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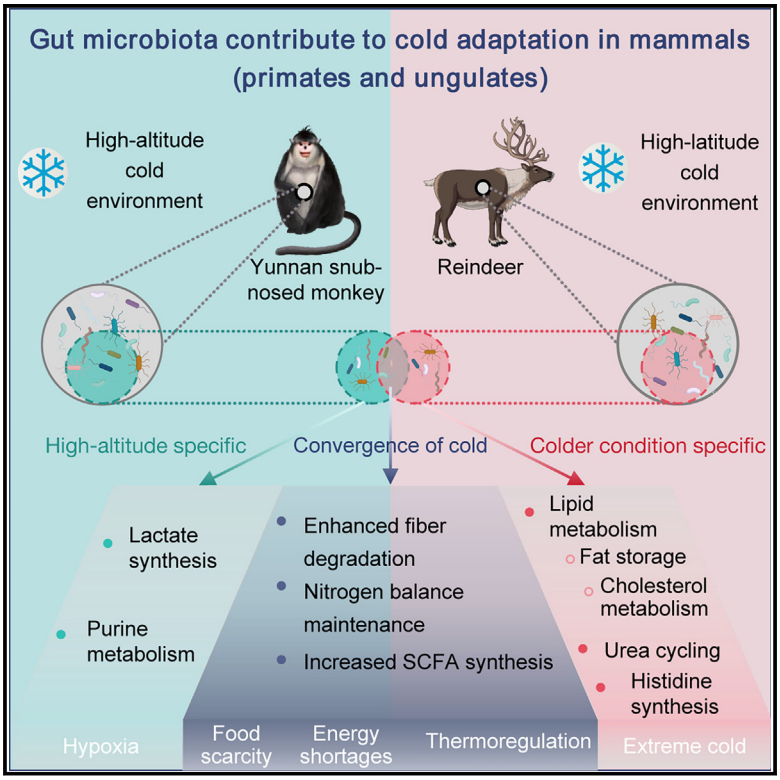
Gut microbiota contribute to cold adaptation in mammals—primates and ungulates

Graphical abstract



Highlights

- Yunnan snub-nosed monkey and reindeer gut microbiota show convergent cold adaptation
- Cold-adapted microbiota add fiber degradation, nitrogen balance, and SCFA synthesis
- Monkey microbiota regulate lactate and purine metabolism for high-altitude condition
- Reindeer microbiota regulate lipid, urea, and histidine synthesis for colder condition

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In brief

Ecology; Zoology; Genomics; Evolutionary biology

Yao et al., 2025, iScience 28, 112245
April 18, 2025
https://doi.org/10.1016/j.isci.2025.112245

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Gut microbiota contribute to cold adaptation in mammals—primates and ungulates

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https://doi.org/10.1016/j.isci.2025.112245

SUMMARY

Gut microbiota play an influential role in how animals adapt to extreme environments. Two phylogenetically distant mammals, Yunnan snub-nosed monkey and reindeer both adapted to frigid environments. Metagenomic analyses revealed they developed similar cold adaptation strategies in response to food scarcity (enhanced fiber degradation and nitrogen balance maintenance), energy shortages (increased short-chain fatty acid [SCFA] synthesis), and a constant body temperature sustainment (stimulation of non-shivering thermogenesis [NST]). Moreover, they evolved distinct adaptation strategies to cope with different cold ecosystems. Yunnan snub-nosed monkey adapt to high-altitude hypoxia environment through enhancing ability to synthesize lactate and metabolize purine, while reindeer adapt to extreme cold environment through increasing blood flow, strengthening urea cycling, and enriching fat storage associated bacteria. Notably, reindeer microbiota uniquely enriched cholesterol-degrading bacteria, potentially mitigating cardiovascular risks from lipid storage. Our study expands the knowledge of how gut microbiome promotes cold adaptation through shared and specialized mechanisms shaped by different phylogenetic and ecological contexts.

INTRODUCTION

An animal must adapt to the environment in which it lives for survival, and this is especially true for extreme environments. Among these, cold environments pose severe challenges to animals due to limited food resources and increased requirements for thermoregulation.[1] The Yunnan snub-nosed monkey and reindeer are examples of mammals, despite having a distant phylogenetic relationship, that have adapted to cold environments. Yunnan snub-nosed monkey inhabits cold environments at high altitudes (>3,000 m), with the annual average temperature of reported populations not exceeding 10°C, and winter nighttime temperatures dropping as low as −10°C.[2–6] Reindeer live in the frigid environment of the Circum-Arctic region, where winter temperatures can drop below −40°C and may even reach −50°C in extreme cold conditions.[7,8] Both mammals have shown phenotypic adaptations to the cold environment. Yunnan snub-nosed monkey has larger body size, allowing them to store more substances and consume less energy for effective thermal regulation,[9] and a diet comprising mainly cold-resistant lichens to cope with the limited food resources in cold environment.[2–4,10,11] In addition, they have been reported to engage in "sleep clusters" by huddling together to conserve heat[12] and choose wind-sheltered, sun-exposed resting sites to maintain thermal balance.[13] Reindeer demonstrates increased fur, fat thickness, lichen-rich diets,[14–16] as well as can restrict heat loss by cooling the peripheral tissues and maintaining a low resting metabolic rate to reduce energy expenditure.[8] Furthermore, they also have evolved genetic adaptations to the cold environment. In Yunnan snub-nosed monkey, the expansion of gene families and upregulation of gene expression associated with oxidative phosphorylation during energy metabolism regulation.[17,18] Reindeer possess the ability to regulate lipid metabolism through specific mutations in genes (*APOB* and *FASN*) and/or changes in gene expression (*UCP1* and *COX4*).[19–23] This suggests that both of these mammalian species, although living in different ecosystems, have developed similar cold-adaptation strategies, including lichen-rich diets, body temperature, and metabolic regulation. In addition to low temperatures, the Yunnan snub-nosed monkey also inhabits regions at high altitudes, which involves the stresses of hypoxia and ultraviolet radiation,[17,18] while reindeer living in the Circum-Arctic region have to develop strategies to cope with extremely cold temperatures and photoperiod pressure.[23]

An increasing number of studies have shown that gut microbiota influence animals' adaptation to the surrounding environment, including cold environments.[24–31] They can help hosts cope with cold environments by enhancing nutrient utilization and optimizing energy acquisition. Studies have shown that cold stress can change the α-diversity and overall composition of gut microbiota in mammals.[28,32–35] In addition, some microbial taxa can help the host maintain energy balance and adapt to harsh conditions through various metabolic pathways.[24,25,28,31,36]

For example, the presence of *Succinivibrio* species in Tibetan macaques has been suggested to enhance fiber degradation efficiency and increase the utilization of limited food resources in cold environments.[37] In yaks, gut microbes such as *Akkermansia* help them survive with the low levels of protein available in cold environments.[26,38] Bacterial species belonging to the families Lachnospiraceae or Prevotellaceae can produce more short-chain fatty acids (SCFAs) to compensate for energy deficits[31,39,40] in most cold-adapted species. In addition to improving metabolism and energy storage, gut microbiota can regulate host's body temperature through the production of microbe-derived metabolites. For instance, the SCFAs, lipopolysaccharides (LPS), and secondary bile acids synthesized by gut microbes, including those belonging to the *Lactobacillus*, *Oscillospira*, or *Prevotella* genera, can activate non-shivering thermogenesis in brown adipose tissue (BAT).[24,30,41,42]

