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Fecal Bacteriome and Mycobiome in Bats with Diverse Diets in South China

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Abstract

Bats can be divided into frugivory, nectarivory, insectivory, and sanguivory based on their diets, and are therefore ideal wild animal models to study the relationship between diets and intestinal microflora. Early studies of bat gut bacteria showed that the diversity and structure of intestinal bacterial communities in bats are closely related to dietary changes. Worthy of note, intestinal microbes are composed of bacteria, fungi, protozoa, and archaea. Although the number of gut fungi is much lower than that of gut bacteria, they also play an important role in maintaining the host homeostasis. However, there are still few reports on the relationship between the gut mycobiota and the dietary habits of the host. In addition, bats have also been shown to naturally transmit pathogenic viruses and bacteria through their feces and saliva, but fungal infections from bat are less studied. Here, we used high-throughput sequencing of bacterial 16S and eukaryotic 18S rRNA genes in the V4 and V9 regions to characterize fecal bacterial and fungal microbiota in phytophagous and insectivorous bats in South China. The results show that the gut microbiota in bats were dominated by bacterial phyla Proteobacteria, Firmicutes, Tenericutes and Bacteroidetes, and fungal phyla Ascomycota and Basidiomycota. There was a significant difference in the diversity of bacterial and fungal microbiota between the groups, in addition to specific bacteria and fungi populations on each of them. Of note, the number of fungi in the feces of herbivorous bats is relatively higher. Most of these fungi are foodborne and are also pathogens of humans and other animals. Thus, bats are natural carriers of fungal pathogens. The current study expands the understanding of the bat gut bacterial and fungal mycobiota and provides further insight into the transmission of fungal pathogens.

Introduction

The intestinal microbiome is one of the most complex microbial ecosystems, which consists of trillions of microorganisms [60, 61]. Microbiota can mutually cooperate with host and promote functional stability and metabolic balance in the gut [56]. For example, gut bacterial microbiota could facilitate the energy storage and consumption of the host, and is also considerably shaped by dietary habits [55]. Although bacteria dominate microfloral communities, fungi and archaea also present [61]. Like the commensal bacteria, gut fungi also play pivotal roles in host homeostasis [53, 61]. Remarkably, recent studies have found that fungi may be also closely related to the feeding behavior of the host, and some of them may even be directly food-derived [18, 30]. In addition, there should be fierce competitions among these commensals in the intestine due to limited food resources and living space. However, compared to gut bacteria, few

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studies focus on the relationship between gut mycobiota and diets, and diet-related bacteria–fungi interactions [53].

Chiroptera is the second most species-rich order in Mammalia, and has notably evolutionarily diverse feeding strategies, which can be divided into insectivory, sanguivory, frugivory, and nectarivory [1, 26]. Thus, bats with diverse diets can be ideal animal models for further studies of those interactions in gut microbiota. Previous studies, if any, just have demonstrated that gut bacterial microbiota of bats present obvious variations in the composition and structure owing to the discrepancies of their diets and life histories [6, 13, 39, 49]. Nevertheless, the typical gut bacterial microbiota of bats closely associated with diets remain to be determined, not to mention gut fungal microbiota.

Bats also catch much attention as natural reservoirs and carriers of viruses without illness and mortality [34, 65]. Recent studies have showed that bats are the potential disseminators of pathogenic bacteria through droppings (urines, saliva, and feces), for example, *Leptospira, Rickettsia, Bartonella, Coxiella*, and *Escherichia* [5, 17, 20, 44, 62]. It is noteworthy that in several previous studies, some human and other animals morbific fungi (*Candida albicans*, etc) were also isolated from bat excreta [3, 9, 10]. Nonetheless, the number of culturable fungi is as smaller as that of bacteria [32], and many undetectable intestinal fungi therefore occur in bats. Furthermore, the fungus *Pseudogymnoascus destructans* infection is responsible for the lethal white-nose syndrome, a disease that has dramatically reduced the bat populations in Europe and American countries [31]. This lethal disease has recently been detected in bats in northeast China [31], but it is unclear whether this pathogen has spread to South China.

The advent of sequencing technologies and the continuous updating of online databases have enabled us to fully understand these non-culturable microorganisms. Thus, in this study, we performed 16S and 18S ribosomal RNA gene sequencing to characterize the gut bacterial and fungal microbiota of healthy phytophagous (both frugivorous and nectarivorous) and insectivorous bats. We aim to (1) determine how the bat diet exerts an influence on the gut mycobiota and bacteria–fungi interactions and (2) survey the prevalence of pathogenic fungi in bats.

Materials and Methods

Ethics Statement

All experimental animal protocols in this study were approved by the committee on the Ethics of Animal Experiments of the Guangdong Institute of Applied Biological Resources and followed basic principles (GIABR20170323).

Fecal Sample Collection

Fourteen healthy adult bats with different diets were randomly collected in Guangdong, Guangxi, and Yunnan provinces in China. Then, the samples were divided into two groups according to eating habits (six for phytophagous (frugivorous and nectarivorous) group; eight for insectivorous group), as shown in Table 1.

Here, we chose to sample fresh bat feces; in addition, to avoid seasonal variations in lifestyle, the samples were collected from spring to summer, when the amount of food resources available to bats were similar (Table 1). Because it is extremely difficult to find bat fecal pellets in the wild and to avoid contamination, we used hoop nets to trap bats when they flew out of the caves at nightfall. Then, we placed the bat individuals into separate sterilized sacks. After the bats excreted, fresh fecal pellets in each bag were collected in a sterile fecal tube and quickly stored in liquid nitrogen. The samples were transferred to the laboratory and then stored in a − 80 °C freezer until the nucleic acids were extracted.

DNA Extraction, PCR Amplification, and Sequencing

For each sample, the total genomic DNA was extracted using the PowerFecal DNA Isolation Kit (MOBIO, USA). Using the universal primer sets 515F/806R and 1380F/1510R, variable region 4 (V4) of bacterial 16S rRNA genes and variable region 9 (V9) of eukaryotic 18S rRNA genes were, respectively, amplified with TransStart Fastpfu DNA Polymerase (TransGen Biotech, China). Sterilized water was used as the negative control. Finally, the 16S rRNA and 18S rRNA amplicons were sequenced on an Illumina HiSeq sequencing platform using paired-ends sequencing according to the manufacturer's protocol.

