

Invasion and defense of the basic social unit in a nonhuman primate society leads to sexual differences in the gut microbiome

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Abstract

Multilevel society is one of the most complex social systems in natural ecosystems and is a typical feature among some primates. Given the potential connection between social behavior and gut microbiome composition, the multilevel social system could affect the primate gut microbiome. Here, based on long-term observation (e.g. social unit dynamics, transfer, and behavior), we investigated this potential integrating 16S rRNA gene amplicon sequencing and behavior data in Yunnan snub-nosed monkeys (*Rhinopithecus bieti*), which possess a multilevel social group based on one male units (OMUs, each unit with several breeding females and their offspring) and all-male unit (AMU, several bachelor males residing together). We found that the mean unweighted Unifrac distance between adult males from different OMUs was significantly lower than that between adult females from different OMUs (paired Wilcoxon test, $P = 0.007$). There was no significant difference in the mean unweighted Unifrac distance between females within the same OMU or between females from different OMUs. These findings indicated the potential connection between the defense and invasion of social units and the gut microbiome community in wild Yunnan snub-nosed monkeys. We speculated that the resident males of OMUs displaying a significantly higher similarity in the gut microbial community than that of adult females in separate OMUs might be associated with the sexual differences in their interactions and from previously having cohabitated together in the AMU. Therefore, this study suggested that multilevel societies might have an effect on the gut microbial community in this wild nonhuman primate species.

Key words: defense and invasion, gut microbial transfer, multilevel society, sexual differences, Yunnan snub-nosed monkeys

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INTRODUCTION

The animal gut microbiome, influenced by host phylogeny and diet (Ley *et al.* 2008a; Youngblut *et al.* 2019), plays an important role in host nutrition uptake and health (Ley *et al.* 2008b; Alberdi *et al.* 2016). Recently, a number of studies have revealed that host social behavior (e.g. physical contacts, grooming, and mating) also shapes the gut microbiome through potential microbiota transmissions (e.g. bees (Vernier *et al.* 2020),

amphibians (Xu *et al.* 2020), birds (Escallón *et al.* 2019), musk deer (Li *et al.* 2021), nonhuman primates (Tung *et al.* 2015; Moeller *et al.* 2016; Amato *et al.* 2017), and humans (Brito *et al.* 2019). For an example, the grooming includes frequent hand-to-mouth contact and ano-genital region contact (fecal-oral contacting) in wild baboon groups, which will facilitate gut microbial transfer between social partners (Tung *et al.* 2015). Nonhuman primate gut microbiomes have been widely investigated due to their close phylogenetic relationship of the hosts to humans (Browne *et al.* 2017; Kuthyar *et al.* 2019). Sexual bias in gut microbiota transmission has been observed in newly paired lab common marmosets due to differences in the frequency of the social behaviors (e.g. grooming and physical contact), with male individuals gaining more gut microbes from the females (Zhu *et al.* 2020). Many of the nonhuman primates live in the highly organized society (Grueter *et al.* 2012). This, therefore, raises one question: What occurs on gut microbial transfer among wild primates living in multilevel societies.

The endangered colobine, Yunnan snub-nosed monkey (*Rhinopithecus bieti*), inhabit high-altitude temperate forests throughout northwestern Yunnan Province and southeastern Tibet in China, subsisting on lichens and the leaves of angiosperms (Ren *et al.* 2012a), and display genomic level adaptations to high altitude (Yu *et al.* 2016). These primates are organized as a multilevel social group based on one-male units (OMUs, each unit with several breeding females and their offspring) and an all-male unit (AMU, several bachelor males residing together) (Kirkpatrick *et al.* 1998; Li *et al.* 2014). The multilevel social system refers to a social model in which individuals in a society gather together through relations at 2 or more levels (Grueter *et al.* 2012; Kirkpatrick 2016). The basic structures of the multilevel social are the OMUs and AMU, which aggregate with each other to form a larger band (Kirkpatrick *et al.* 1998; Ren *et al.* 2012b; Li *et al.* 2014). Grooming between individuals within an OMU and aggressive behavior between the adult males of different OMUs (bite, snatch, catch, threaten, and displacement) are common in Yunnan snub-nosed monkey (Kirkpatrick & Grueter 2010; Ren *et al.* 2012a; Li *et al.* 2013; Zhu *et al.* 2016). The males from OMUs and AMUs occasionally invade other OMUs, and the males in these OMUs defend their units. Thus, the physical contact between the adult breeding males among different units is highly frequency. However, physical contact between the adult breeding females among different units is rare.