These previous findings show that the gut microbiome plays a critical role in the cold adaptation of animals. However, the extent to which the gut microbiota of two phylogenetically distant mammals, the Yunnan snub-nosed monkey and reindeer, contribute to cold adaptation has not yet been reported. These species offer an interesting opportunity to investigate whether distantly related mammals that can thrive in cold conditions share similar gut microbiome adaptive mechanisms, as well as identify differences between the adaptive mechanisms due to their living in different cold environmental regions. We hypothesize that the gut microbiomes of the Yunnan snub-nosed monkey and reindeer may have developed convergent cold-adaptive features, and moreover have developed specific features to cope with the high-altitude and the more colder pressures respectively. In this study, we thus performed metagenomic analyses to compare the gut microbiota of these two cold-adapted mammals with the microbiota of related mammals inhabiting warmer environments to address these issues. These findings enhance our understanding of how the gut microbiota can contribute to mammalian cold adaptation across different phylogenetic and ecological contexts.

RESULTS AND DISCUSSION

Sequencing data statistics

This study was approved by the Laboratory Animal Welfare Ethics Committee at Yunnan University (approval number: YNU20241056) and all procedures complied with ethical guidelines. We collected fecal samples from Yunnan snub-nosed monkey (*Rhinopithecus bieti*, $n = 30$) and reindeer (*Rangifer tarandus valentinae*, $n = 26$) specimens, as well as fecal samples from various specimens of Tonkin snub-nosed monkey (*Rhinopithecus avunculus*, $n = 30$), a relative of the Yunnan snub-nosed monkey that inhabits tropical regions of Vietnam, for metagenomic sequencing. The publicly available metagenomic data for Père David's deer (*Elaphurus davidianus*, $n = 30$), a species that resides in subtropical regions of China and is closely related to reindeer,[43] is also included. A total of 116 samples were gathered for subsequent analysis (Table S1). Metagenomic sequencing yielded 586,822,753 raw reads, with 552,393,895 clean reads remaining after quality control and the removal of host sequences; the clean reads represent 94.6% of the raw data. The assembly of these reads resulted in 22,633,747 contigs (Table S2). According to the 95% average nucleotide identity (ANI) threshold, a non-redundant genome set consisting of 10,192 species-level genome bins (SGBs) was constructed based on these data and a previously published ungulates microbial data.[44] We used at least 40% genome coverage to identify SGBs in each sample, and 2,771 representative SGBs were finally obtained, of which 1,267 SGBs (42.67%) were high-quality genomes (>90% completeness and <5% contamination; Figures 1A and 1B; Tables S3 and S4).

Gut microbiota taxonomic diversity in cold-adapted mammals and relatives from warmer regions

Based on the abundance of SGBs, we conducted α and β diversity analyses on four mammalian species. The results indicated that the two cold-adapted mammals exhibited lower α-diversity compared to their relatives inhabiting warmer climates (Figure 1C; Shannon index, Wilcoxon rank-sum test, false discovery rate [FDR] < 0.05). This finding corroborates previous gut microbiota studies in cold-exposed (experimental rats) and cold-adapted mammals (Tibetan pigs), which generally show reduced gut microbial diversity.[45,46] This may be related to the low diversity of plant-based food resources in the cold habitats of the Yunnan snub-nosed monkey and the reindeer, both of which heavily rely on cold-tolerant lichens as their primary food source.[11]

The hierarchical clustering (UPGMA) and principal coordinate analysis (PCoA) were used to assess the β-diversity of the four mammalian species. The results of UPGMA revealed that all of the samples cluster based on species (Figure 1D) and that species with closer phylogenetic relationships have shorter between-sample distances (Figure 1E; Adonis test, $R^2 = 0.59$, FDR < 0.001). The PCoA analysis also corroborated this result (Figure 1E).

Convergent and species-specific microbial signatures in cold-adapted mammals

To investigate microbes that may have a potential link to cold adaptation in the Yunnan snub-nosed monkey and reindeer, we conducted taxonomic differential analysis by comparing these species to relatives representing warm environments. Phylum-level analyses identified 20 bacterial phyla from the samples, with Firmicutes_A and Bacteroidota being the most abundant across the four studied species (Figure 2A). We identified six phyla that were significantly enriched in the Yunnan snub-nosed monkey compared to the Tonkin snub-nosed monkey and eight phyla that were significantly enriched in reindeer compared to the Père David's deer (Table S5; Wilcoxon rank-sum test, FDR < 0.05). Both species showed increased abundance of Spirochaetota (Figure 2C), with members of this phylum previously reported to contribute to the catabolism of carbohydrates, like pectin and cellulose, in ruminants.[47] Thus, the significant enrichment of Spirochaetota in both the Yunnan snub-nosed monkey and reindeer suggests a greater capacity to extract nutrients from lichen-rich diets. Interestingly, we found that the Yunnan snub-nosed monkey specifically enriched with Fibrobacterota, a phylum also found enriched in Tibetan pig intestines.[48] This phylum is thought to aid in high-altitude hypoxia

