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Letter to the editor Open Access

Unique characteristics of gut microbiota in black snub-nosed monkeys (*Rhinopithecus strykeri*) reveal an enzymatic mechanism of adaptation to dietary vegetation

DEAR EDITOR,

The Myanmar or black snub-nosed monkey (*Rhinopithecus strykeri*) is a recently discovered and critically endangered colobus primate with an unknown gut microbiota. Here, we characterized and compared the gut microbiota of *R. strykeri* with those of two closely related snub-nosed monkey species, *R. roxellana* and *R. bieti*. Results showed that *R. strykeri* exhibits a unique gut microbiota composition, with higher bacterial and fungal diversity and greater abundance of carbohydrate-active enzymes (CAZymes) related to pectate and glucose metabolism. In addition, we identified core microbial taxa shared among the three snub-nosed monkey species, involved in the digestion of carbohydrates that cannot be digested by the host, such as cellulose, hemicellulose, and lignin. Our findings provide insights into the important role of the gut microbiota in facilitating adaptation to dietary vegetation in snub-nosed monkeys, and enabling the expansion of their dietary niches.

Rhinopithecus strykeri is a recently discovered snub-nosed monkey species located in the Gaoligong Mountains on the Sino-Myanmar border (Geissmann et al., 2011). All snub-nosed monkeys are highly folivorous, exploiting diets composed predominantly of leaves, seeds, bark, and lichen (Zhou et al., 2014). Despite exhibiting a similar dietary structure to other snub-nosed monkeys, *R. strykeri* consumes more fruits, seeds, buds, and flowers than *R. roxellana* and *R. bieti* (Yang, 2019). The gut microbiota plays a crucial role in foregut fermentation during leaf consumption in *R. roxellana* (Zhou et al., 2014). Coker (2022) recently reported that the fungal and viral communities are also critical components of the animal gut microbiota, essential for maintaining intestinal homeostasis and promoting animal survival. To date, however, these microbial communities have not been characterized in any snub-nosed monkey species, and there are no reports on the gut microbiota of *R. strykeri*.

In the current study, to clarify the potential uniqueness of the *R. strykeri* gut microbiota and elucidate the dietary adaptive mechanisms of the three species, we collected fecal samples of 42 individuals in the wild, including 13 *R. strykeri* (Rst), 15 *R. roxellana* (Rro), and 14 *R. bieti* (Rbi) monkeys, from seven different locations (Supplementary Table S1). We adopted shotgun metagenomic sequencing to explore the similarities and differences in the bacterial, fungal, and viral communities and to detect the carbohydrate hydrolases among the three species.

Sequence taxonomy was resolved using Kraken2 to estimate microbial abundance at each taxonomic level. Alpha and beta diversities were calculated based on the abundance of each genus. Bacterial, fungal, and viral genera that generally existed in at least one monkey group were filtered to identify coexisting microbial clusters, and then the unique characteristics of each group were detected. Single-sample metagenomic assembly and carbohydrate-active enzyme (CAZyme) annotation were used to compare differences in carbohydrate metabolism capacity among the three groups. Core microbes and CAZymes from the three groups were then selected to explore the general characteristics of the gut microbiota in the three snub-nosed monkey species. Further details on methods are available in the Supplementary Materials.

Our results indicated that the gut microbiota structure at the phylum level was similar among the three species, primarily composed of bacteria, fungi, and viruses (Supplementary Figure S1A). The most abundant bacterial phyla detected in the three groups were Firmicutes (mean 56.17%), Proteobacteria (mean 16.19%), and Bacteroidetes (mean 14.32%). The most abundant fungal and viral phyla were Ascomycota (mean 88.91%) and Uroviricota (mean 77.04%), respectively. The Venn diagram showed that most microbial genera and carbohydrate hydrolases were shared among the three monkey species (Supplementary Figures S1B, S2A). However, beta and alpha diversities showed significant differences between Rst and the other two groups. Beta diversity analyses, including non-metric multidimensional scaling (NMDS) and principal coordinate analysis (PCoA), demonstrated that Rbi and Rro were clustered together and significantly distant from Rst (Figure 1A; Supplementary Figures S3A, S2B; $P<0.005$). Alpha diversity was calculated using the Chao1 and Shannon indices at the genus level across the three monkey groups. Results showed that the gut microbiota diversity of Rst was significantly lower than that of Rbi and Rro (Figure 1B; Supplementary Figure S3B; $P<0.0001$), with no significant differences detected between the latter two species.