In this study, we focus on a group of habituated wild *R. bieti* displaying multilevel society features in the Baimaxueshan Nature Reserve, which had been under observa-

tion for approximately 11 years (Fig. 1). A majority of the individuals could be identified using distinctive physical characteristics such as body size, hair pattern, scars, and facial features. Here, based on the sexual differences in the frequency of social behavior between adults among the different social units, we hypothesized that the gut microbial communities among adult males of different units would show higher similarity than those among the breeding females among different units. In addition, we hypothesized that grooming within an OMU might increase the similarity in the gut microbiome among individuals of that OMU.

MATERIALS AND METHODS

Study area

This study was carried out at Xiangguqing (99°22'E, 27°37'N) in the Baimaxueshan Nature Reserve, located on the southern slopes of the Hengduan Mountains, Yunnan Province, China (Fig. 1a,b). The study area is characterized by a plateau monsoon climate, where temperature and precipitation are strongly seasonal (Xia *et al.* 2020). The research site was located in an evergreen broad-leaved forest, evergreen needle-leaved forest, and shrub wood at an altitude of 2600–3200 m.

Yunnan snub-nosed monkeys (*R. bieti*) form large, multilevel social groups consisting of many OMUs and an associated AMU (Xia *et al.* 2020). The research group was a habituated wild group from May 2008 (Ren *et al.* 2012b; Xia *et al.* 2016). Since the end of 2009, all individuals were identified using distinctive physical characteristics such as body size, hair pattern, scars, and facial features (Xia *et al.* 2020). From June 18, 2020, to September 18, 2020, a total of 10 OMUs and 1 AMU (63 individuals) were recorded in the research group. Individuals sometimes transferred among OMUs, AMUs, and other *R. bieti* groups (Xia *et al.* 2020).

Interaction data and analysis

To understand the social interaction between individuals, we collected interaction data from June 18, 2020 to September 18, 2020 (effective observation time was 390.79 h), including grooming (total of 127.14 h) and fighting (total of 1.85 h) using an all-occurrence sampling method, and the interactions between each individual within and between the OMUs and were measured with the interaction index (II). On any given observation day, we stayed with the study subjects and collected interaction data on individuals in all visible OMUs and