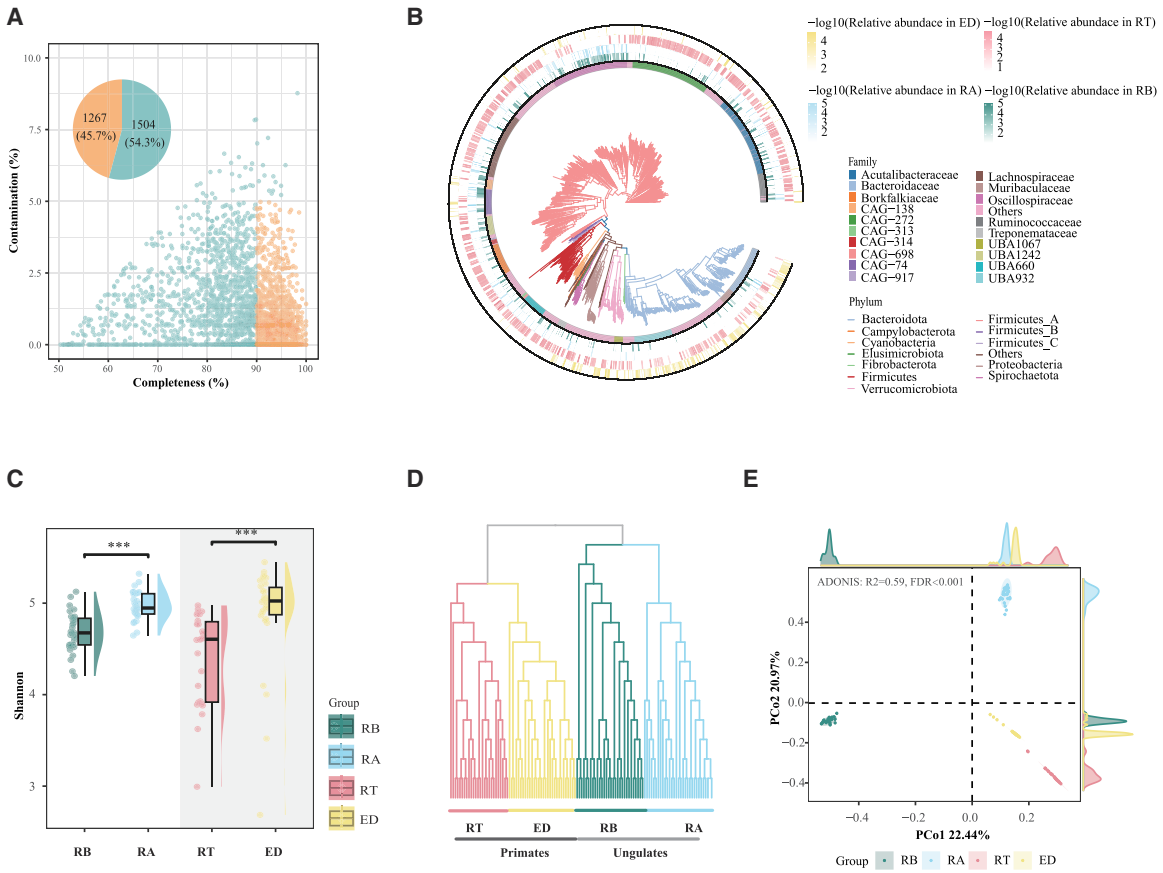

Figure 1. Assessment of SGBs quality and gut microbial diversity

(A) Estimated completeness and contamination of 2,771 genomes identified from two cold-adapted mammals and their relatives. Medium-quality genomes are shown in yellow and high-quality genomes in green.

(B) Phylogenetic tree of gut representative SGBs. Inner circle is a phylogenetic tree of 2,771 representative SGBs colored according to GTDB phylum-level taxonomic classifications (see color legend). Concentric circles from the inside to the outside represent the family-level taxonomic classification, and the mean relative abundance of SGBs in the RB, RA, RT, and ED samples.

(C) Alpha (α) diversity analyses of SGBs between two cold-adapted mammals and their relatives, data are represented as median±SD. The analyses were conducted using the Wilcoxon rank-sum test based on Shannon diversity indices, with * representing FDR<0.05, ** representing FDR <0.01, and *** representing FDR <0.001.

(D and E) Results from (D) UPGMA trees and (E) PCoA, both of which used the Bray-Curtis distance to calculate the relative abundance of SGBs. The 95% confidence ellipses are shown by circular shadows.

RB, Yunnan snub-nosed monkey; RT, reindeer; RA, Tonkin snub-nosed monkey; ED, Père David's deer.

adaptation because of their lactate synthesis potential, which can promote angiogenesis by binding with the NDRG3 protein.[49] Therefore, the enriched Fibrobacterota in Yunnan snub-nosed monkeys may help them adapt to the hypoxic environment of high-altitude regions.

We annotated 623 bacterial genera, with *Cryptobacteroides* being the dominant genus across the four studied species, followed by *Faecousia* and *Prevotella* (Figure 2B; Table S4). We identified 121 significantly enriched genera in the Yunnan snub-nosed monkey compared to the Tonkin snub-nosed monkey and 181 in reindeer relative to the Père David's deer (Table S5); furthermore, 40 genera were elevated in both cold-adapted species, including *Acetatifactor* and *Treponema_D* (Figure 2D). Bacteria representing *Acetatifactor*, which belong to the Lachnospiraceae family, are known to produce SCFAs, i.e., acetate and butyrate.[50] Both acetate and butyrate serve as energy sources for intestinal cells and stimulate NST in BAT.[24,51–54] Moreover, acetate also promotes insulin and ghrelin secretion in order to enhance food intake and energy accumulation.[42,55] Thus, microbes from the genera *Acetatifactor* may help provide the host with a sustained energy source via the production of SCFAs. In addition, the abundance of the genera *Treponema_D*, which include bacteria with fiber-degrading properties,[56–58] may help the Yunnan snub-nosed monkey and reindeer extract nutrients from the high-fiber lichen that is part of their habitats.

In addition to the convergent enrichment of certain genera in the Yunnan snub-nosed monkey and reindeer, instances of species-specific enrichment were also found (Figure 2E; Table S5). Among the 30 significantly enriched core genera (relative

