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Specialized digestive adaptations within the hindgut of a colobine monkey

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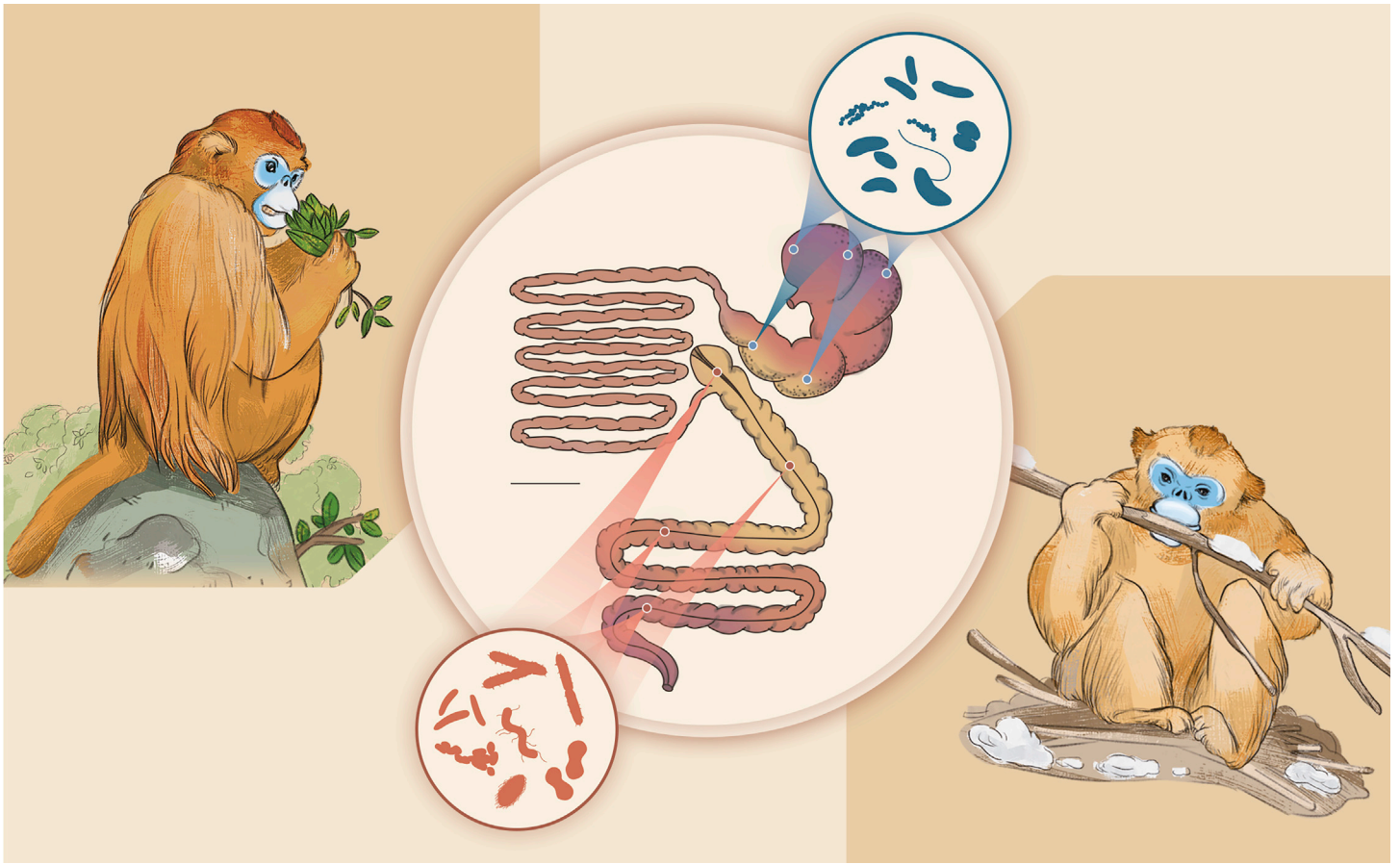
GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- How folivores extract adequate nutrition from their ultra-high-fiber diets remains unclear
- We studied the morphology, microbiome and digestive efficiency of gut for *R. roxellana* (GSM)
- Both fore- and hind-gut regions of GSM play important function of digesting complex carbohydrates
- An enlarged colon of GSM likely accommodates a high throughput of fiber-rich food during winter

Report

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In mammal herbivores, fiber digestion usually occurs predominantly in either the foregut or the hindgut. Reports of mechanisms showing synergistic function in both gut regions for the digestion of fiber and other nutrients in wild mammals are rare because it requires integrative study of anatomy, physiology, and gut microbiome. Colobine monkeys (Colobinae) are folivorous, with high-fiber foods fermented primarily in their foreguts. A few colobine species live in temperate regions, so obtaining energy from fiber during the winter is essential. However, the mechanisms enabling this remain largely unknown. We hypothesized that such species possess specialized mechanisms to enhance fiber digestion in the hindgut and studied microbial and morphological digestive adaptations of golden snub-nosed monkeys (GSMs), *Rhinopithecus roxellana*. which is a temperate forest colobine from central China that experiences high-thermal-energy demands while restricted to a fibrous, low-energy winter diet. We tested for synergistic foregut and hindgut fiber digestion using comparisons of morphology, microbiome composition and function, and digestive efficiency. We found that the GSM colon has a significantly greater volume than that of other foregut-fermenting colobines. The microbiomes of the foregut and hindgut differed significantly in composition and abundance. However, while digestive efficiency and the expression of microbial gene functions for fiber digestion were higher in the foregut than in the hindgut, both gut regions were dominated by microbial taxa producing enzymes to enable active digestion of complex carbohydrates. Our data suggest that both the GSM foregut and hindgut facilitate fiber digestion and that an enlarged colon is likely an adaptation to accommodate high throughput of fiber-rich food during winter.