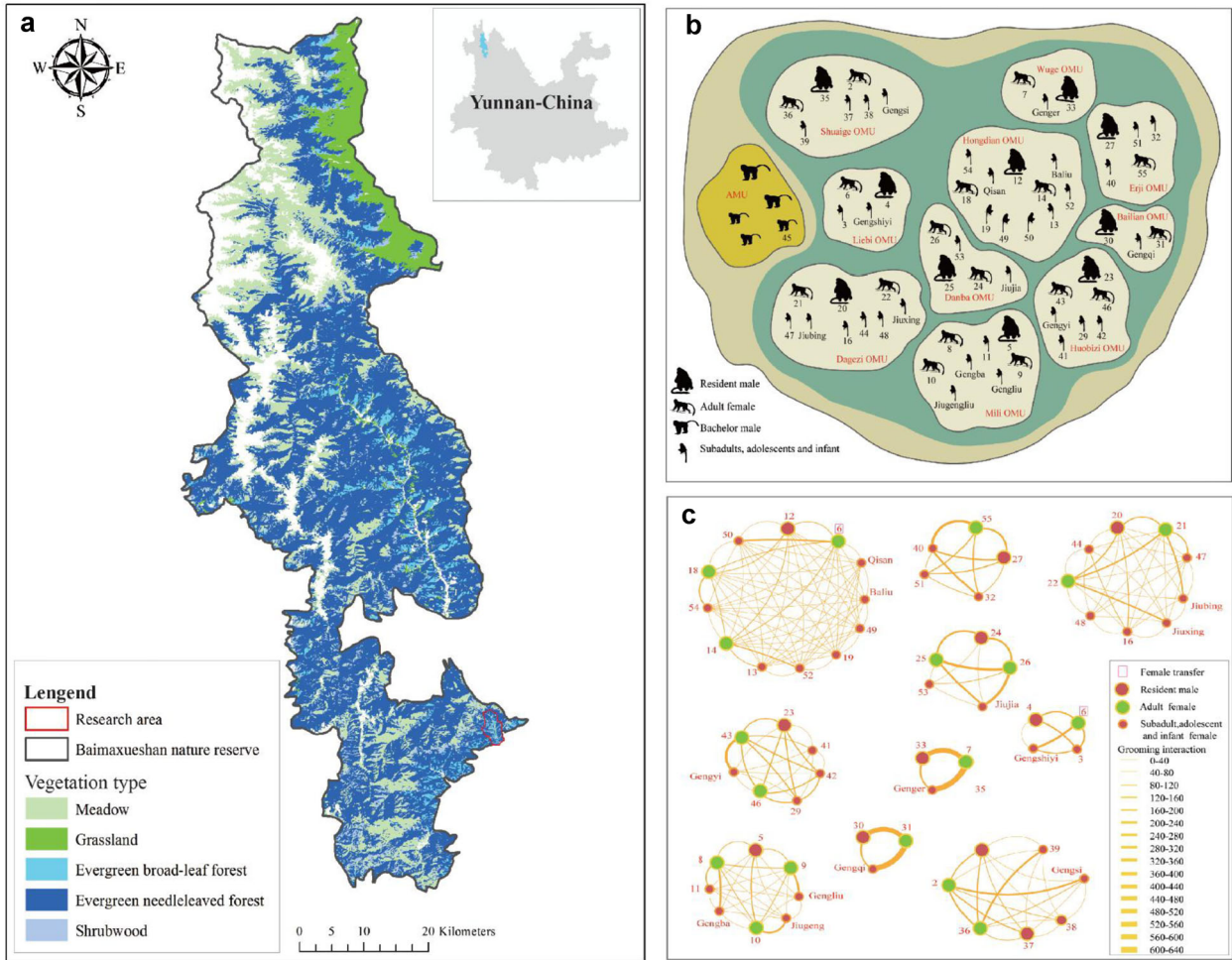


Figure 1 The multilevel society in the Yunnan snub-nosed monkeys (*Rhinopithecus bieti*). (a) Study area; (b) the social organization of the study group. The light gray boxes represent OMUs, which contains a single breeding male, several breeding females, and their offspring. The yellow box represents the AMU, in which several bachelor males reside together. Many of these are juvenile and sub-adult males who transferred directly into the AMU from their natal OMU and other groups; the numbers and letters under each silhouette represent an individual's name. (c) Sociograms for individuals grooming interactions of each OMU based on social network analysis: Line thickness indicates the strength of the grooming interaction. Numbers and letters next to nodes represent individual names. The individuals of the collected fecal sample are represented by a number corresponding to the fecal sample number. If an individual is given a nonnumber name, such as Gengshiyi from the Liebi OMU, no fecal sample was collected from that individual.

AMU. The criteria for selecting focal OMUs and AMU were: being in close proximity to the observer, being clearly visible, and not having any missing OMUs and AMU members. To ensure balanced and comprehensive data collection, we collected data on each OMUs and AMU at least twice a week.

$$II = \frac{\sum G_{ij} + \sum G_{ji}}{T}$$

where G_{ij} is the total time grooming was initiated by individual i to j , and T is the observation time.