Data Processing

QIIME V1.90 was used to filter the raw reads to obtain high-quality effective sequences [22]. The effective sequences for each sample were clustered into operational taxonomic units (OTUs) at 97% similarity using UPARSE V7.0.1.1001 [21]. The representative sequence for each OTU was annotated after alignment with the Green genes database for the 16S rRNA gene and the Silva database for the 18S rRNA gene [19, 51].

Statistical Analysis

To compare the samples, the abundance data for each sample were standardized, followed by the construction of phylogenetic trees in KronaTools-2.7. The standardized abundance

Table 1 Characteristics of study bat sample in China in 2016

Species	Code[a]	Sex[b]	Diet	Location	Time[c]
Myotis ricketti	Mr	F	Insectivore	China: Guangdong, Huizhou, Lianghua town	2016-06
Myotis ricketti	Mr2	M	Insectivore	China: Guangdong, Shaoguan, Ruyuan county	2016-07
Myotis ricketti	Mr3	M	Insectivore	China: Guangdong, Shaoguan, Ruyuan county	2016-07
Hipposideros larvatus	Hl1	M	Insectivore	China: Guangdong, Shaoguan, Ruyuan county	2016-07
Tylonycteris pachypus	Tp	M	Insectivore	China: Guangxi, Chongzuo, Ningming county	2016-05
Pipistrellus abramus	Pp	M	Insectivore	China: Yunnan, Jinghong, Menghai county (1)[d]	2016-05
Scotophilus heathi	Sh	F	Insectivore	China: Yunnan, Jinghong, Menghai county (1)[d]	2016-05
Hipposideros armiger	Ha	F	Insectivore	China: Yunnan, Jinghong, Menghai county (2)[e]	2016-05
Rousettus leschenaultii	Rl	M	Frugivore	China: Yunnan, Xishuangbanna, Mengla county	2016-05
Rousettus leschenaultii	Rl2	F	Frugivore	China: Yunnan, Xishuangbanna, Mengla county	2016-05
Cynopterus sphinx	Cs	M	Frugivore	China: Guangdong, Guangzhou, Haizhu district	2016-04
Cynopterus sphinx	Cs2	F	Frugivore	China: Guangdong, Guangzhou, Haizhu district	2016-04
Eonycteris spelaea	Es	M	Nectarivore	China: Yunnan, Xishuangbanna, Mengla county	2016-05
Eonycteris spelaea	Es2	F	Nectarivore	China: Yunnan, Xishuangbanna, Mengla county	2016-05

[a]Code of each sample

[b]Sex of sample: Male (M), Female (F)

[c]Time of sample collection

[d]China: Yunnan, Jinghong, Menghai county, Manzhang village

[e]China: Yunnan, Jinghong, Menghai county, Manxishang village

data and phylogenetic trees were used to calculate alpha diversity indices using QIIME V1.90 [12, 14]. We compared the difference between the groups with One-way variance analysis (ANOVA).

Beta diversity analysis was preformed based on UniFrac distances using QIIME V1.90. Principal coordinate analysis (PCoA) was carried out with the R package "ape," and a clustering dendrogram was constructed using the unweighted pair-group method with arithmetic mean (UPGMA) [15].

Based on the standardized abundance data, multi-response permutation procedures (MRPP) were performed to identify significant inter- and intra-group differences in community structure using the R package "vegan." Metastats, which relies on non-parametric tests, was used to detect differentially abundant features in this study using Fisher's exact test at each level of classification using the "vegan" package in R [63].

Sequence Data Accession Number

The sequencing data generated from the fourteen bats described in this study are available in a sequence read archive (SRA) at the NCBI under the accession numbers SRP101532.

Results

The sequencing results showed that each sample contained at least 30,000 effective sequences. Rarefaction curves indicated that most of the diversity presented in these fecal samples had already been captured (Fig S1 in Supplementary Materials).

Analysis of the Fecal Eukaryotic Microbiota of Bats

A total of 507,092 high-quality 18S rRNA gene reads were classified into 17 phyla, 48 class, 99 order, 166 family, 207 genera, and 293 species. Fungi were detected in all the fecal samples, with 141 species primarily from two phyla: Ascomycota (56.71% in phytophages vs. 15.72% in insectivores) and Basidiomycota (7.03 vs. 2.48%). Other fungal taxa in low abundance were, in decreasing order, Fungi incertae sedis, Glomeromycota, and Entomophthoromycota (Fig. 1a).

Comparison of the Diversity of Fecal Eukaryotic Microorganisms Between Groups

ANOVA was used to compare the alpha diversity between the groups. Plant-eating group had a high diversity than insect-eating group based on the Shannon index (Fig S1d), although there was no significant difference in alpha diversity between the groups.

Beta diversity of each sample was analyzed based on unweighted UniFrac distance. The PcoA results showed that except a *Cynopterus sphinx* (Cs2), herbivorous bats and insectivorous bats were divided into two sets (Fig. 2a). UPGMA cluster analysis also reflected the difference in eukaryotic microbial community structure between the two groups (Fig. 2b).