INTRODUCTION

Mammals rely on microbes in the gastrointestinal (GI) tract to metabolize dietary structural carbohydrates, such as cellulose and hemicellulose, and produce host-available energy in the form of short-chain fatty acids (SCFAs).[1] GI microbes also neutralize dietary toxins and digestive inhibitors,[2,3] further improving host digestive efficiency. These functions are particularly important for host species that consume food containing relatively high proportions of fiber and toxins, such as grass, bark, and leaves. As a result, a wide variety of host adaptations have evolved to optimize the digestive efficiency of symbiotic gut microbiota, including sacculated stomachs, enlarged ceca and colons, and rumination[4,5] in ungulates and coprophagous mammals.[6]

Among primates, lineages have evolved either a large, multi-chambered foregut (stomach)[7] or a voluminous hindgut (cecum-colon),[8] in which high volumes of fiber are fermented by symbiotic microorganisms. Colobine primates have a sacculated foregut and a relatively small hindgut compared with hindgut-fermenting primates.[7,8] The foregut has thus been suggested as the most important GI chamber for microbial fermentation and subsequent contributions to host nutrition in colobines.[9–11] Nevertheless, colobines are likely to be less efficient at foregut microbial fermentation than ruminants.[7,11] Although often termed "ruminant-like,"[7] with the exception of the proboscis monkey (*Nasalis larvatus*),[5] colobines do not ruminate (regurgitate and re-masticate a bolus of previously consumed food). Moreover, although colobines typically exhibit three or four stomach fermentation chambers, these chambers do not exhibit the same strong functional division as do ruminants,[7,8] and increased mixing of food particles occurs.[12] As a result, although only the smallest, most completely digested particles are allowed to pass from the rumen into the rest of the ruminant digestive tract, particles of any size and stage of digestion can pass from the colobine foregut to the rest of the digestive tract. Given this reduced efficiency of the colobine foregut, it is still not fully understood how colobines extract sufficient energy and nutrients from their high-fiber, high-toxin diets.[13]

For mammals consuming diets high in fiber and toxins, it has been posited that the most efficient digestive anatomy should include both a foregut and hindgut of similar size.[14] It is known that many ruminants benefit nutritionally from microbial fermentation in both the rumen and the hindgut.[1,2,4] In these species, the hindgut continues to ferment structural carbohydrates that pass undigested from the foregut and to also digest proteins that have been released from compounds, such as tannins, by microbial metabolism.[2,15] The hindgut also digests microbial material originating in the foregut.[9] However, in most ruminants, the hindgut exhibits a reduced volume compared with the rumen, likely as a result of reduced food volume in the distal GI tract as well as the incomplete mixing of food particles in the rumen that allows only the most completely digested food particles to pass on to the rest of the GI tract. In short, the increased digestive efficiency that would be conferred by an enlarged hindgut is unnecessary.

Because colobines do not ruminate and exhibit particle mixing in the foregut, more undigested structural carbohydrates and proteins are likely to reach the hindgut compared with other foregut-fermenting mammals, such as ruminants. Therefore, in colobines, microbial fermentation in the hindgut may play a more important role in complementing the function of the foregut. Although hindgut microbial community composition is distinct in colobines compared with hindgut-fermenting primates, the colobine hindgut still harbors microbial taxa and genes typically associated with fermentation in other primate hindguts.[16,17] Additionally, high SCFA concentrations in both the foregut and hindgut of king colobus monkeys (*Colobus polykomos*) indicate substantial microbial fermentation of fiber in both compartments.[7] However, like ruminants, most colobines possess a hindgut with a smaller volume than the foregut, suggesting that the hindgut plays a secondary role to the foregut in the digestion of high-fiber and high-toxin foods. Importantly, though, most previous studies have not directly compared microbial activity in both the foregut and hindgut.[18] Without integrated data describing the gut morphology, microbiome, and enzymatic activity in the same individuals, it is difficult to determine the extent to which microbial functions in the colobine hindgut repeat and/or complement microbial functions in the foregut.

The relationship between the foregut and hindgut may also vary among colobine species in response to the nutritional landscapes within which they evolved. Although all colobines consume hard-to-digest diets high in fiber and toxins, some inhabit more seasonal environments in which shifts in food availability and climate can make meeting energy demands more difficult. For these species, a hindgut of increased volume with substantial microbial activity to complement the digestive function of the foregut could be advantageous to survival.