Sociograms to describe the patterns of individual association and interaction were constructed using SOCPROG v.2.1. The Independent sample t -test was used to examine interaction differences: (1) male and female individuals of different OMUs, (2) resident males and females versus adult females within the same OMU, and (3) resident males and offspring versus adult females and offspring within the same OMU. All tests were 2-tailed with $P \leq 0.05$ as the threshold for significance. Average values are expressed as mean \pm SD.

Transfer data collection

Transfer data was recorded over 11 years (2010.1.1–2020.9.31) for this group as part of long-term population monitoring. Each day at feeding time, the number of individuals in each OMU and AMU were counted; other information was also recorded, including (i) demographic changes and composition of OMUs and the AMU, including birth, death, and transfer, and (ii) details of the transfer process (name, time, age, transfer path). All transfer events were confirmed to have occurred within 1 to 3 days. Individuals that immigrated into an OMU or AMU and were accepted by the members in the unit were considered successful transfer events. We were unable to follow individuals out of the study group, so we assumed that all monkeys that disappeared had emigrated out of the focal group, with the exception of individuals that stayed away for fewer than 2 years and severely injured individuals.

Fecal sample collection

The Yunnan snub-nosed monkeys (*R. bieti*) have the habit of defecation after midday feeding. Every day from 1300 to 1500, we observed the *R. bieti* at distances about 20–30 m identifying individuals in advance and waiting for them to defecate. Fecal samples were collected into sterile centrifuge tubes, sealed, labeled, and retained in a mobile refrigerator until being taken to the laboratory for final storage at -80°C . On September 1–18, 2020, a total of 50 fresh fecal samples from 50 individuals (1 sample per individual, 26 males and 24 females) was collected (Fig. 1b).

Analysis of microbiota composition by 16S rRNA gene amplicon sequencing

The fecal samples were snap-frozen and stored at -80°C after collection. Bacterial DNA was isolated from the fecal contents using the DNeasy PowerSoil kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA concentration and integrity were measured by a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. PCR amplification of the V3-V4 hypervariable regions of the bacterial 16S rRNA gene was carried out in a $25\ \mu\text{L}$ reaction using universal primer pairs (343F: 5'-TACGGRAGGCAGCAG-3'; 798R: 5'-AGGGTATCTAATCCT-3') (Nossa *et al.* 2010). The reverse primer contained a sample barcode, and

both primers were connected with an Illumina sequencing adapter. The PCR products were purified with Agencourt AMPure XP beads (Beckman Coulter Co., USA), and the concentrations were adjusted for sequencing. Sequencing was performed on an Illumina Miseq with 2 paired-end read cycles of 300 bases each. (Illumina Inc., San Diego, CA; OE Biotech Company; Shanghai, China).

Paired-end reads were preprocessed using Trimmomatic software (Bolger *et al.* 2014) to detect and remove ambiguous bases (N) and low-quality sequences with an average quality score below 20 using the sliding window trimming approach. After trimming, paired-end reads were assembled using FLASH software (Reyon *et al.* 2012). Parameters of the assembly were: 10 bp of minimal overlapping, 200 bp of maximum overlapping, and 20% of maximum mismatch rate. Additional sequence denoising was performed as follows: reads with ambiguous, homologous sequences, or shorter than 200 bp were filtered out. Reads with 75% of bases above Q20 were retained for further analysis using QIIME software (version 1.8.0) (Caporaso *et al.* 2010). Potential chimeras were detected and removed using VSEARCH (Rognes *et al.* 2016). Clean reads were subjected to primer sequence removal and clustering to generate operational taxonomic units (OTUs) using Vsearch software with a 97% similarity cutoff (Rognes *et al.* 2016). A representative read of each OTU was selected using QIIME package. All representative reads were annotated and blasted against Silva database (Version 123) using RDP classifier (confidence threshold was 70%) (Wang *et al.* 2007). Thus, taxonomic summaries for each fecal sample were gained using OTUs table. Circos (Krzywinski *et al.* 2009) was used to display the relative abundance of the dominant OTUs in each OMU.