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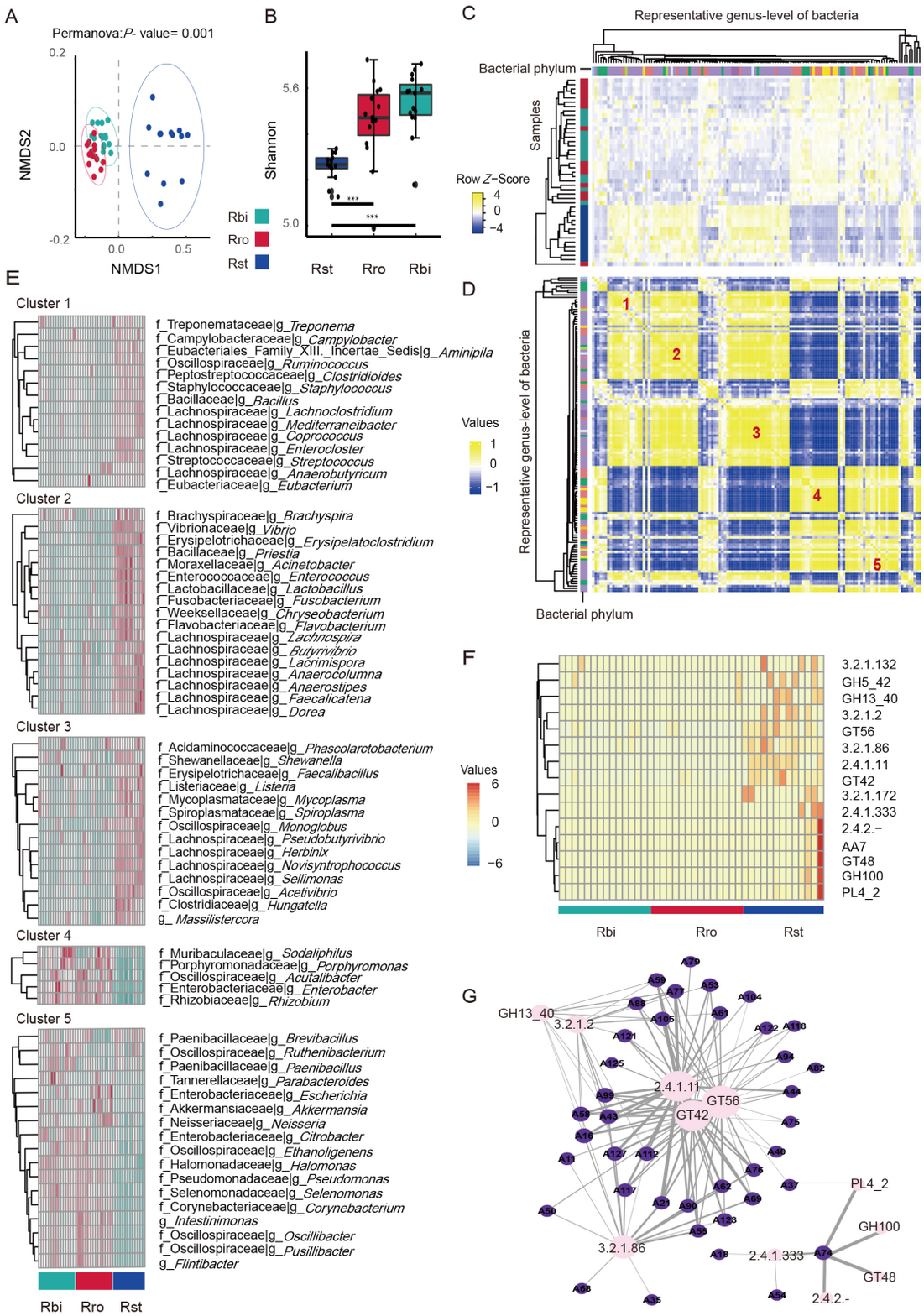

Figure 1 Gut microbial diversity and uniqueness in three snub-nosed monkey species

A: Representative NMDA plots of gut microbiota community composition of three snub-nosed monkey species at the genus level. B: Representative alpha diversity of gut microbiota of three snub-nosed monkey species at the genus level. C: Heatmap representing relative abundance of each bacterial genus in each sample. Heatmap was hierarchically clustered using average linkage based on Euclidean distance and showed the coexistence of several groups of bacterial genera within specific groups. D: Correlation matrix calculated among vectors of abundances of bacterial genera across all samples. Bacterial genera were hierarchically clustered using average linkage based on Euclidean distance. E: Five clusters of coexisting bacterial genera were visually selected, and their abundances in corresponding hosts are depicted in Figure 1D. Clusters 1, 2, and 3 coexisted in Rst; whereas Clusters 4 and 5 coexisted in Rro and Rbi. F: Heatmap of CAZymes that were significantly more abundant in Rst compared with Rbi and Rro. G: Correlation network diagram of coexisting bacteria and significantly more abundant CAZymes detected in Rst.