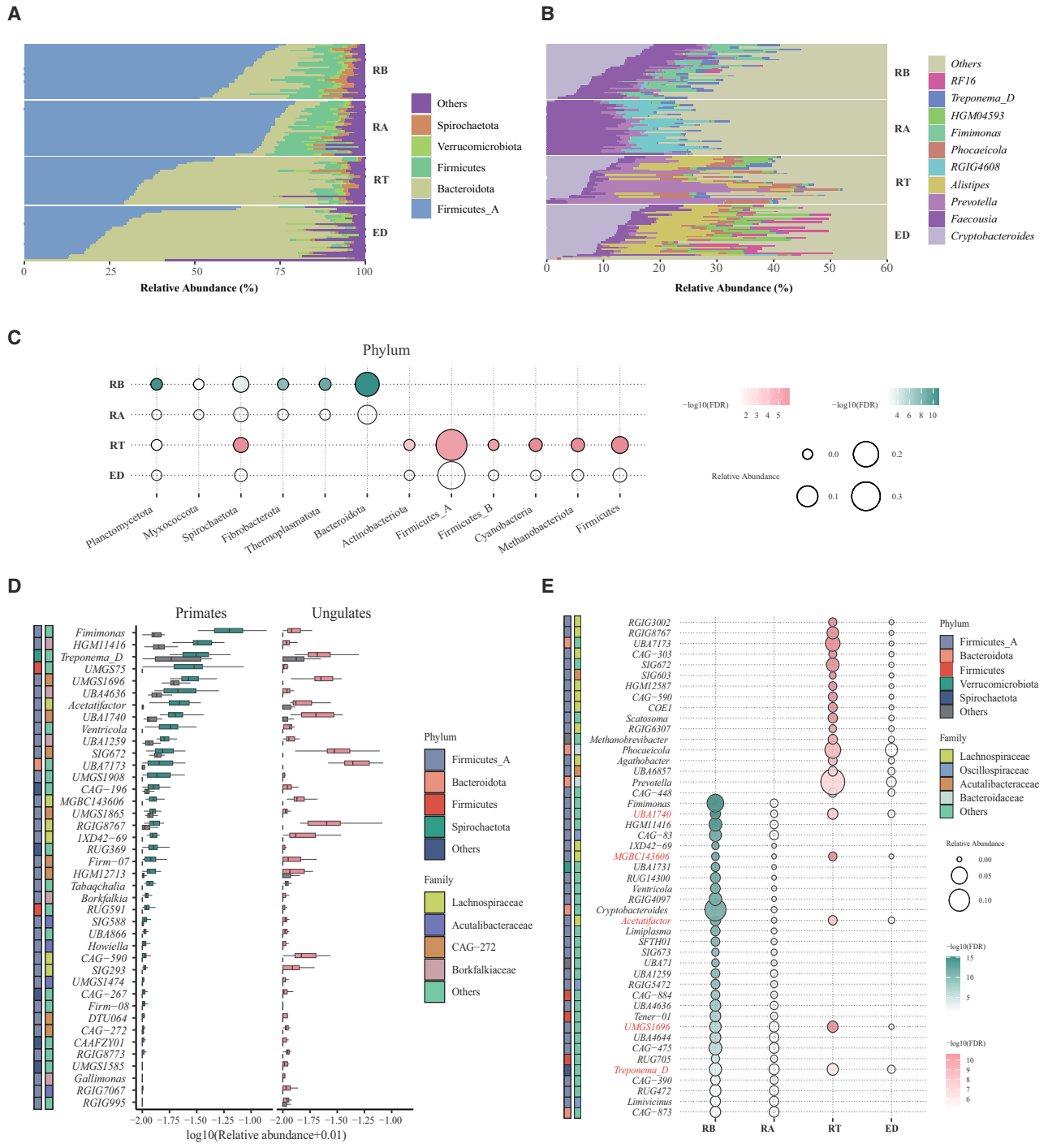

Figure 2. Taxonomic composition of cold-adapted mammals and relatives from warmer climates

(A) Relative abundances of the top 5 phylum in each sample.

(B) Relative abundances of the top 10 genera in each sample.

(C) The differentially enriched phylum in the Yunnan snub-nosed monkey and reindeer (Wilcoxon rank-sum test, FDR <0.05).

(D) Genera demonstrating enrichment in both Yunnan snub-nosed monkey and reindeer (Wilcoxon rank-sum test, FDR <0.05), data are represented as median±SD. In the boxplots, green and pink boxes indicate the abundances observed in the Yunnan snub-nosed monkey and reindeer, respectively, while the gray box indicates the abundances in the Tonkin snub-nosed monkey and Père David's deer.

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abundance >0.1% and prevalence >90%) unique to the Yunnan snub-nosed monkey, *Cryptobacteroides*, and several Oscillospiraceae genera (e.g., *RGIG5472* and *CAG-83*) showed the high degree of enrichment. Previous studies have suggested that *Cryptobacteroides* species in the gut microbiome of plateau ungulates have a higher density of CAZymes, which are predominantly involved in SCFA synthesis. This enables them to maintain a highly efficient energy metabolism, which is essential for survival in the high-altitude harsh environment.[44] Interestingly, Oscillospiraceae is also recognized for helping the Skywalker hoolock gibbon adapt to a high-leave diet during the winter in high-altitude areas by encoding CAZymes for fiber digestion and utilization.[59] Therefore, our study suggests that these microbial communities may play a significant role in helping mammals cope with limited food resources in cold, high-altitude environments.

Samples from reindeer identified the enrichment of 21 core genera, with *Prevotella* and *Phocaeicola* (both from the family Bacteroidaceae) demonstrating the highest relative abundance, while members of the family Lachnospiraceae (e.g., *RGIG3002*, *RGIG8767*, *CAG373*, and *Agathobacter*) were also enriched (Figure 2E; Table S5). Genera from these two families are widely recognized as significant producers of SCFAs.[60,61] In particular, the Bacteroidaceae family can synthesize propionate.[62] Previous studies have shown that propionate promotes hepatic gluconeogenesis and enhances NST in Mongolian gerbil to help them maintain body temperature under cold treatment conditions.[42] Notably, studies on reindeer revealed that the thermogenic protein UCP1, which is implicated in NST, underwent positive selection and showed increased expression in BAT during winter months.[21] This indicates that not only the host mammal, but also the gut microbiota, may contribute to enhanced NST in reindeer. In addition, the abundance of *Prevotella* and *Agathobacter* is positively correlated with fat-storage.[63–65] For example, *Prevotella copri* has been reported to increase fat accumulation in pigs.[63] Fat also entails excellent insulation through high energy density, and is considered to be crucial in cold adaptation.[25,42] Moreover, the genus *Phocaeicola* (*Bacteroides*) has been found to metabolize cholesterol efficiently.[66]

Hence, the comparison of cold-adapted mammals and relatives inhabiting warmer climates identified the convergent enrichment of microbes potentially linked to cold adaptation, i.e., those involved in fiber degradation and SCFAs production, in both the Yunnan snub-nosed monkey and reindeer; some species-specific enrichment characteristics were also identified. Animals primarily obtain the energy required to sustain physiological processes from food.[67] In the case of mammals inhabiting cold environments, the scarcity of food resources and the additional energy required to maintain body temperature make the acquisition and utilization of food particularly crucial. The lichen-rich diet shared by the Yunnan snub-nosed monkey and reindeer presents significant challenges in terms of fiber degradation, while the gut microbes can ferment fiber and produces substantial amounts of SCFAs.[68] We observed that both species exhibit a significant enrichment in fiber-degrading and SCFA-producing bacteria, which suggests that both mammals demonstrate an enhanced ability to extract nutrients from lichen-rich diets under cold conditions. SCFAs produced by bacteria not only provide energy to the host but also stimulate NST to offer additional heat sources necessary for life in cold environments. Notably, SCFA-producing bacteria significantly enriched in Yunnan snub-nosed monkeys have also been found in previous studies of gut microbes in other high-altitude species.[44,59] This suggests that these bacteria may be key species that help mammals adapt to the harsh high-altitude environment. In addition to convergent enrichment, we also identified some species-specific enrichment features. In Yunnan snub-nosed monkeys, we found that some bacteria associated with high-altitude hypoxic adaptation were specifically enriched. In reindeer, our results observed the fat-storing microbes' enrichment, which may be related to reindeer adaptation to the extremely cold temperatures at high latitudes. The high fat storage observed among cold-adapted animals is a typical adaptation for satisfying the high energy demands associated with life in these environments.[8,30,69,70] Previous studies have reported that reindeer partly rely on high body fat (27%–40%) for energy supply.[15,71] However, an excess of fat can be detrimental to cardiovascular health. Studies have indicated that lipid metabolism genes (e.g., *SCARB1* and *APOB*) in cold-adapted animals have undergone adaptive evolution to counteract the adverse effects of fat accumulation.[23,72,73] Interestingly, the enrichment of cholesterol-metabolizing bacteria in reindeer may effectively mitigate the risk of cardiovascular disease associated with increased fat storage due to adaptation to extreme cold, which is contributed by both host and gut microbes.[72,74]

Functional responses of gut microbiota in cold-adapted mammals

We annotated 2,771 nonredundant SGBs using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and investigated the prevalence of differentially enriched SGBs in both cold-adapted mammals (Table S6). Finally, Yunnan snub-nosed monkey showed significant enrichment (Wilcoxon rank-sum test, FDR < 0.05) in 51 pathways, 67 modules, and 550 KOs (Tables S7–S9); reindeer exhibited significant enrichment in 65 pathways, 61 modules, and 482 KOs (Tables S7–S9).