To explore this possibility, we collected data describing the digestive function of the foregut and hindgut of wild golden snub-nosed monkeys (GSMs; *Rhinopithecus roxellana*). The GSM is an ideal model species to examine the digestive relationship between the colobine foregut and hindgut. The GSM is endemic to

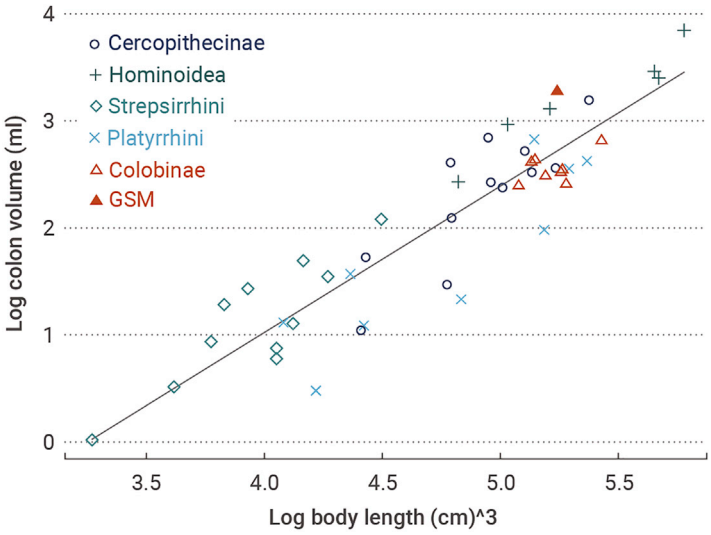

Figure 1. Relationship between the raw values of body size in centimeters (log of cubed value) and colon volume (log of volume in milliliters) in 48 primate species
Ten members of the Colobinae are shown as empty red triangles; the datum point for *R. roxellana* is shown as a filled red triangle. The high positive residual (raw data) for *R. roxellana* shows that this species has a relatively large colon for its size compared with other species included in the analysis. The results of a least-squares regression (with the intercept set at zero) using the phylogenetically independent contrasts of these data are b = 1.44, $F_{1, 44}$ = 74.32, $p < 0.001$, and R^2 = 0.62 (see materials and methods). The relationship thus remains significant when the effects of phylogeny have been removed. Data used are as presented in Table S2.

a few temperate forests in China, where it endures the longest winters and lowest temperatures of most non-human primates, except a few macaques.[19] Although the GSM diet is dominated by leaves during much of the year, leaves are unavailable during winter. GSMs are therefore forced to switch to a fibrous diet of lichens, buds, and bark.[20,21] Thermal demands also result in GSMs requiring twice as much energy during winter compared with the spring. Winter thus presents these monkeys a considerable challenge to extract the required amount of energy from an enforced ultra-high-fiber diet.[22,23] Balancing the energy cost during the winter is partially achieved by increasing daily food consumption,[22] but GSMs are also likely to depend heavily on microbial functions to extract sufficient nutritional resources from their diets during the winter. This harsh nutritional environment increases the likelihood that GSMs are reliant on the complementary functions of the foregut and hindgut microbiomes compared with other (tropical/sub-tropical) colobines that do not experience such pronounced seasonal challenges to their nutritional demands.

We tested the hypothesis that the hindgut plays an important digestive role for GSMs despite the presence of a sacculated foregut. Specifically, we predicted that the hindgut of GSMs would be relatively large, compared with other colobines, to allow for increased microbial fermentative activity and subsequent nutrient absorption. Because the foregut is exposed to the highest concentrations of dietary structural carbohydrates and toxins, we expected that it would be enriched in microbial genes and taxa associated with structural carbohydrate degradation and xenobiotic metabolism, compared with the hindgut. However, given that the morphology of the colobine foregut reduces the efficiency of digestion of these compounds, we expected to detect similar microbial genes and taxa in the hindgut, albeit at lower relative abundances. Furthermore, we expected the hindgut to be enriched in microbial genes and taxa associated with protein metabolism to aid in the digestion of microbially liberated dietary proteins as well as microbial proteins from the foregut. Finally, we predicted that enzymatic activity targeting structural carbohydrates would be present in both the foregut and hindgut, although at a reduced capacity in the hindgut.

RESULTS

Gut morphology

The raw data for each species used in the analysis show that GSMs have a typically enlarged colobine stomach (Figures S1 and S2A; Table S2), with a cecum volume that would be expected for a primate of the same size (Figure S2B). However, the GSM also possesses a particularly large colon for its size (Figure 1), averaging 68.8% of the volume of the foregut. Using the residuals from a linear regression of the raw data for colon volume (Log volume(ml)) on body size (Log body lenghth(cm)^3) for all 48 species, the residual for the GSM (0.558) was significantly higher than the mean residual value of the 10 other colobine species included in the analysis (−0.143 [SE = 0.04]; range −0.360 to 0.047; one-sample t test: t_8 = 16.44; $p < 0.001$). Thus, the GSM has a larger colon for its size than the other colobines included in the analysis (Figure 1).