Based on the above results, although the gut microbiota of the three species shared many of the identified microbial genera and enzymes, gut microbiota structure and function were more similar in Rro and Rbi than in Rst. Furthermore, gut microbial diversity was significantly higher in Rbi and Rro than in Rst. Both Rbi and Rro inhabit mixed deciduous broad-leaved and coniferous forests, where lichens are an important dietary component (Zhou et al., 2014). In contrast, Rst inhabits humid mixed evergreen broad-leaved, coniferous, and bamboo forests (Chen et al., 2022; Ma et al., 2014), where fruits, flowers, and buds are more abundant (Yang, 2019). In addition, the Rst samples were only collected from one location (Gaoligong Mountains), whereas the Rro and Rbi samples were collected from three different habitat locations, which may explain their greater gut microbial diversity. Previous research has suggested that host lifestyle can influence gut microbiota more than geographic location in shaping the microbiomes of primates, including humans (Campbell et al., 2020). Thus, we speculate that the similar lifestyles of Rro and Rbi contribute to the similarities in their gut microbiota.

Based on the distinctive structure of the Rst gut microbiota, we analyzed the relationship between the gut microbiome and phylogeny in the three species. We identified 130 bacterial, 43 fungal, and 121 viral genera in more than 20% of individuals in each monkey group (Supplementary Table S2). Based on cluster and correlation analyses of the microbial genera, we identified several clusters of coexisting gut microbes within each group (Figure 1C, D; Supplementary Figures S4, S5). Notably, bacterial Clusters 1, 2, and 3 coexisted in Rst, whereas Clusters 4 and 5 coexisted in Rbi and Rro (Figure 1C, D). Most of the 45 bacterial species coexisting in Rst are related to pectate and glucose degradation, including the Lachnospiraceae (a typical pectin-decomposing family) and Oscillospiraceae families, as well as the *Massilistercora*, *Faecalibacillus*, and *Vibrio* genera (Figure 1E). In contrast, among the 22 bacterial species coexisting in Rro and Rbi (Figure 1E), *Oscillibacter* and *Parabacteroides* are involved in fiber digestion. Similar results were obtained following analysis of coexisting fungal genera among all monkeys. Yeast fungal clusters associated with pectate and glucose fermentation were more abundant in the Rst gut microbiota (Supplementary Figure S4), with most yeast genera belonging to the Saccharomycetaceae family, including *Lachancea*, *Saccharomyces*, and *Kazachstania*. No obvious viral genera clusters were detected for the different monkey groups (Supplementary Figure S5). We also compared CAZymes among the three species and identified 15 CAZymes significantly enriched in Rst (Figure 1F; Supplementary Table S3), with most involved in starch and glucose metabolism, such as glycogen (starch) synthase (2.4.1.11), phospho-beta-glucosidase A (3.2.1.86), and glycosyltransferase (GT42). Furthermore, the CAZymes and coexisting bacteria were correlated in Rst (Figure 1G; correlation coefficient>0.6; P>0.005). Notably, several families of Firmicutes, including Lachnospiraceae; Bacillaceae, Enterococcaceae, Oscillospiraceae, and Clostridiaceae, were highly correlated with the CAZymes. These findings demonstrated a strong correlation between the bacteria and CAZymes clustered and enriched in Rst, indicating consistent distribution and functional trends of the bacterial and functional genes.

A greater number of microbes and carbohydrate-digesting enzymes related to pectin and glucose metabolism were detected in the gut microbiota of Rst (Figure 1E–G; Supplementary Figure S4). Compared with Rro and Rbi, the Rst diet contains a higher proportion of flowers (Rst: ~15.2%; Rbi: 1.1%–1.9%; Rro: 1.13%–1.3%), buds (Rst: ~13.8%; Rbi: ~3%; Rro: 5.36%–5.8%), and fruits/seeds (Rst: ~15.7%; Rbi: 1.1%–10.5%; Rro: ~9.5%) (Yang, 2019). These plant parts contain a higher proportion of pectin and glucose compared to leaves. Thus, we suggest that the enhanced ability of the gut microbiota in *R. strykeri* to digest pectin and glucose may be an adaptation to the higher proportion of these nutrients in the diet, enabling the monkeys to stabilize in their unique habitat (Chen et al., 2022; Ma et al., 2014). Diet can rapidly and reproducibly alter the human gut microbiome, thus reflecting high variability in the gut microbiota in feeding adaptation (David et al., 2014). Multiple studies have also revealed the important role of the gut microbiota in maintaining adaptation to different habitats and in expanding diets (Xiang et al., 2012; Zhou et al., 2014). Similar dietary patterns have also been observed in the Tonkin snub-nosed monkey (*R. avunculus*) and gray snub-nosed monkey (*R. brelichi*), which consume relatively higher proportions of flowers, buds, and fruits in their diets (Xiang et al., 2012; Yang, 2019). We deduce that their gut microbes may play a similar role in feeding adaptation; however, the characteristics of their gut microbiota remain to be explored.