The pathways and modules that were significantly enriched in the cold-adapted mammals were related to amino acid metabolism as well as lipid metabolism (Figures 3A and 3B; Tables S7 and S8).

Amino acid metabolism

The herbivores that inhabit cold regions often face low-nitrogen stress due to limited protein intake and a lack of essential amino acids (EAAs).[26,38] For instance, both the Yunnan snub-nosed monkey and reindeer have developed a unique lichen-rich diet to adapt to the extreme climates that they inhabit. Lichens

(E) Bubble chart shows the enrichment of core genera in Yunnan snub-nosed monkey and reindeer samples (Wilcoxon rank-sum test, FDR <0.05). The red font indicates the core genera enriched in both cold-adapted mammals.
Colored bands in (D) and (E) represent phylum and family level classification information. RB, Yunnan snub-nosed monkey; RT, reindeer; RA, Tonkin snub-nosed monkey; ED, Père David's deer.

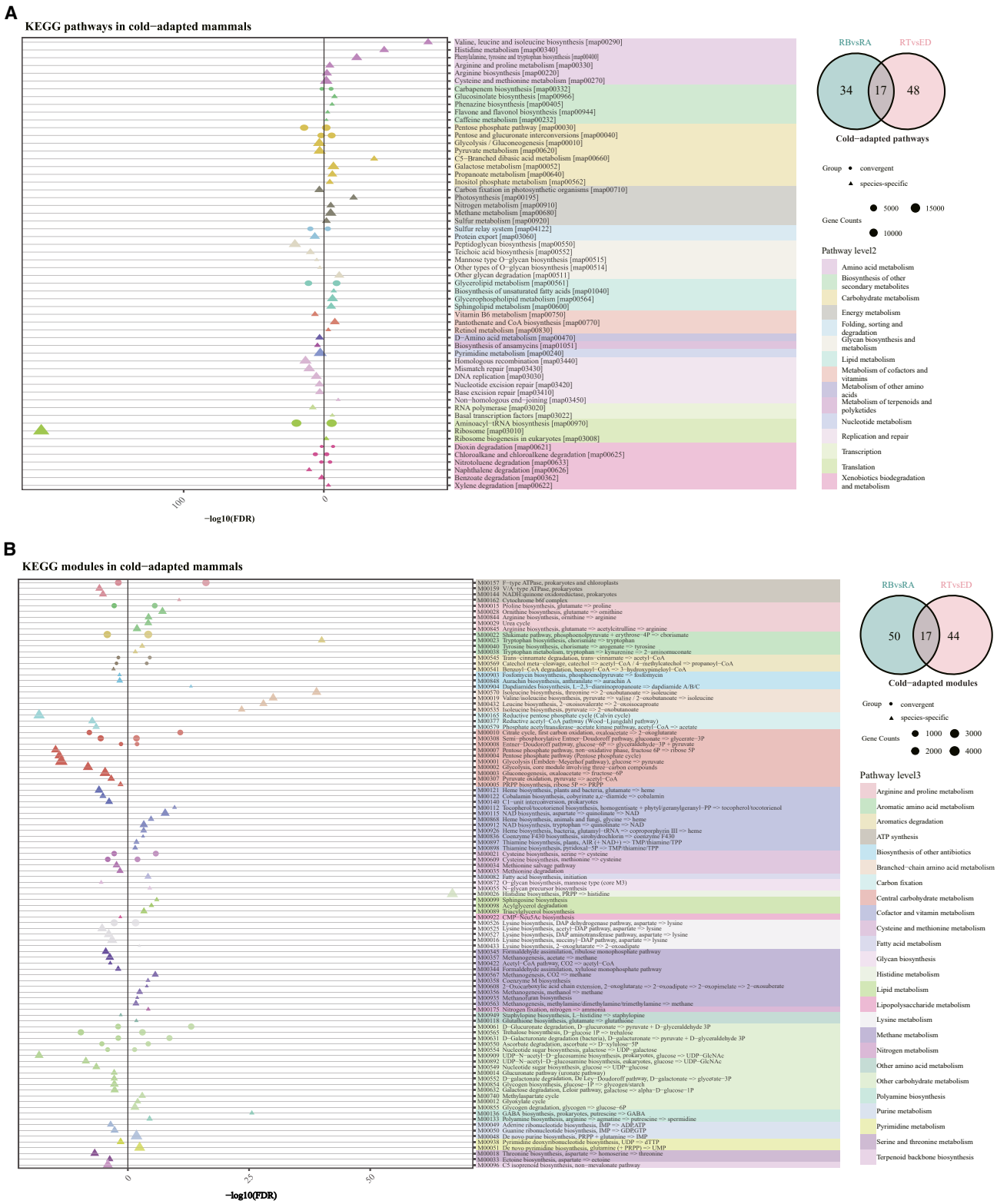
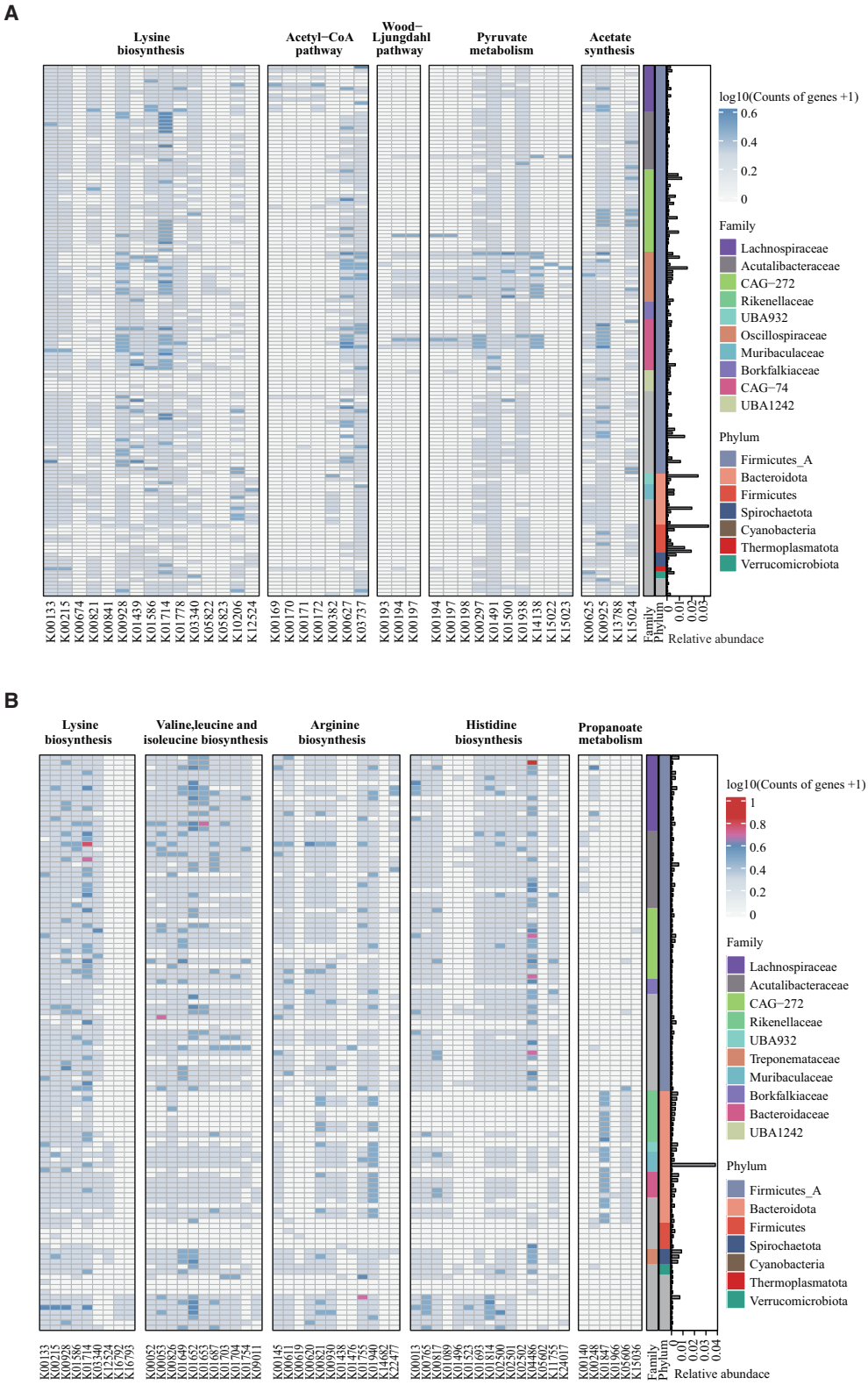