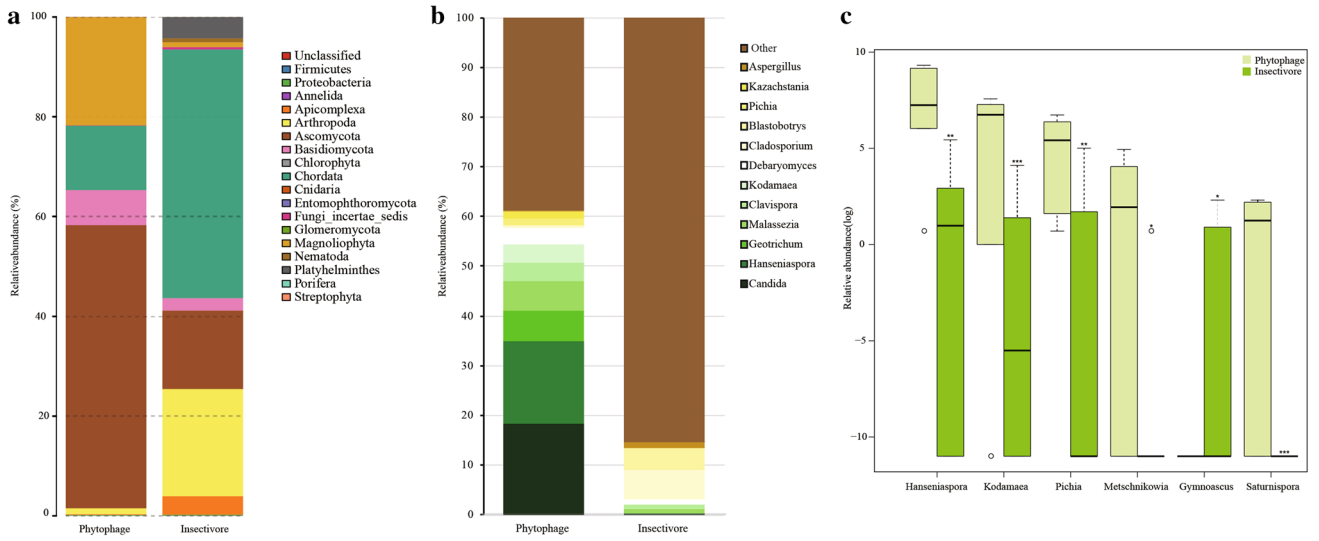

Fig. 1 Eukaryotic taxonomic representation in the feces of phytophagous (Phytophage) and insectivorous (Insectivore) bats. **a** Eukaryotic taxonomic representation in every group at the phylum level; **b** fungal taxonomic representation in every group at the genus level; **c** boxplots depict the range of log relative abundance of diet-dependent genera. $*P < 0.05$; $**P < 0.01$; $***P < 0.0001$

Different Fungi Between Groups

The compositional dissimilarities between the groups were greater than those within the groups (Table S1A in Supplementary Materials). Ascomycota from the fecal mycobiota of phytophagous bats were significantly more numerous than those in the fecal mycobiota of insectivorous bats ($P = 0.0135$). The most abundant genera in kingdom Fungi included Candida (18.24%, only *C. albicans*), Hanseniaspora (16.73%), Geotrichum (6.00%, only G. candidum), Malassezia (5.96%), Clavispora (3.80%), Kodamaea (3.56%), and Debaryomyces (3.39%) in the phytophagous group and Cladosporium (5.86%) and Blastobotrys (4.39%, only B. terrestris) in the insectivorous group (Fig. 1b and Table S2 in Supplementary Materials). There were significant differences in the genera Hanseniaspora, Kodamaea, Pichia, Metschnikowia, Saturnispora, and Gymnoascus, which were more abundant in herbivorous bats, with the exception of Gymnoascus (Fig. 1c, and Table S2 in

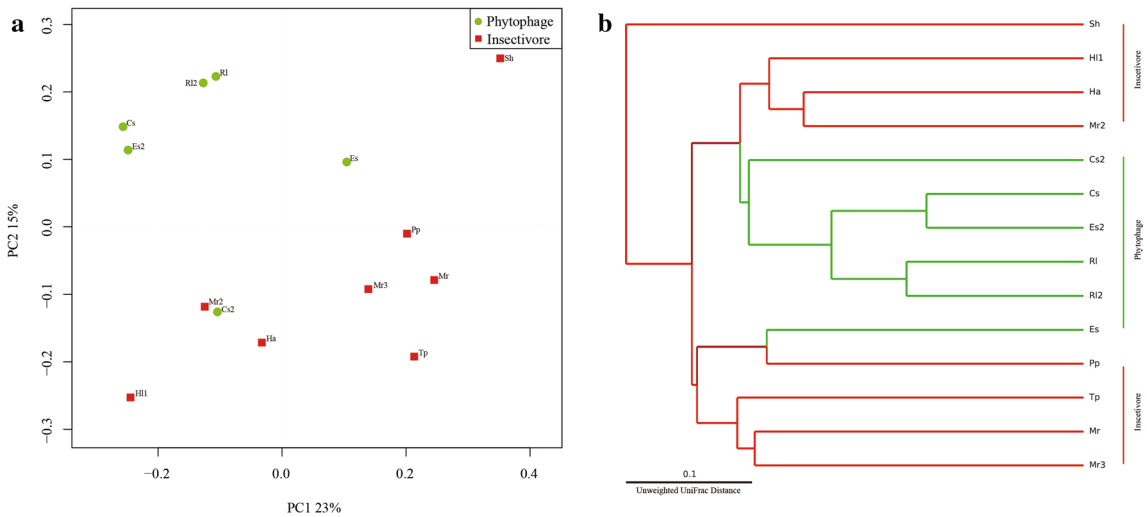

Fig. 2 Beta diversity of fecal eukaryotic microbiota from phytophagous and insectivorous bats by unweighted UniFrac distance; **a** Principal coordinate analysis (PcoA) from every sample. **b** Clustering dendrogram of bat fecal eukaryotic (***) microorganisms using UPGMA (Unweighted Pair-group Method with Arithmetic Mean)

Supplementary Materials). Pseudogymnoascus destructans was not detectable in any of the fecal samples.

Association Between Eukaryotic Composition and Diet

Both Magnoliophyta (21.72% in phytophages vs. 0.98% in insectivores) and Arthropoda (1.20 vs. 21.51%) appeared in two groups (Fig. 1a). The feces of phytophagous bats had a higher representation of Magnoliophyta and a lower representation of Arthropoda; this trend was reversed in the insectivorous group (Fig. S2 in Supplementary Materials).

Analysis of the Fecal Bacteria of Bats

A total of 526,159 16S rRNA gene sequences were classified into 672 OTUs belonging to 18 different phyla across all 14 samples. In descending order, the dominant phyla were Proteobacteria (42.97 vs. 51.67% in phytophagous and insectivorous bats), Firmicutes (27.93 vs. 34.39%), Tenericutes (26.05 vs. 0.61%), and Bacteroidetes (2.47 vs. 7.96%) (Fig. 3a). Notably, at the family level, the Enterobacteriaceae species were found to be dominant bacteria in both groups (Fig. 3b).

Comparison of the Diversity of Fecal Bacteria Between Groups

A comparison of alpha diversity indices between groups is presented in Fig. S1 a and b. The plant-eating group (fruit and nectar) harbored an obviously lower diversity than the insect-eating group based on the Shannon index (Fig S1b), despite an undetectable difference between the phytophagous and insectivorous bats.