Microbial compositions and functional genes

To further investigate the digestive functions of the GSM gut, we performed 16S analysis for both the foregut and hindgut and found that the compositions of the bacterial communities in the two gut regions differed significantly (permutational multivariate ANOVA [PERMANOVA], F = 40.53, R2 = 0.522, $p < 0.001$; Figure 2A). At both the amplicon sequence variant (ASV) and genus levels, the hindgut microbiome community was more diverse than that of the foregut (Shannon index; χ^2 = 91.1 [ASV], 94.4 [genus], degree of freedom [df] = 1, $p < 0.001$; Figure S3A). Co-occurrence network analysis shown that although the foregut has fewer microbial taxa, they are more closely associated, as demonstrated by multiple network metrics (Figure 3; Table 1). The relative abundance of every prevalent microbial ASV (19 ASVs present in at least 10 samples) differed significantly between the foregut and hindgut, as did 129 of the 132 genera that collapsed from all ASVs. When we considered only the genera with at least a 10-fold change in relative abundance, we found that 39 were more abundant in the hindgut whereas only 7 were more abundant in the foregut (Table S4). Taxa, including the known carbohydrate degraders *Clostridium*, *Roseburia*, *Faecalibacterium*, *Blautia*, *Dorea*, *Bacteroides*, and *Ruminococcus*, were more abundant in the hindgut whereas *Atopobium*, *Acidaminococcus*, *Syntrophococcus*, *Shuttleworthia*, two unknown Selenomonadaceae, and an unknown Lachnospiraceae, were more abundant in the foregut (Figure 2B). The same pattern was observed at the family level. Metagenomic analysis showed similar taxonomic patterns with 16S (Figures 2B and S4).

Using metagenomic data, we identified 356 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways present in the foregut and/or the hindgut. Among these, 137 differed significantly in relative abundance between the two gut regions (Table S5, $p < 0.05$; Figures S5 and S6A; supplemental information). Although the hindgut exhibited richer and more abundant functional genes (Figure S5), the metabolic pathways represented in both gut regions appeared to be complementary. For example, genes for carbohydrate digestion and absorption as well as glycan, pentose, and glucuronate metabolism were enriched in the foregut, whereas genes for the metabolism of starch and sucrose, peptidoglycan, pyruvate, fructose and mannose, amino sugar and nucleotide sugar, carbon and glycosaminoglycan were all more abundant in the hindgut. With respect to xenobiotic metabolism, genes for monoterpenoid and phenylpropanoid biosynthesis, geraniol degradation, and xenobiotic metabolism by cytochrome were enriched in the foregut. In contrast, genes for the biosynthesis of ansamycins, zeatin, flavone, and flavonol as well as genes for the metabolism of other compounds, such as caffeine, were enriched in the hindgut; styrene, nitrotoluene, and polycyclic aromatic hydrocarbon degradation genes were also enriched in the hindgut. Finally, although some pathways associated with protein and amino acid metabolism were more abundant in the hindgut (ribosome ko03010, non-ribosomal peptide structures ko01054, aminoacyl-tRNA biosynthesis ko00970, ko03020, ko00270), many more pathways were more abundant in the foregut (protein digestion and absorption ko04974, protein processing in endoplasmic reticulum ko04141, ko00480, ko00290). Genes for the metabolism of vitamins and lipids also showed the same pattern (Table S5A, Wilcoxon rank-sum test, $p < 0.05$). Because of our focus on cellulose and hemicellulose degradation, we also specifically tested for differences in the relative abundance of genes involved in the metabolism of cellulose and hemicellulose between gut sections. Of the 18 relevant KEGG Orthology that we detected in both gut sections, the relative abundances of all but one were significantly higher in the foregut than in the hindgut (Table S6).

Using a carbohydrate-active enzyme (CAZyme) analysis, overall, we identified gene modules from 107 glycoside hydrolase (GH) families. The bulk of these enzymes are involved in the digestion of oligosaccharides (25%), starch (16%), cellulose and hemicellulose (8%), debranching enzymes (5%), and pectin digesting (GH28) (4%) (Table S7). Although the relative abundances of those GH families in both the foregut and the hindgut show similar patterns (Table S8), the composition of these CAZyme arrays differed between the two gut regions