Figure 3. Enrichment of functional characteristics in the gut microbiota of cold-adapted mammals

Significantly enriched metabolism-related pathways (A) and modules (B) in the Yunnan snub-nosed monkey and reindeer (Fisher's test, FDR <0.05); pathways/modules on the left of the Y-axis (X = 0) indicate significant enrichment in the Yunnan snub-nosed monkey, while those on the right indicate significant enrichment in the reindeer; Circular and triangular bubbles represent convergent and species-specific pathways/modules in two cold-adapted mammals, respectively.



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have far lower protein contents than other plants, and this can make the animals that rely on them for nutrition subject to great low-nitrogen stress.[11,16,71,75] Hence, deciphering how the Yunnan snub-nosed monkey and reindeer manage low-nitrogen stress is key to understanding their cold adaptation strategies. We observed a high levels of enrichment of EAA synthesis function in cold-adapted mammals, particularly lysine (Tables S7 and S8). The lysine biosynthesis modules were significantly enriched in both species (M00016, M00525, M00526, and M00527 in the Yunnan snub-nosed monkey; M00526 and M00433 in reindeer) (Figures 3A and 3B; Table S8). This corroborates previous findings from studies on rats subjected to protein-restricted diets, more specifically, that the gut microbiota—when subjected to nitrogen-limited conditions—can effectively assist the host in synthesizing lysine to supplement the lack of protein.[76] Our results showed that in both cold-adapted mammals, genes of lysine biosynthesis modules had a high abundance in SGBs from Acutalibacteraceae (Figures 4A and 4B). So Acutalibacteraceae may play an important role in coping with low-nitrogen stress in cold environment. Additionally, reindeer exhibited a specific synthetic potential for branched-chain amino acids (BCAAs: valine, leucine, and isoleucine), which is reflected in the enrichment of the synthesis pathway (map00290), modules (M00019, M00432, M00535, and M00570), and the key gene *leuB* (K00052) (Figure 3B; Tables S7–S9). BCAAs synthesis-related genes were more abundant in Lachnospiraceae, especially an SGB from *VSOB0* (W04.bin.111). In addition, the abundance of several genera in Lachnospiraceae was significantly higher in reindeer than in Peer David deer, suggesting that members of Lachnospiraceae may be the main source of gut microbes for BCAAs in reindeer under low-nitrogen stress (Figure 4B). In addition to utilizing gut microbiota to supplement low protein levels, efficient nitrogen conservation represents another strategy to cope with low-nitrogen stress. We observed that reindeer showed significant enrichment in urea cycle related pathway (map00220) and modules (M00029, M00028, M00844, and M00845) (Figure 3B; Tables S7 and S8), and these pathway and modules are prevalent in reindeer differentially enriched SGBs (Figure 4B). This mechanism enables the reindeer to recycle nitrogen that would otherwise be lost via the urine. Therefore, our study shows that the two cold-adapted mammals can cope with low-nitrogen stress by both increasing the sources of EAAs, and in the case of reindeer, by also reducing nitrogen expenditure. Compared to Yunnan snub-nosed monkeys, the more extreme cold habitat of reindeer leads to higher energy expenditure and protein turnover demands, thereby exacerbating nitrogen demand pressure in reindeer. This may explain why reindeer have evolved enhanced nitrogen-balancing strategies. Gut microbial synthesis of amino acids not only aids the host in adapting to low-nitrogen stress under cold environments, but also produces functional amino acids, such as histidine, which play an important role in host physiological regulation.[48] Our results suggest that reindeer significantly enriched SGBs from the family CAG-272 have more genes encoding the histidinol-phosphatase (PHP family), and these SGBs may provide more histidine to reindeer (Figure 4B). Histidine can be converted into histamine, which promotes vasodilation to increases blood flow and help dissipate heat.[77] Interestingly, previous studies have shown that reindeer's thick fur may cause overheating risks while protecting against cold, and peripheral vascular dilation can effectively reduce this risk.[8]

Lipid metabolism

Animals residing in cold environments are often challenged by a limited energy supply due to restricted food availability and the need to maintain body temperature; as such, these animals must be able to effectively synthesize and utilize energy to adapt to cold environments. Lipids, as molecules with a function in energy storage, play a crucial role in species' adaptation to cold environments.[52] As an important lipid substance, SCFAs synthesized by gut microbes can provide energy for the host. The Yunnan snub-nosed monkey showed species-specific significant enrichment in pathway (map00620), module (M00579), and KOs (K15024, K00627, K01500, and K14138) relevant for acetate synthesis (Figure 3A; Tables S7–S9). Meanwhile, modules associated with acetyl-CoA synthesis (M00307, M00377, M00422)—a key intermediate in acetate biosynthesis—were also enriched (Figure 3A; Table S8). It was also shown that the SGBs of CAG-74, Lachnospiraceae, and Oscillospiraceae had more genes involved in acetyl-coA and acetate synthesis. Interestingly, in high-altitude rhesus macaques, it also been found the acetyl-CoA and acetate synthesis were enriched.[40] These observations suggested that acetate and synthesis-related bacteria may be important for Yunnan snub-nosed monkey dealing with the challenges of energy shortages in cold, high-altitude environments. Reindeer showed a species-specific enrichment in propionate metabolism pathway (map00640) (Figure 3A; Table S7), while the genes related to propionate synthesis were mainly found in Bacteroidetes (Figure 4B). Our results suggest that both cold-adapted mammals can respond to energy shortage by increasing SCFA synthesis, but there are some differences in the types of SCFA synthesis, which may be related to their adaptation to different cold habitats.