For beta diversity, despite the high variation between insect-eating bats, the PCoA results also showed that samples from plant-eating and insect-eating bats were unambiguously dispersed into two sets (Fig. 4a and Fig S3 in Supplementary Materials). Similarly, based on the UPGMA clustering, two groups also were distinguished. The insectivorous bats clustered first, followed by the phytophagous ones (Fig. 4b, and Fig S3 in Supplementary Materials).

Different Composition Between Groups

We found that 20 species, 19 genera, 16 families, 19 orders, 19 classes, and 16 phyla were enriched or reduced in different groups using multivariate statistical analysis (Fig. S4 in Supplementary Materials).

A comparison between groups showed that the representative profile in phytophagous bats was families Mycoplasmataceae, Leuconostocaceae and Clostridiaceae, and genera *Enterobacter, Fructobacillus, Ureaplasma, Klebsiella*, and *Weissalla* (Fig. 3b, c, Fig. S4 in Supplementary Materials). However, insectivorous bats presented a significant increase mainly in families Enterococcaceae, Flavobacteriaceae, Lactobacillaceae, Streptococcaceae, Peptostreptococcaceae, Bacillaceae, and Fusobacteriaceae, and genera *Plesiomonas, Enterococcus, Lactobacillus*, and *Bacillus* (Fig. 3b, c, Fig. S4 in Supplementary Materials).

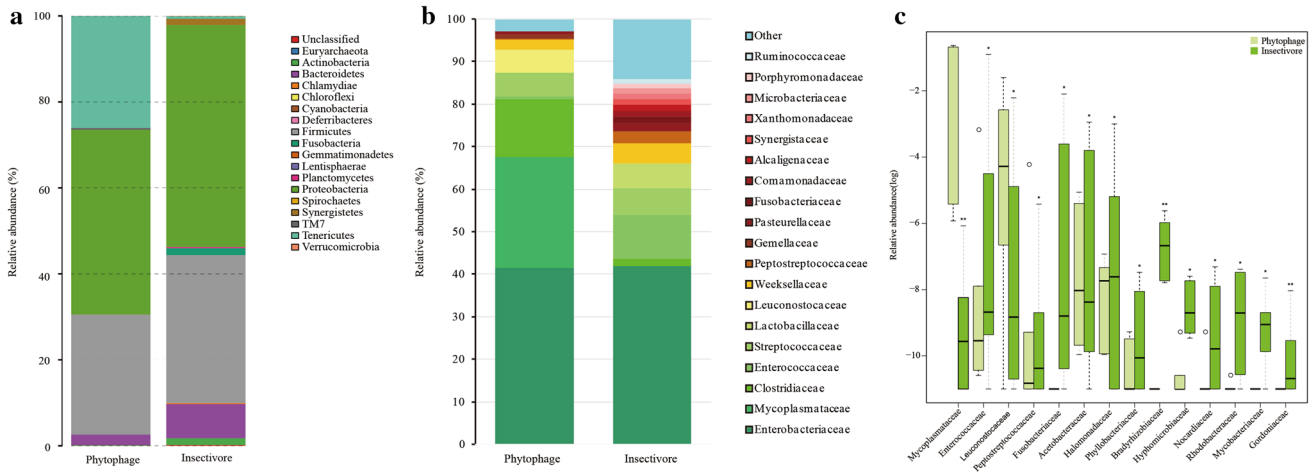

Fig. 3 Bacterial taxonomic representation in the feces of phytophagous (Phytophage) and insectivorous (Insectivore) bats. **a** Bacterial taxonomic representation in every group at the phylum level; **b** bacterial taxonomic representation in every group at the family level; **c** boxplots depict the range of log relative abundance of diet-dependent families. $*P < 0.05$; $**P < 0.01$; $***P < 0.0001$.

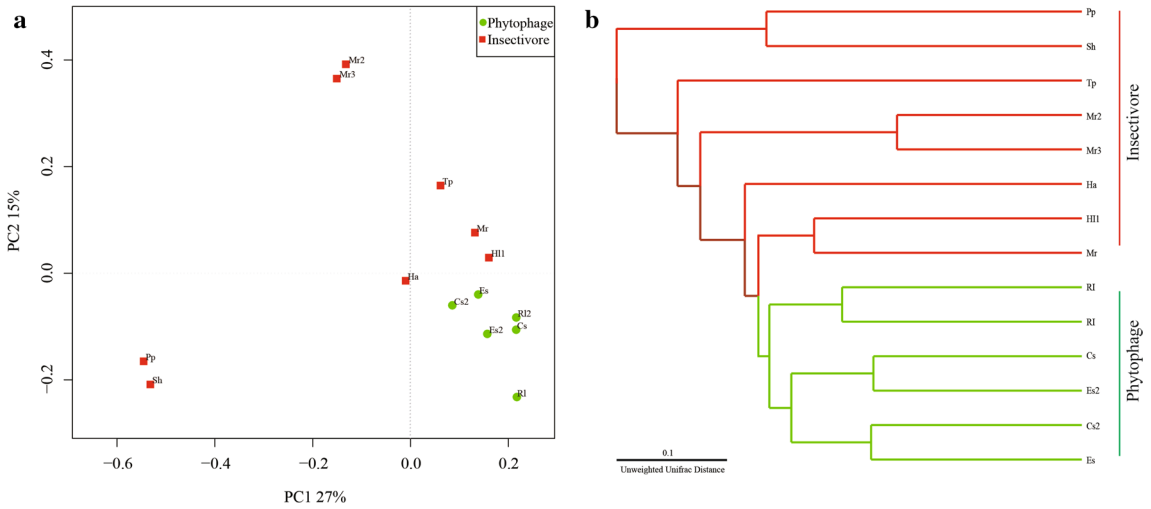

Fig. 4 Beta diversity of fecal microbiota from phytophagous and insectivorous bats by unweighted UniFrac distance; **a** Principal coordinate analysis (PcoA) from every sample. **b** Clustering dendrogram of bat fecal bacteria using UPGMA (Unweighted Pair-group Method with Arithmetic Mean)

Discussion

Composition and Diversity Analysis of Bat Fecal Microbiota

The composition of the bat gut bacterial microbiota is unique. Proteobacteria overwhelmingly dominated bat guts across plant- and animal-feeding bats, and was followed by Firmicutes and Tenericutes, along with a few Bacteroidetes, which is typically one of the most abundant bacterial phyla found in the vertebrate intestine [16] (Fig. 3a). In particular, we observed that the Enterobacteriaceae in phylum Proteobacteria was the most prevailing family for bats (Fig. 3b). The high level of presence of this family in the gut was also found in previous studies of bats [4, 6, 13]. Thus, we could speculate that the core bacterial microbiota in bats are similar, despite variations in diets, geography, species [13, 49], and life history [6, 39]. For fungal community, yeasts from Ascomycota and Basidiomycotina are the main fungal components of bat feces, which is consistent with early studies of gut mycobiota [27, 29, 45] (Fig. 1a).