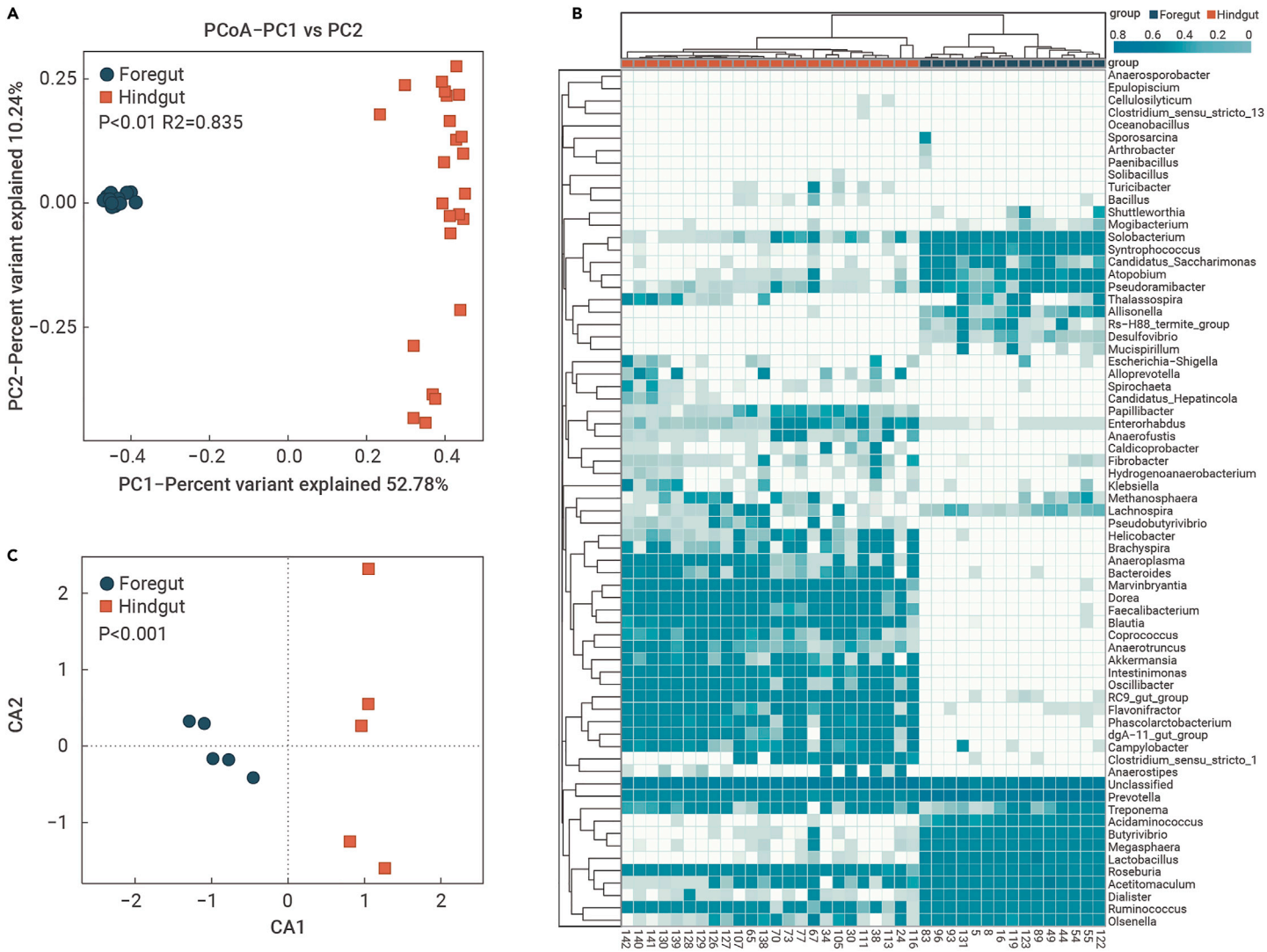

Figure 2. Bacterial community composition and metagenome of the foregut and hindgut of *R. roxellana* (A) Principal coordinates analysis showing different bacterial community compositions in each sample of the two gut regions (PERMANOVA, $p < 0.001$; hindgut data from 24 samples of gastrointestinal sites of 5 individuals; foregut data from 15 samples of gastrointestinal sites of 5 individuals). (B) Bidirectional clustering heatmap of 16S data showing the relative abundances of indicator genera (top 70 relative abundances genera) that characterize each gut region. (C) Plot of the results of a correspondence analysis based on the abundances of different GH families in the foregut (gray points) and hindgut (red squares), which shows that bacterial communities in each gut section exhibit different functional arrangements.

(PERMANOVA, $F = 40.54$, $R2 = 0.83$, $p < 0.01$; Figure 2C). Specifically, the relative abundances of 40 out of 107 identified GH gene families that digest starch, cellulose, and oligosaccharides differed significantly between the foregut and the hindgut (Welch's t test, $p < 0.01$; Figure S6B; Table S7). For example, β-mannosidase, β-glucuronidase (GH2) had higher relative abundance in the foregut whereas amylomaltase (GH77) had higher relative abundance in the hindgut.

Activity of fiber-degrading enzymes

The activity of both β-glucosidase and xylanase was significantly higher in the foregut than in the hindgut (Table S9). The endo-β-1,4-glucanase that digests lignin was not detected in either the foregut or the hindgut.

Cellulose and hemicellulose digestibility

Cellulose and hemicellulose were digested in both the foregut and the hindgut. Although not statistically significant, both cellulose and hemicellulose exhibited a trend of being digested more completely in the foregut than in the hindgut (Tables S10A and S10B).

DISCUSSION

To evaluate the digestive roles of the foregut and hindgut in the GSM, we gathered data describing the anatomy, microbiome compositions and functions, and enzyme presence and activity in the foreguts and hindguts of five wild individuals. Recent work on four *Rhinopithecus* species, including the GSM, shows that these colobines possess derived genetic adaptations associated with an efficient ability to metabolize fatty acids and xenobiotics and to enable the digestion of high levels of RNA derived from their stomach microbiome.[24] Our investigations demonstrate that a specialized gut morphology and microbiome accompany these genetic adaptations, enabling GSMs to subsist on a diet very high in cellulose, hemicellulose, and lignin. As predicted, the hindgut appears to play an important digestive role. The relative size of the GSM hindgut is larger than that of other colobines included in our study. Additionally, while the microbial communities of the GSM foregut and hindgut differ, those of the hindgut appear to perform key digestive functions. Specifically, the foregut microbiota appears to initiate the degradation of dietary fiber and toxins, whereas the hindgut is enriched with a specific subset of microbiota that may target dietary compounds that have been incompletely digested in the foregut.

