C., Caderni, G., Brigidi, P., & Turroni, S. (2022). The core human microbiome: Does it exist and how can we find It? A critical review of the concept. *Nutrients*, 14(14): 2872. DOI:10.3390/nu14142872

Taudière, A. (2023). MiscMetabar: Miscellaneous functions for metabarcoding analysis. Retrieved January 16, 2024, from https://github.com/adrientaudiere/MiscMetabar.

Teng, Y., Yang, X., Li, G., Zhu, Y., Zhang, Z. (2022). Habitats show more impacts than host species in shaping gut microbiota of sympatric rodent species in a fragmented forest. *Frontiers in Microbiology*, 13: 811990. DOI:10.3389/fmicb.2022.811990

Turnbaugh, P.J., Ley, R.E., Hamady, M., Fraser-Liggett, C.M., Knight, R., & Gordon, J.I. (2007). The human microbiome project. *Nature*, 449(7164): 804-810. DOI:10.1038/nature06244

Valdes, A.M., Walter, J., Segal, E., & Spector, T.D. (2018). Role of the gut microbiota in nutrition and health. *BMJ*, 361: k2179. DOI:10.1136/bmj.k2179

Viney, M. (2019). The gut microbiota of wild rodents: Challenges and opportunities. *Laboratory Animals*, 53(3): 252-258. DOI:10.1177/0023677218787538

Wang, J., Lang, T., Shen, J., Dai, J., Tian, L., & Wang, X. (2019). Core gut bacteria analysis of healthy mice. *Frontiers in Microbiology*, 10: 887. DOI:10.1128/AEM.00794-10

Wang, Z., Zhang, C., Li, G., & Yi, X. (2022). The influence of species identity and geographic locations on gut microbiota of small rodents. *Frontiers in Microbiology*, 13: 983660. DOI:10.3389/fmicb.2022.983660

Weldon, L., Abolins, S., Lenzi, L., Bourne, C., Riley, E.M., & Viney, M. (2015). The gut microbiota of wild mice. *PLoS ONE*, 10(8): e0134643. DOI: 10.1371/journal.pone.0134643

Wexler, H. M. (2007). *Bacteroides*: The good, the bad, and the nitty-gritty. *Clinical Microbiology Reviews*, 20(4), 593-621. DOI:10.1128/cmr.00008-07

Wickham, H. (2011). ggplot2. *Wiley Interdisciplinary Reviews: Computational Statistics*, 3(2): 180-185. DOI:10.1002/wics.147

Wortelboer, K., Koopen, A.M., Herrema, H., de Vos, W.M., Nieuwdorp, M., & Kemper, E.M. (2022). From fecal microbiota transplantation toward next-generation beneficial microbes: The case of *Anaerobutyricum soehngenii*. *Frontiers in Medicine*, 9: 1077275. DOI:10.3389/fmed.2022. 1077275

Zheng, J., Wittouck, S., Salvetti, E., Franz, C.M., Harris, H.M., Mattarelli, P., O'Toole, P.W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G.E., Gänzle, M.G., & Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. *International Journal of Systematic and Evolutionary Microbiology*, 70(4): 2782-2858. DOI:10.1099/ijsem.0.004107