We found that species diversity of bacterial community showed an increase from phytophagous bats to insectivorous bats; this trend was opposite for that of fungal community (Fig. S1b and d). The composition of fecal bacteria and eukaryotic microorganisms between the groups was distinctly separated depending on feeding strategies, as demonstrated by PCoA and UPGMA clustering (Figs. 2a, b, 4a, b). Interestingly, the discrepancy in the alpha diversity of gut bacteria in bats with different diets obviously differed from that observed in non-flying mammals, in which alpha diversity decreases from herbivores to omnivores to carnivores [38]. In the wild, the preference for fermented fruit may cause that more fungi thus easily access the digestive tract of phytophagous bats than that of insectivorous bats. The emergence of a large number of exotic fungi may lead to more intense ecological competition in the intestine of phytophagous bats, and therefore negatively affect the gut bacterial community.

Differential Microoganisms Between the Groups

For fecal bacteria, the plant-feeding group exhibited a gut bacterial community enriched in Mycoplasmataceae, Clostridiaceae, and *Fructobacillus* compared with insect-feeding group (Fig. 3b, c and S4 b, c). *Fructobacillus* comprises fructophilic lactic acid bacteria, and adapts to fructose-rich niches such as flowers or fruits [23]. The increase in this genus may be explained by food source, and their capacity to survive fructose-rich intestinal environments of phytophagous bats. Clostridiaceae species are main consumers of plant-derived carbohydrate [64], thus potentially indicating their role in degradation of plant-derived nutrients. Mycoplasmataceae bacteria frequently correlate with illness in the urogenital or respiratory tracts [54]. In previous studies, Mycoplasmataceae have been found in the blood and guts of bats [6, 40, 42]. However, it is still unclear whether phytophagous bats are natural carriers of those pathogens or were just infected. For insectivorous group, there was a higher abundance in Enterococcaceae, Lactobacillaceae, and Fusobacteriaceae (Fig. 3b, c and Fig. S 4b). Enterococcaceae are the dominant symbiotic bacteria in several insects [11, 43]. Therefore, bats may have acquired them presumably through ingestion of insects. Fusobacteriaceae in the gut

are positively associated with amino acid metabolism and are negatively associated with sugar breakdown [36, 52]. The nutritional value of insects is high fat and high protein [24]. Hence, Fusobacteriaceae bacteria may play a role in the digestion of fat and protein in the intestine of bats. However, these distinctions in those biomarkers and the diversity were not found in previous studies among bats [6]. This may be because the present study used bat fresh feces instead of intestinal tissues and not all foodborne bacteria can colonize in the host gut.

The gut mycobiota of phytophagous bats were enriched in *Candida* (only *C. albicans*), *Hanseniaspora, Geotrichum* (only *G. candidum*), *Malassezia, Kodamaea* (only *K. ohmeri*), *Pichia, Metschnikowia*, and *Saturnispora* (Fig. 1b, c). *Candida* is reported to be positively correlated with food high in carbohydrates [30]. *Hanseniaspora* [33], *G. candidum* [28, 33], *Kodamaea* [59], *Pichia* [57], *Metschnikowia* [35], and *Saturnispora* are yeasts that involve in fruit fermentation and decay. Preexisting reports of bat ethology have demonstrated that frugivorous and nectarivorous bats prefer ripe fruit and nectar with fermented odors [37, 47]. The fermented fruits and nectar may be the primary source of the fecal fungal community of phytophagous bats, and significantly affected the gut mycobiota. *Cladosporium* and *Blastobotrys* (only *B. terrestris*) presented with higher proportions in insectivorous bats than they did in phytophagous bats (Fig. 1b, c). Most of *Cladosporium* species are usually phytopathogens, some of which were also later discovered to be human pathogens [41, 46, 67]. *B. terrestris* has been isolated from the intestine of carnivorous snakes [8]. However, it remains unclear whether the higher level of presence in insectivorous bats is related to their special diets.

Fungal Pathogens from Bats

Of note, many fungi detected at higher levels in plant-feeding bat feces have been reported to be also human pathogens [3, 9, 10, 25, 50, 58, 66]. *C. albicans* is the major pathogenic *Candida* species and with pathogenic determinants and adaptation mechanism. This fungi is able to damage the immune barriers of hosts and cause invasive candidiasis and candidemia [66]. Previously, *C. albicans* have been isolated from bat excreta and showed potential virulence in vitro and in vivo [9, 10]. *G. candidum*, except as a plant pathogen, can infect pulmonary, bronchopulmonary, cutaneous and oral site, and blood of the humans and result in geotrichosis or fungemia [3]. *Malassezia* species have frequently been found to be responsible for skin disorders with characterized inflammations, though the commensal members constituting skin or gut microbiota [25, 58]. Recent studies have showed that this pathogen is even closely associated with skin cancer and HIV/AIDS. Moreover, fungaemia, endocarditis, cellulitis, funguria, and peritonitis have recently been confirmed to be also likely due to *Kodamaea ohmeri* infection [3].

Importantly, bats are a group of nocturnal mammals that can fly long distances, which gives them more chances to spread these pathogens widely. More crucially, the reduction of natural habitats of bats gradually causes the overlapping of human and bat living areas [2, 7, 48]. Thus, humans are more likely to live in environments that were contaminated by the pathogens from bats. Additionally, in this study, we did not detect the *Pseudogymnoascus destructans* in all feces, potentially indicating that bats in south China have not yet been infected by this pathogen probably due to the geographical isolation.