It has been suggested that the most efficient gut anatomy for the digestion of high-fiber, high-toxin diets is one in which the hindgut has a similar volume as the foregut.[14] Although this anatomy has not been observed in any foregut-fermenting animal, including both ruminants and colobines, the hindgut of the GSM is relatively large compared with other colobine species from which there are data. This enlarged hindgut is consistent with the GSM's nutritional ecology. The GSM consumes large amounts of high-fiber food during winter, when thermal demands are also increased. An enlarged hindgut also enables a longer food retention time and increased microbial activity as well as potentially increased energy and vitamin absorption.[25] These traits likely facilitate survival

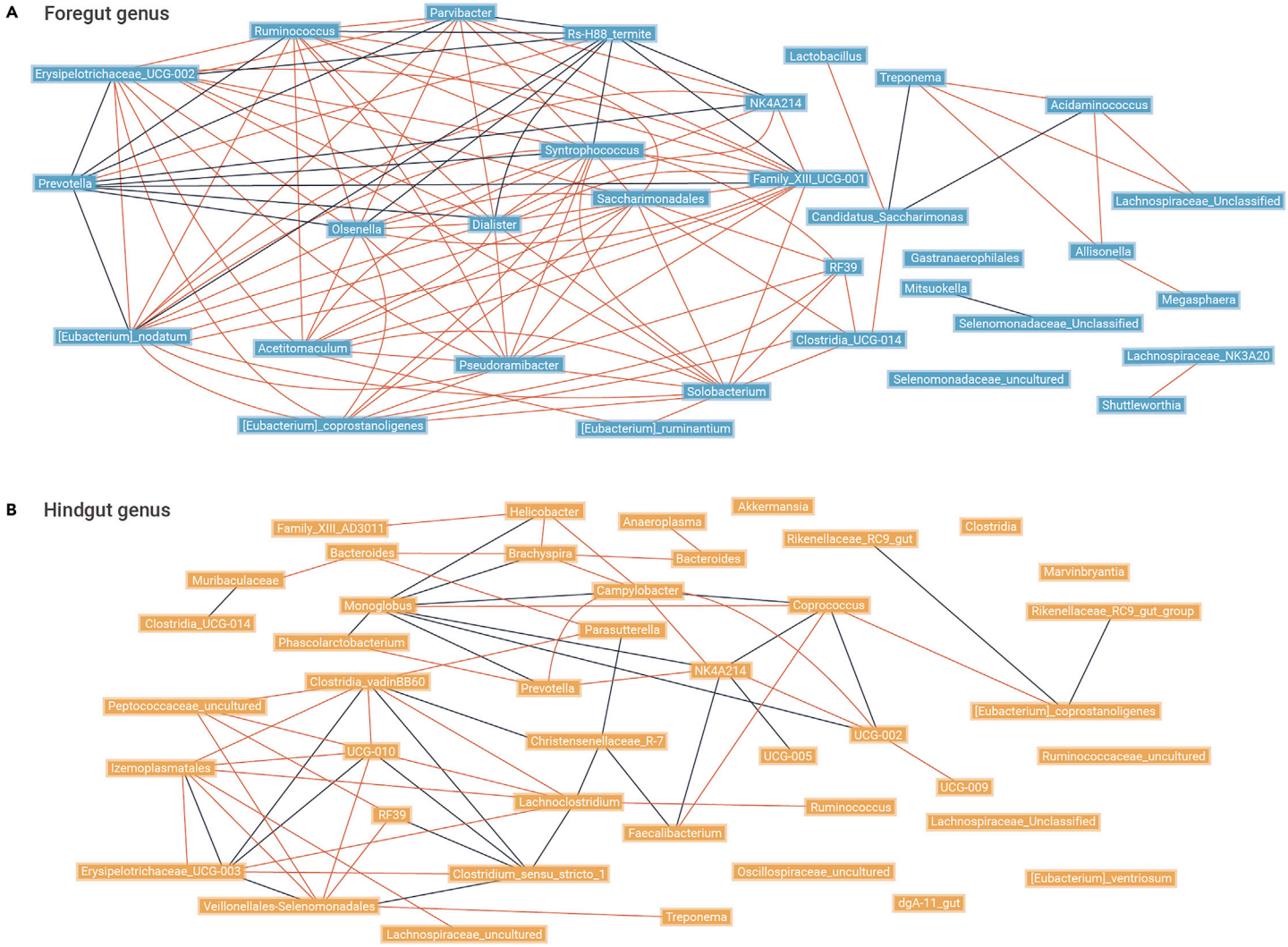

Figure 3. Co-occurrence network constructed from the relative abundances of genus level 16S data for the (A) foregut and (B) hindgut of golden snub-nosed monkeys in the Qinling Mountains Positive/negative correlations are presented as red/blue lines, respectively.

in such a seasonally variable environment. Data describing the food retention time, microbiome composition and function, and enzymatic activity of both the foregut and hindgut in other colobine species are required for a more systematic comparison.