Besides those related to amino acid metabolism as well as lipid metabolism, we observed the specific enrichment of purine metabolism in the Yunnan snub-nosed monkey. In Yunnan snub-nosed monkeys, we observed a unique enrichment of purine metabolism-related genes (*deoD*, K03784, EC 2.4.2.1) (Table S9). It has been shown that under hypoxic condition, the ATP degradation acceleration and the enucleation of mature red blood cells lead to elevated purine metabolite production, resulting in increased synthesis and accumulation of blood uric acids.[78–80] The rise of uric acid level can increase the risk of gout and other metabolic diseases.[81] Therefore, the gut microbiota enrichment related to purine metabolism may help the host reduce purine and uric acid accumulation under hypoxic stress, which is advantageous for high-altitude survival. Indeed, a

Figure 4. Metabolic reconstruction of SGBs enriched in the gut microbiota of cold-adapted mammals

Heatmaps showing counts of genes associated with selected significantly enriched functional modules among high-quality SGBs (completeness >90% and contamination <5%) that are significantly enriched in both the Yunnan snub-nosed monkey (A) and reindeer (B) (Fisher's test, FDR <0.05). Colored bands represent the phyla and families associated with these SGBs. See also Table S9.

recent study found that the gut microbiota of high-altitude human populations can effectively degrade accumulated purines and uric acids, helping the host adapt to hypoxic environments.[82]

Our findings highlight that the gut microbiota of two phylogenetically distant mammals, the Yunnan snub-nosed monkey and reindeer, are characterized by similar cold adaptation strategies in response to food scarcity (enhanced fiber degradation and nitrogen balance maintenance), energy shortages (increased SCFA synthesis), and the need to maintain a constant body temperature (stimulation of NST). Nevertheless, it is notable that both species also evolved distinct adaptation strategies to cope with different cold ecosystems. The species-specific high-altitude hypoxia environment adaptations of the Yunnan snub-nosed monkey include an enhanced ability to synthesize lactate and metabolize purine. The species-specific extreme colder environment adaptations of the reindeer include the increased blood flow, strengthened urea cycling, and enriched fat storage associated bacteria, underscoring that the gut microbiota developed divergent evolutionary responses to specific environmental demands. Notably, we propose the enrichment of cholesterol-degrading bacteria in the guts of reindeer could be associated with mitigating the risk of cardiovascular disease caused by lipid storage. Our study is the first to investigate the contribution of gut microbiota to the cold adaptation of the Yunnan snub-nosed monkey and reindeer. It expands the current knowledge of how the gut microbiome promotes cold adaptation through shared and specialized mechanisms that are shaped by different phylogenetic and ecological contexts.

Limitations of the study

Future phenotypic validation experiments, including the comparison of SCFA content and NST capacity between cold-adapted mammals and their relatives and the fecal microbiota transplantation into germ-free mice to recapitulate cold adaptation phenotypes, would confirm the causal contribution of gut microbiota to cold adaptation.

RESOURCE AVAILABILITY

Lead contact

Further information and requests should be directed to and will be fulfilled by the lead contact, Prof. Li Yu (yuli@ynu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Raw sequence data are available at the China National Center for Bioinformation (CNCB):CRA020417.
- This paper does not report any original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

ACKNOWLEDGMENTS

We thank Professor Lifeng Zhu for providing the raw metagenomic data of the Père David's deer. We thank the Management Bureau of Baimaxueshan National Nature Reserve for collecting fecal samples from Yunnan snub-nosed monkeys. We thank native-English speaker Dr John Blackwell and his colleagues for polishing the English of the manuscript. The Graphical abstract was created in BioRender, X.Q.Y. (2025) https://BioRender.com/e82p537, with icons and templates provided by the platform.

The work was supported by the National Natural Science Foundation of China (32360121), Xingdian Talent Fund Project of Yunnan Province (202305AB350001), and a grant (no.2024KF006) from State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan and Key Laboratory of Industrial Microbial Fermentation Engineering of Yunnan Province, Yunnan University, Central Government Guidance Fund for the Development of Local Science and Technology (202407AB110004).

AUTHOR CONTRIBUTIONS

L.Y. and C.B.L. designed the project; X.Q.Y. performed the data analyses; L.Y., C.B.L., and X.Q.Y. wrote the manuscript; H.B., P.H.Z., G.S.J., and N.T.L. collected the samples.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2025.112245.

Received: November 16, 2024
Revised: February 4, 2025
Accepted: March 14, 2025
Published: March 19, 2025

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Fecal samples from different populations of Yunnan snub-nosed monkeys, Siberian forest reindeer, and Tonkin snub-nosed monkeys were collected for microbiome analysis	This paper	NA
Deposited data		
Raw sequencing data	This paper	CNCB:CRA02041
Père David's deer metagenomic data	Zhu et al.[43]	https://doi.org/10.6084/m9.figshare.6303713
Reference genome of Yunnan snub-nosed monkey	Wu et al.[83]	CNCB:PRJCA007648
Reference genome of reindeer	Dussex et al.[84]	NCBI:PRJEB60852
Reference genome of Père David's deer	Chen et al.[85]	NCBI:PRJNA438286
Genome Taxonomy Database, release 202	Parks et al.[86]	https://gtdb.ecogenomic.org/
KEGG database	Kanehisa et al.[87]	https://www.kanehisa.jp/
Experimental models: Organisms/strains		
Yunnan snub-nosed monkey (*Rhinopithecus bieti*)	This paper	NCBI: txid61621
Tonkin snub-nosed monkey (*Rhinopithecus avunculus*)	This paper	NCBI: txid66062
Reindeer (*Rangifer tarandus*)	This paper	NCBI: txid9870
Père David's deer (*Elaphurus davidianus*)	This paper	NCBI: txid43332
Software and algorithms		
Fastp	Chen et al.[88]	https://github.com/OpenGene/fastp
Bowtie2	Langmead et al.[89]	http://bowtie-bio.sourceforge.net/bowtie2/
seqtk	Li et al.[90]	https://github.com/lh3/seqtk
MEGAHIT	Li et al.[91]	https://github.com/voutcn/megahit
Metabat2	Kang et al.[92]	https://bitbucket.org/berkeleylab/metabat/src/master/
MetaWRAP	Brown et al.[93]	https://github.com/bxlab/metaWRAP
CheckM	Parks et al.[94]	https://ecogenomics.github.io/CheckM/
RefineM	Parks et al.[95]	https://github.com/dparks1134/RefineM
dRep	Olm et al.[96]	https://github.com/MrOlm/drep
GTDB-Tk	Chaumeil et al.[97]	https://github.com/Ecogenomics/GTDBTk
Prodigal	Hyatt et al.[98]	https://github.com/hyattpd/Prodigal
DIAMOND	Buchfink et al.[99]	https://github.com/bbuchfink/diamond
KEGGREST package	NA	https://bioconductor.org/packages/release/bioc/html/KEGGREST.html
Rstudio	RStudio Team	https://github.com/rstudio/rstudio
vegan package	Oksanen et al.[100]	https://vegandevs.github.io/vegan/
ggplot2 package	Wickham et al.[101]	https://ggplot2.tidyverse.org/

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Ethics statement

All necessary research permits and ethical approvals for this study were granted by the Laboratory Animal Welfare Ethics Committee at Yunnan University (Approval number: YNU20241056).