Dietary Overlap Among Bats with Different Eating Habits

Previous study has speculated the dietary overlap within bats of different diets according to the gut bacterial microbiota [6]. In this study, we sequenced the 18S rRNA gene from insectivorous and phytophagous bat stools to more precisely assess their dietary patterns. The results suggest that except that phytophagous bats can consume insects [6], insectivorous bats could also eat plants (Fig. S2). It appears that It seems that accidental ingestion may be one of the reasons for the appearance of Magnoliophyta in the feces of insectivorous bats. Furthermore, it may also be that a long-term single diet caused a lack of nutrients, which led to spontaneous feeding.

Conclusion

In this study, for the first time, we used high-throughput sequencing of 16S and 18S rRNA genes to analyze the bacterial and fungal microbiota of feces of bats in South China. Our results demonstrated that (1) intestinal bacterial and fungal community are significantly affected by the dietary habits of the host, especially the gut mycobiota of phytophagous bats, which may mainly consist of those food-derived fungi; (2) gut bacteria and fungi might interact with each other; (3) bats, especially phytophagous bats, are also communicators of various fungal pathogens. We have discovered the interactions between bat gut bacteria and fungi, and their relationship with dietary habits with 16S and 18S rRNA genes sequencing. However, in order to further explain the potential function and role of bat intestinal bacteria and fungi, metagenomic sequencing technology with larger sequencing throughput will be needed.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

References

1. Altringham JD, Hammond L, Mcowat T (1996) Bats: biology and behaviour. Oxford University Press, Oxford
2. Ancillotto L, Ariano A, Nardone V, Budinski I, Rydell J, Russo D (2017) Effects of free-ranging cattle and landscape complexity on bat foraging: implications for bat conservation and livestock management. Agric Ecosyst Environ 241:54–61. https://doi.org/10.1016/j.agee.2017.03.001
3. Arendrup MC, Boekhout T, Akova M, Meis JF, Cornely OA, Lortholary O, European Society of Clinical M, Infectious Diseases Fungal Infection Study G, European Confederation of Medical M (2014) ESCMID and ECMM joint clinical guidelines for the diagnosis and management of rare invasive yeast infections. Clin Microbiol Infect 20(Suppl 3):76–98. https://doi.org/10.1111/1469-0691.12360
4. Avena CV, Parfrey LW, Leff JW, Archer HM, Frick WF, Langwig KE, Kilpatrick AM, Powers KE, Foster JT, McKenzie VJ (2016) Deconstructing the bat skin microbiome: influences of the host and the environment. Front Microbiol 7:1753. https://doi.org/10.3389/fmicb.2016.01753
5. Banskar S, Bhute SS, Suryavanshi MV, Punekar S, Shouche YS (2016) Microbiome analysis reveals the abundance of bacterial pathogens in *Rousettus leschenaultii* guano. Sci Rep 6:36948. https://doi.org/10.1038/srep36948
6. Banskar S, Mourya DT, Shouche YS (2016) Bacterial diversity indicates dietary overlap among bats of different feeding habits. Microbiol Res 182:99–108. https://doi.org/10.1016/j.micres.2015.10.006
7. Berthinussen A, Altringham J (2012) The effect of a major road on bat activity and diversity. J Appl Ecol 49(1):82–89. https://doi.org/10.1111/j.1365-2664.2011.02068.x
8. Bhadra B, Singh PK, Rao RS, Shivaji S (2008) *Blastobotrys serpentis* sp. nov., isolated from the intestine of a Trinket snake (*Elaphe* sp., Colubridae). FEMS Yeast Res 8(3):492–498. https://doi.org/10.1111/j.1567-1364.2008.00356.x
9. Botelho NS, de Paula SB, Panagio LA, Pinge-Filho P, Yamauchi LM, Yamada-Ogatta SF (2012) *Candida* species isolated from urban bats of Londrina-Parana, Brazil and their potential virulence. Zoonoses Public Health 59(1):16–22. https://doi.org/10.1111/j.1863-2378.2011.01410.x
10. Brilhante RS, Maia-Junior JE, Oliveira JS, Guedes GM, Silva AL, Moura FB, Sales JA, Castelo-Branco DS, Sidrim JJ, Cordeiro RA, Pereira-Neto WA, Rocha MF (2016) Yeasts from the microbiota of bats: a focus on the identification and antimicrobial susceptibility of cryptic species of *Candida*. J Med Microbiol 65(10):1225–1228. https://doi.org/10.1099/jmm.0.000340
11. Brown BP, Wernegreen JJ (2016) Deep divergence and rapid evolutionary rates in gut-associated *Acetobacteraceae* of ants. BMC Microbiol 16(1):140. https://doi.org/10.1186/s12866-016-0721-8
12. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7(5):335–336. https://doi.org/10.1038/nmeth.f.303
13. Carrillo-Araujo M, Tas N, Alcantara-Hernandez RJ, Gaona O, Schondube JE, Medellin RA, Jansson JK, Falcon LI (2015) Phyllostomid bat microbiome composition is associated to host phylogeny and feeding strategies. Front Microbiol 6:447. https://doi.org/10.3389/fmicb.2015.00447
14. Chao A (1987) Estimating the population size for capture-recapture data with unequal catchability. Biometrics 43(4):783–791. https://doi.org/10.2307/2531532
15. Chen J, Bittinger K, Charlson ES, Hoffmann C, Lewis J, Wu GD, Collman RG, Bushman FD, Li H (2012) Associating microbiome composition with environmental covariates using generalized UniFrac distances. Bioinformatics 28(16):2106–2113. https://doi.org/10.1093/bioinformatics/bts342
16. Colston TJ, Jackson CR (2016) Microbiome evolution along divergent branches of the vertebrate tree of life: what is known and unknown. Mol Ecol 25(16):3776–3800. https://doi.org/10.1111/mec.13730
17. Daniel DS, Ng YK, Chua EL, Arumugam Y, Wong WL, Kumaran JV (2013) Isolation and identification of gastrointestinal microbiota from the short-nosed fruit bat *Cynopterus brachyotis*. Microbiol Res 168(8):485–496. https://doi.org/10.1016/j.micres.2013.04.001
18. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ (2014) Diet rapidly and reproducibly alters the human gut microbiome. Nature 505(7484):559–563. https://doi.org/10.1038/nature12820
19. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72(7):5069–5072. https://doi.org/10.1128/AEM.03006-05
20. Dietrich M, Kearney T, Seamark EC, Markotter W (2017) The excreted microbiota of bats: evidence of niche specialisation based on multiple body habitats. Fems Microbiol Lett. https://doi.org/10.1093/femsle/fnw284
21. Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10(10):996–998. https://doi.org/10.1038/nmeth.2604
22. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27(16):2194–2200. https://doi.org/10.1093/bioinformatics/btr381
23. Endo A, Tanizawa Y, Tanaka N, Maeno S, Kumar H, Shiwa Y, Okada S, Yoshikawa H, Dicks L, Nakagawa J, Arita M (2015) Comparative genomics of *Fructobacillus* spp. and *Leuconostoc* spp. reveals niche-specific evolution of *Fructobacillus* spp.. BMC Genom 16:1117. https://doi.org/10.1186/s12864-015-2339-x
24. Finke MD (2013) Complete nutrient content of four species of feeder insects. Zoo Biol 32(1):27–36. https://doi.org/10.1002/zoo.21012
25. Gaitanis G, Magiatis P, Hantschke M, Bassukas ID, Velegraki A (2012) The *Malassezia* genus in skin and systemic diseases. Clin Microbiol Rev 25(1):106–141. https://doi.org/10.1128/CMR.00021-11
26. Gardner AL (1976) Biology of bats of the New World family *Phyllostomatidae*. Texas Tech Press, Lubbock
27. Gouba N, Drancourt M (2015) Digestive tract mycobiota: a source of infection. Med Mal Infect 45(1–2):9–16. https://doi.org/10.1016/j.medmal.2015.01.007
28. Hafeez R, Akhtar N, Shoaib A, Bashir U, Haider MS, Awan ZA (2015) First report of *Geotrichum candidum* from Pakistan