Our microbiome data suggest synergistic food processing functions of the foregut and hindgut in the GSM. For example, the GSM foregut is dominated by *Prevotella* and *Selenomonadales*, which are also both abundant in the foreguts of ruminants (Figures 2B and S4).[26,27] These microbes facilitate hemicellulose and pectin digestion as well as lactate and succinate transformations that increase energy production from fiber degradation by reducing methane production.[4] However, KEGG analysis also showed that genes associated with methane metabolism were enriched in the hindgut. This may indicate that microbes in the hindgut help metabolize methane that exits the foregut (Figure S5, e.g., ko00680). A similar relationship is apparent for xenobiotic degradation, increasing digestive ability. While some genes for degradation of plant secondary metabolites, such as tannins, were enriched in the foregut,[28–30] more were enriched in the hindgut.

Some of the microbial patterns we identified in the GSM hindgut are similar to those described in other colobines. For example, the feces of *Rhinopithecus bieti*, a congeneric species to the GSM that inhabits higher forests (4,500 m above sea level [asl]) and feeds mainly on high-fiber lichens during winter,[31,32] contains a microflora with high relative abundances of *Fibrobacteres*, a lignin degrader, and a wide diversity of GH enzymes for degrading fiber. Similarly, a study of the GSM microbiome found high relative abundances of fiber-degrading *Prevotella* and Ruminococcaceae[33] (Figures 2B and S4). The fecal microbiomes of gray (*Semnopithecus priam*) and purple-faced (*S. vetulus*) langurs contain many of the same microbial taxa that we detected in the GSM.[34] Finally, proboscis monkey (*N. larvatus*) foreguts have high abundances of *Prevotella*, which likely function in structural carbohydrate degradation.[35] However, the extent to which these microbial taxa and genes vary between the foregut and hindgut of different colobine species has yet to be systematically determined. Furthermore, although feces samples are often used to represent the distal gut, our results showing differences in the composition and function of the microbiome, enzyme activity, and degradation between the foregut and hindgut show that feces samples are insufficient to fully understand the biology and ecology of the entire GI tract.[36]

Finally, although the microbiome analysis shows that the hindgut contained a more diverse microbial community than the foregut, the cellulose and hemicellulose digestibility ratios and the activity of fiber-degrading enzymes were both highest in the foregut. These patterns suggest the microbial community of the foregut may be more specialized than that of the hindgut. This is also supported by our co-occurrence analysis, which showed the foregut harboring more specialized functional groups of microbes; although the foregut's network has fewer vertices than the hindgut, they interact more intensively and have more positive edges and correlations (Figure 3; Table 1). However, we still observed substantial cellulose and hemicellulose degradation in the hindgut, suggesting that both gut regions play important roles in fiber metabolism. Furthermore, the abundances and distributions of GH functional genes within the foregut and the hindgut are similar (Table S8). Compared with the GH profiles of cows, pandas, termites, and wallabies, microbiota associated with pectin digestion at the family level are most abundant in the GSM. The GSM GH profile is more similar to the wallaby and the cow rumen and contains a higher proportion of cellulases/endohemicellulases than the other two species known to specialize in fiber digestion.

Table 1. Summary data of genus level co-occurrence network in foregut and hindgut of golden snub-nosed monkeys in Qinlin Mountains

	Number of positive correlations	Number of negative correlations	Number of nodes (genus taxon)	Connectivity	Average degree	Average path length	Betweenness centralization	Degree centralization
Foregut	92	19	32	0.224	6.938	2.713	0.241	0.292
Hindgut	39	30	42	0.080	3.285	3.666	0.155	0.115

Visualization of this network were presented in Figure 3.

However, this needs to be confirmed with more precise evaluation of the enzyme activities within the GSM digestive system.

CONCLUSION

An enlarged foregut enables effective fiber digestion for most leaf-eating colobines. An enlarged specialized hindgut and key complementary microbial functions in both the foregut and hindgut are likely to contribute to digestive efficiency in some foregut-fermenting mammals. Our observation of these traits in our study GSM population is consistent with the hypothesis that the GSM possesses digestive adaptations to enable individuals to optimize energy and nutrient acquisition from a highly fiber-rich diet, especially during winter. This capacity likely helps enable the GSM to balance energy acquisition and expenditure by microbially extracting energy from cellulose and hemicellulose. Our results support our predictions that both the GSM foregut and hindgut play important roles in fiber and toxin digestion. Additional morphological and microbiome data from other colobine and mammal species will clarify the functional importance of the hindgut relative to the foregut in folivores.

MATERIALS AND METHODS

Sample collection

Over the last 15 years, the GSMs of the Zhouzhi National Nature Reserve (ZNNR) in the Qinling Mountains have been studied.[23] During the winters of 2012–2013, we found and then dissected the bodies of a total of 5 GSM individuals that had each recently died of injuries incurred from fighting and/or falling out of a tree (supplemental information). Immediately upon discovery, we carefully slit open each dead body to take samples of the contents from different regions of the digestive tract. We took a total of 39 samples from 5 individuals in the foregut (saccus gastric I, saccus gastric II, proximal gastric, tubus gastric, pistol gastric, and pylorus sinus regions) and the hindgut (cecum, colon, and rectum) (Figure S1; Table S1). Each sample was placed into a 2 mL centrifuge tube and then taken to the laboratory and stored in liquid nitrogen prior to DNA extraction.