We extracted the core microbes and CAZymes from 42 individuals to explore common gut microbiota functions among the three species of snub-nosed monkeys (Supplementary Table S4). The core bacteria mainly belonged to the phyla Firmicutes, Bacteroidetes, and Proteobacteria, with *Paenibacillus* (5.22%), *Clostridium* (3.68%), and *Faecalibacterium* (3.25%) being the most abundant genera (Supplementary Table S4A). The core fungi mainly belonged to the phylum Ascomycota, with *Fusarium* (9.59%), *Aspergillus* (8.06%), and *Eremothecium* (7.38%) being the most abundant genera (Supplementary Table S4B). The core viruses mainly belonged to the phyla Nucleocytoviricota and Uroviricota, with *Mimivirus* (9.6%) being the most abundant genus (Supplementary Table S4C). Core CAZymes primarily belonged to 10 glycoside hydrolase families and seven glycosyl transferase families (Supplementary Table S4D). Glycoside hydrolases are a widespread group of enzymes that hydrolyze the glycosidic bond between two or more carbohydrates, or between carbohydrate and noncarbohydrate moieties. Glycosyltransferases enzymes are involved in the biosynthesis of disaccharides, oligosaccharides, and polysaccharides, as well as the metabolism of various carbohydrates, such as xylan, starch, mannose, and galactose.

Although the gut microbiota of *R. strykeri* reflects advantages in pectin and glucose digestion, as a typical leaf-eating nonhuman primate, it also shares similar dietary adaptations to those of other snub-nosed monkeys. At our sampling sites, *R. roxellana* and *R. bieti* predominantly consume lichens (Rro: 38.4%–43.28%; Rbi: 50.6%–82.1%) and leaves (Rro: 22.4%–32.22%; Rbi: 9.1%–24.7%) (Yang, 2019). Similarly, *R. strykeri* consumes a large proportion of leaves (37.9%) and twigs (7.8%), including total nonstructural carbohydrates of 34.9% and neutral detergent fiber of 34.68% (Yang, 2019). Our results related to core microbes and CAZymes indicated the three snub-nosed monkey species have a strong ability to digest complex carbohydrates, including cellulose, hemicellulose, starches, and pectin, in

addition to simple carbohydrates, such as glucose and oligosaccharides. Consistent with our results, Xu et al. (2015) detected numerous glycoside hydrolases enriched in the gut microbiota of *R. bieti*. Glycoside hydrolases have also been found in abundance in the rumen microbiome of bovines and in the gut of termites (*Trinervitermes trinervoides*) (Brulc et al., 2009), with abundant glycosyltransferases also detected in the cow rumen. Thus, these dietary adaptations are not only widely observed in the gut microbiota of snub-nosed monkeys, but also in other herbivores, reflecting potential convergent evolution of gut microbiota during dietary adaptation (Muegge et al., 2011).

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Before conducting sampling in the wild, we obtained approval from the State Forestry and Nature Reserves and the Institutional Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences.

DATA AVAILABILITY

Sequencing data can be found in the Sequence Read Archive (NCBI, nlm.nih.gov/sra) under BioProjectID PRJNA916620, GSA (https://ngdc.cncb.ac.cn/gsa/) under BioProjectID PRJCA014890, and Science Data Bank (https://www.scidb.cn/c/zoores) databases DOI:10.57760/sciencedb.07331.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

M.L. and Z.F.X. designed the project. X.C.W., J.L.Z., and H.J.P. compiled the data and conducted the analyses. Y.X.C. and Z.F.X. conducted the sampling. X.C.W., J.L.Z., H.J.P., and M.L. wrote the paper. S.X.M., J.W.Q., Y.S., and M.Y.Z. provided help in analysis. All authors read and approved the final version of the manuscript.

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