causing postharvest sour rot in loquat (*Eriobotrya Japonica*). J Anim Plant Sci 25(6):1737–1740
29. Hallen-Adams HE, Suhr MJ (2017) Fungi in the healthy human gastrointestinal tract. Virulence 8(3):352–358. https://doi.org/10.1080/21505594.2016.1247140
30. Hoffmann C, Dollive S, Grunberg S, Chen J, Li H, Wu GD, Lewis JD, Bushman FD (2013) Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. PLoS ONE 8(6):e66019. https://doi.org/10.1371/journal.pone.0066019
31. Hoyt JR, Sun K, Parise KL, Lu G, Langwig KE, Jiang T, Yang S, Frick WF, Kilpatrick AM, Foster JT, Feng J (2016) Widespread bat white-nose syndrome fungus, Northeastern China. Emerg Infect Dis 22(1):140–142. https://doi.org/10.3201/eid2201.151314
32. Huffnagle GB, Noverr MC (2013) The emerging world of the fungal microbiome. Trends Microbiol 21(7):334–341. https://doi.org/10.1016/j.tim.2013.04.002
33. Hussain M, Hamid MI, Ghazanfar MU, Akhtar N, Raza M (2016) First report of fruit rot of strawberry caused by *Geotrichum candidum* in Pakistan. Plant Dis 100(9):1948. https://doi.org/10.1094/pdis-03-16-0277-pdn
34. Jiang H, Li J, Li L, Zhang X, Yuan L, Chen J (2017) Selective evolution of Toll-like receptors 3, 7, 8, and 9 in bats. Immunogenetics 69(4):271–285. https://doi.org/10.1007/s00251-016-0966-2
35. Kajdácsi E, Fazekas M, Antunovics Z, Sipiczki M (2008) *Antagonistic metschnikowia* strains isolated from fruits. Acta Microbiol Immunol Hung 55(3):363–363. https://doi.org/10.1556/AMicr.55.2008.3.7
36. Keenan SW, Engel AS, Elsey RM (2013) The alligator gut microbiome and implications for archosaur symbioses. Sci Rep 3:2877. https://doi.org/10.1038/srep02877
37. Korine C, Sanchez F, Pinshow B (2011) Effects of ethanol on food consumption and skin temperature in the Egyptian fruit bat (*Rousettus aegyptiacus*). Integr Comp Biol 51(3):432–440. https://doi.org/10.1093/icb/icr012
38. Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI (2008) Worlds within worlds: evolution of the vertebrate gut microbiota. Nat Rev Microbiol 6(10):776–788. https://doi.org/10.1038/nrmicro1978
39. Maliničová L, Hrehová Ľ, Maxinová E, Uhrin M, Pristaš P (2017) The dynamics of mediterranean horseshoe bat (*Rhinolophus euryale*, Chiroptera) gut microflora during hibernation. Acta Chiropterol 19(1):211–218. https://doi.org/10.3161/15081109acc2017.19.1.017
40. Mascarelli PE, Keel MK, Yabsley M, Last LA, Breitschwerdt EB, Maggi RG (2014) Hemotropic mycoplasmas in little brown bats (*Myotis lucifugus*). Parasit Vectors 7:117. https://doi.org/10.1186/1756-3305-7-117
41. Mercier E, Peters IR, Billen F, Battaille G, Clercx C, Day MJ, Peeters D (2013) Potential role of *Alternaria* and *Cladosporium* species in canine lymphoplasmacytic rhinitis. J Small Anim Pract 54(4):179–183. https://doi.org/10.1111/jsap.12049
42. Millan J, Lopez-Roig M, Delicado V, Serra-Cobo J, Esperon F (2015) Widespread infection with hemotropic mycoplasmas in bats in Spain, including a hemoplasma closely related to "*Candidatus Mycoplasma hemohominis*". Comp Immunol Microbiol Infect Dis 39:9–12. https://doi.org/10.1016/j.cimid.2015.01.002
43. Morrow JL, Frommer M, Shearman DC, Riegler M (2015) The microbiome of field-caught and laboratory-adapted Australian Tephritid fruit fly species with different host plant use and specialisation. Microb Ecol 70(2):498–508. https://doi.org/10.1007/s00248-015-0571-1
44. Muhldorfer K (2013) Bats and bacterial pathogens: a review. Zoonoses Public Health 60(1):93–103. https://doi.org/10.1111/j.1863-2378.2012.01536.x
45. Nash AK, Auchtung TA, Wong MC, Smith DP, Gesell JR, Ross MC, Stewart CJ, Metcalf GA, Muzny DM, Gibbs RA, Ajami NJ, Petrosino JF (2017) The gut mycobiome of the human microbiome project healthy cohort. Microbiome 5(1):153. https://doi.org/10.1186/s40168-017-0373-4
46. Okmen B, Collemare J, Griffiths S, van der Burgt A, Cox R, de Wit PJ (2014) Functional analysis of the conserved transcriptional regulator CfWor1 in *Cladosporium fulvum* reveals diverse roles in the virulence of plant pathogenic fungi. Mol Microbiol 92(1):10–27. https://doi.org/10.1111/mmi.12535
47. Orbach DN, Veselka N, Dzal Y, Lazure L, Fenton MB (2010) Drinking and flying: does alcohol consumption affect the flight and echolocation performance of phyllostomid bats? PLoS ONE 5(2):e8993. https://doi.org/10.1371/journal.pone.0008993
48. Pauli BP, Zollner PA, Haulton GS, Shao G, Shao G (2015) The simulated effects of timber harvest on suitable habitat for Indiana and northern long-eared bats. Ecosphere. https://doi.org/10.1890/es14-00336.1
49. Phillips CD, Phelan G, Dowd SE, McDonough MM, Ferguson AW, Delton Hanson J, Siles L, Ordonez-Garza N, San Francisco M, Baker RJ (2012) Microbiome analysis among bats describes influences of host phylogeny, life history, physiology and geography. Mol Ecol 21(11):2617–2627. https://doi.org/10.1111/j.1365-294X.2012.05568.x
50. Pottier I, Gente S, Vernoux JP, Gueguen M (2008) Safety assessment of dairy microorganisms: *Geotrichum candidum*. Int J Food Microbiol 126(3):327–332. https://doi.org/10.1016/j.ijfoodmicro.2007.08.021
51. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41(Database Issue):D590–D596. https://doi.org/10.1093/nar/gks1219
52. Roggenbuck M, Baerholm Schnell I, Blom N, Baelum J, Bertelsen MF, Sicheritz-Ponten T, Sorensen SJ, Gilbert MT, Graves GR, Hansen LH (2014) The microbiome of new world vultures. Nat Commun 5:5498. https://doi.org/10.1038/ncomms6498
53. Sam QH, Chang MW, Chai LY (2017) The fungal mycobiome and its interaction with gut bacteria in the host. Int J Mol Sci. https://doi.org/10.3390/ijms18020330
54. Sasaki Y, Ishikawa J, Yamashita A, Oshima K, Kenri T, Furuya K, Yoshino C, Horino A, Shiba T, Sasaki T, Hattori M (2002) The complete genomic sequence of Mycoplasma penetrans, an intracellular bacterial pathogen in humans. Nucleic Acids Res 30(23):5293–5300. https://doi.org/10.1093/nar/gkf667
55. Sommer F, Stahlman M, Ilkayeva O, Arnemo JM, Kindberg J, Josefsson J, Newgard CB, Frobert O, Backhed F (2016) The gut microbiota modulates energy metabolism in the hibernating brown bear *Ursus arctos*. Cell Rep 14(7):1655–1661. https://doi.org/10.1016/j.celrep.2016.01.026
56. Sonnenburg JL, Backhed F (2016) Diet-microbiota interactions as moderators of human metabolism. Nature 535(7610):56–64. https://doi.org/10.1038/nature18846
57. Spencer DM, Spencer JFT, Defigueroa L, Heluane H (1992) Yeasts associated with rotting citrus-fruits in tucuman, Argentina. Mycol Res 96:891–892
58. Suhr MJ, Banjara N, Hallen-Adams HE (2016) Sequence-based methods for detecting and evaluating the human gut mycobiome. Lett Appl Microbiol 62(3):209–215. https://doi.org/10.1111/lam.12539
59. Tra Bi CY, N'Guessan FK, Kouakou CA, Jacques N, Casaregola S, Dje MK (2016) Identification of yeasts isolated from raffia wine (*Raphia hookeri*) produced in Cote d'Ivoire and genotyping of *Saccharomyces cerevisiae* strains by PCR interdelta. World J Microbiol Biotechnol 32(8):125. https://doi.org/10.1007/s11274-016-2095-3