Morphology of the major components of the GSM digestive system

We measured the length and mass of each body in the field. Each body was then taken to the laboratory and the digestive tract removed in order for the volumes of the stomach, cecum, and colon to be measured using the same methods, as previously described.[11] The resulting data were then added to an existing database consisting of the same measurements taken from 47 other primate species from all major subfamilies.[37] Linear regression analysis enabled us to assess the sizes of the GSM anatomical traits measured independently of body size relative to the other primate species in the database. To determine statistical significance of the three linear regressions, we used phylogenetically independent contrasts (PICs) of the log-transformed values to account for data non-independence due to common descent (Table S2).

Data analysis of 16S rRNA

We sequenced 47 samples and produced a total of 9,298,270 reads; 79,413 reads per sample were retained after quality filtering, with an average length of 448 base pairs (bp). DNA sequences were demultiplexed and quality filtered using MiSeq Control Software. We used the *search* function for chimerism checks to remove low-quality sequences, the *flash* function for splicing, and the *trimmomatic* function for quality control.[38] Sequences were clustered into ASVs using the DADA2 wrapper in QIIME2 (2019.10)[39] (https://benjjneb.github.io/dada2/tutorial.html). Taxonomy was assigned using a pre-trained Bayesian classifier in QIIME2 and the Silva 138 database (https://www.arb-silva.de/documentation/release-138/). Principal coordinates analysis (PCoA) and an unweighted pair group method with arithmetic mean (UPGMA) tree were used to visualize the data based on both weighted and unweighted UniFrac distances.[40]

We tested for differences in microbial diversity and relative abundances of specific microbial ASVs between the hindgut and foregut using a linear mixed effects model (NLME, R version [v.]3.5.4).

Metagenomic data analysis

We obtained a total of 928,965,472 raw reads (150 bp) across 10 samples from the foreguts and hindguts of the 5 individuals. After removing any adapter-polluted reads or N contents >10%, trimming bases with quality value <20, and removing host contamination reads, a total of 417,333,904 reads, with an average length of 146 bp for the 10 samples, remained (Table S3). Sequences from each sample were assembled *de novo* into contigs with SOAPdenovo (v.2),[41] which we used to construct scaffolds. We built scaftigs by extracting the contiguous sequences that is without unknown bases (N) from each scaffold, resulting in an average of 42,846 scaftigs per sample; 831,312 genes were predicted from those scaftigs using MetaGeneMark (v.3.26). We clustered those genes into 560,215 unique genes with 95% identity using CD-HIT (v.4.5.6).

To analyze the relative abundance of scaftigs in each sample, paired-end clean reads were mapped to assembled scaftigs using the Burrows-Wheeler Aligner (BWA v.0.7.12) to generate read coverage information for assembled scaftigs. Paired forward and reverse read alignments were generated in the sequence alignment map (SAM) format using the BWA-SAMPE algorithm with default parameters. The mapped read counts were extracted using SAMtools 0.1.17. The corresponding scaftigs were mapped to the bacterial data extracted from the Nucleotide (NT) database of the National Center for Biotechnology Information (NCBI). An LCA algorithm (lowest common ancestor, applied in MEtaGenome ANalyzer [MEGAN] software system) was used to ensure the annotation significance by picking out the lowest common classified ancestor for final display.

We conducted a detailed metagenomic study of carbohydrate digestion from the functional genes in the foregut and the hindgut and used an online database to identify CAZymase (for more detail, see supplemental information).

Enzyme activity of fiber digestion in the gut

Endo-cellulase (endo-β-1,4-glucanase) and hemi-cellulase (endo-β-1,4-xylanase) activities were assayed by measuring the amount of reducing sugar released from 2% carboxymethylcellulose (CMC) sodium salt (Sigma-Aldrich, US) and 2% xylan (Sigma-Aldrich, US), respectively, using the dinitrosalicylic acid (DNS) method.[42] β-glucosidase activity on *p*-nitrophenyl-*d*-glucopyranoside (pNPG; Sigma) was assayed.[43]

Digestion ratio

For wild monkeys, it is impossible to use artificial markers to indicate a dry matter diet. We therefore used fecal acid-detergent lignin (ADL) as an internal marker to estimate the digestibility of hemicellulose and cellulose (more detail in the supplemental information).

DATA AND MATERIAL AVAILABILITY

The 16S and metagenomic data can be accessed in NCBI:PRJNA726190, SUB9554749, SUB9557011.

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AUTHOR CONTRIBUTIONS

S.G. designed the research and wrote the manuscript. S. G., D.W.D., R.L., K.A., A.G., C.A.C., P.A.G., B.L., and N.R. contributed to the improvement of our ideas and to the revision of the manuscript. S.G., J.Z., R.H, G.F., and G.H. carried out the data collection and research experiments.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION

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