Animals

A total of 86 fecal samples were collected in this study, including 30 samples from wild Yunnan snub-nosed monkeys, 26 samples from Siberian forest reindeer, and 30 samples from wild Tonkin snub-nosed monkeys (Table S1). The Yunnan snub-nosed monkey fecal samples were collected in December 2017 from the Xiangguqing population in the Yunnan Baima Snow Mountain National Nature Reserve (elevation: 3,166 meters, 99°21′87″E, 27°38′87″N); the average temperature during the sampling period was -2°C.[102] The Siberian forest reindeer fecal samples were collected in December 2020 from individuals in the Hanma National Nature Reserve, which is located in the Greater Khingan Mountains, China (122°36′E, 51°45′N); the winter temperature in this area ranges from -23.4°C to -12.7°C.[103] The Tonkin snub-nosed monkey fecal samples were collected in collaboration with the Forest Bureau of Ha Giang Province, Vietnam, from April to May 2021. The samples were collected from the Khau Ca forest in northern Ha Giang Province, Vietnam (105°12′E, 22°84′N); the average temperature during the sampling season ranged from 26.0°C to 29°C.[104] In addition, publicly available metagenomic data for the Père David's deer ($n = 30$) were downloaded,[43] including 12 records from Jiangsu Dafeng National Natural Reserve between September and November 2014 (average annual temperature of 14.1°C) and six records from Hubei Shishou National Natural Reserve in November 2014 (average annual temperature of 17.4°C).

METHOD DETAILS

Fecal sample collection

All fecal samples was freshly put into collection containers on dry ice. These samples were then transported to the laboratory and stored at -80°C until the fecal DNA extraction.

DNA extraction and sequencing

DNA from the Yunnan snub-nosed monkey fecal samples was extracted using the Mag-Bind Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA), while DNA from the Tonkin snub-nosed monkey and reindeer fecal samples was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany). DNA concentration and purity was determined via the TBS-380 and NanoDrop2000 approaches, respectively. DNA integrity was assessed through 1% agarose gel electrophoresis. Sequencing libraries for the Yunnan snub-nosed monkey samples were constructed using NEXTFLEX Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA), and sequenced on the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA) to generate 150-bp paired-end reads. Sequencing libraries for the reindeer and Tonkin snub-nosed monkey samples were constructed through DNA cyclization and sequenced on the MGISEQ-2000 platform (MGI Tech, Wuhan, China) using Combinatorial Probe-Anchor Synthesis technology to return 150-bp paired-end reads.

Metagenomic data quality control

The collected raw data were processed using a quality control workflow in Fastp (v0.39, parameters: -W 4 -M 20 -n 0 -q 20 -5 5 -3 5 -l 50) to remove adapters and low-quality reads. Potential host sequences were removed using Bowtie2 (v2.4.4)[89] based on the reference genomes of the two hosts; the reference genomes were obtained from the CNCB: PRJCA007648 (Yunnan snub-nosed monkey; used for both species of snub-nosed monkey), NCBI: PRJEB60852 (reindeer), and PRJNA438286 (Père David's deer). The metagenomic datasets from a total of 116 samples were used for subsequent analysis, with the average data size per sample exceeding 16.1 Gb (Table S2). To ensure the comparability between datasets, we used seqtk (v1.3-r106)[90] to randomly subsample clean reads to match the lowest average number of clean reads for the Père David's deer.

Metagenomic assembly, binning and annotation

Both individual assembly and co-assembly approaches were applied to quality-controlled clean reads from each sample using MEGAHIT (v1.2.9)[91]; contigs longer than 200 bp were retained. These contigs obtained from both individual assembly and co-assembly were binned using Metabat2 integrated in MetaWRAP (v1.3.1)[93] to construct metagenomic-assembled genomes (MAGs). The completeness and contamination of MAGs or species-level genomes (SGBs) were assessed using CheckM[94] (v1.1.3) with the "lineage_wf" options. The medium/high-quality MAGs (completeness > 50% and contamination < 10%) were retained for subsequent analysis. We used RefineM (v0.1.2)[95] with default parameters to filter bins with divergent genomic properties, mismatched taxonomic classifications, and incongruent 16S rRNA genes. CheckM (v1.1.13)[94] was re-run to reassess the quality of the retained MAGs. 1,205 and 1,006 medium/high-quality MAGs were obtained from were obtained from the metagenomic data of Yunnan snub-nosed monkey and reindeer, respectively. Additionally, we integrated previously published ungulates SGB datasets.[44] All 2,207 medium/high-quality MAGs and 8,488 published SGBs were dereplicated using dRep (v3.2.2) with primary clustering at 90% and secondary clustering at 95% ANI. The highest-scoring MAG (according to the default scoring formula) from each secondary cluster was retained as the representative genome. In total, 10,192 SGBs were obtained, with 1,773 derived from this study and 8,419 from previous studies. To achieve a more accurate SGB abundance profile, 2,771 SGBs with genome coverage greater than 40% were retained for further analysis.

Taxonomic annotation and functional annotations of 2,771 representative SGBs

GTDB-Tk (v2.0.0)[97] was used to perform taxonomic assignments of 2,771 representative SGBs based on GTDB (Genome Taxonomy Database, release 202). We predicted open reading frames (ORFs) in each SGB and translated to amino acid sequences (AAs) using

Prodigal (v2.6.3).[98] Furthermore, we aligned the deduced AAs with Kyoto Encyclopedia of Genes and Genomes (KEGG) database (version 92.0, released October 1, 2019)[87] using DIAMOND (v2.0.13.151, parameters: -evalue 0.00001 -max-target-seqs 1)[99] to acquire functional annotations. The R package KEGGREST was applied to obtain the hierarchical structure of KEGG functions from the KEGG website.

QUANTIFICATION AND STATISTICS ANALYSIS

The taxonomic and functional annotations from all of the analyzed samples served as the input for the microbial diversity analysis, performed using the R package vegan (v2.5-6).[100] Alpha (α) diversity was assessed using the Shannon diversity index. Beta (β) diversity was visualized using Principal Coordinates Analysis (PCoA), with UPGMA applied for hierarchical clustering and Permutational Multivariate Analysis of Variance (PERMANOVA, Adonis) used to test the significance of community variation; all of these analyses applied the Bray-Curtis distance matrix to assess differences in microbial community structure among groups. Differential taxonomic features were identified using the Wilcoxon rank-sum test (unpaired). The enrichment analyses were performed by Fisher's exact test. Significance was adjusted using the Benjamin-Hochberg method for false discovery rate (FDR). Core genera were defined as those with a relative abundance exceeding 0.1% and a prevalence over 90%. The results of the diversity and differential taxonomic and functional analyses were visualized using the R package ggplot2 (v2.0.0).[101]