60. Tremaroli V, Backhed F (2012) Functional interactions between the gut microbiota and host metabolism. Nature 489(7415):242–249. https://doi.org/10.1038/nature11552
61. Underhill DM, Lliev LD (2014) The mycobiota: interactions between commensal fungi and the host immune system. Nature Rev Immunol 14(6):405–416. https://doi.org/10.1038/nn3684
62. Veikkolainen V, Vesterinen EJ, Lilley TM, Pulliainen AT (2014) Bats as reservoir hosts of human bacterial pathogen, *Bartonella mayotimonensis*. Emerg Infect Dis 20(6):960–967. https://doi.org/10.3201/eid2006.130956
63. White JR, Nagarajan N, Pop M (2009) Statistical methods for detecting differentially abundant features in clinical metagenomic samples. PLoS Comput Biol 5(4):e1000352. https://doi.org/10.1371/journal.pcbi.1000352
64. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD (2011) Linking long-term dietary patterns with gut microbial enterotypes. Science 334(6052):105–108. https://doi.org/10.1126/science.1208344
65. Wu Z, Yang L, Ren X, He G, Zhang J, Yang J, Qian Z, Dong J, Sun L, Zhu Y, Du J, Yang F, Zhang S, Jin Q (2016) Deciphering the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging infectious diseases. ISME J 10(3):609–620. https://doi.org/10.1038/ismej.2015.138
66. Yan L, Yang C, Tang J (2013) Disruption of the intestinal mucosal barrier in *Candida albicans* infections. Microbiol Res 168(7):389–395. https://doi.org/10.1016/j.micres.2013.02.008
67. Zeller S, Lempert S, Goebeler M, Hamm H, Kolb-Maurer A (2015) *Cladosporium cladosporioides*: a so far unidentified cause of white piedra. Mycoses 58(5):315–317. https://doi.org/10.1111/myc.12311