The analysis of the beta diversity in the gut microbiome

Here, we focused on 3 levels for analysis. First, we used all fecal samples to compare the dissimilarity among 8 OMUs (2 OMUs were not included due to insufficient fecal samples [2 samples each]). PCoA clustering using the unweighted Unifrac distance matrix (Lozupone *et al.* 2011) was performed with QIIME (Caporaso *et al.* 2010). Adonis using unweighted Unifrac distance was used to test the significance of difference in the gut microbiome communities among these 8 OMUs.

Second, we conducted pairwise comparisons using unweighted Unifrac distance across all 10 OMUs.

(1) We performed pairwise comparisons of the unweighted Unifrac distances between each adult male and the adult males from other OMUs. Next, we calculated the mean pairwise unweighted Unifrac distance for per adult male per OMU. (2) We performed pairwise comparisons of the unweighted Unifrac distances between each adult female and the adult females from other OMUs, then calculated the mean pairwise unweighted Unifrac distance per OMU for an adult female. (3) If an OMU had several adult females, we performed pairwise comparisons of the unweighted Unifrac distances between adult females within the same OMU. We then compared the mean pairwise unweighted Unifrac distance per OMU for an adult female. Therefore, k-related sample analysis (Chi-square) in SPSS was used to assess for significant difference among these 3 mean distance matrixes. If significant, the paired Wilcoxon test was performed.

We also compared another 3 types of mean unweighted Unifrac distances: (1) The mean distance between the adult male and its mate (adult female within the same OMU) per OMU; (2) the mean distance between the adult males and unrelated females (adult females belonging to different OMUs) per OMU; and (3) the mean distance between adult females and unrelated males (adult males belonging to different OMUs) per OMU. Therefore, k-related samples analysis (Chi-square) in SPSS was used to assess the significant difference among these 3 types of mean distance matrixes. If significant, the paired Wilcoxon test was performed.

Finally, we compared 4 types of mean unweighted Unifrac distances related to offspring: (1) the mean distance between the mother and its offspring per OMU; (2) the mean distance between the father and its offspring per OMU; (3) the mean distance between females and unrelated offspring (belonging to other OMUs) per OMU; and (4) the mean distance between the male and unrelated offspring (belonging to other OMUs) per OMU. Therefore, k-related samples analysis (Chi-square) in SPSS was used to conduct the significant difference among these 3 types of mean distance matrixes. If significant, the paired Wilcoxon test was performed.

The relationship between the frequencies in the grooming and gut microbiome community

Within each OMU, the relationships between the pairwise unweighted Unifrac distance and the frequencies of pairwise grooming were tested via linear and quadratic models. The better model was selected according to the lower value of Akaike's information criterion

(Yamaoka *et al.* 1978). The above statistical analyses and plotting were conducted in R software (Ihaka & Gentleman 1996). In addition, we also conducted regression analysis for all fecal samples in this study.

RESULTS AND DISCUSSIONS

High similarity in the gut microbiome among the social units of the wild Yunnan snub-nosed monkeys

We collected 50 feces samples from 50 individuals belonging to 11 social units (10 OMUs: member size: 3–11 individuals; 1 AMU: however, we only successfully collected 1 feces collected from a male individual) (Figs 1b,2). We chose to rarefy our sampling depth at ~40 000 reads per sample to equalize the sampling depth across all samples. The dominant phyla included Firmicutes, Bacteroidetes, Spirochaetes, Proteobacteria, and Fibrobacteres (Fig. 3a,b; Fig. S1, Supporting Information). The dominant genera included *Preveotella_7*, *Chirstensenellaceae R-7 group*, *Ruminococcaeae UCG 005*, *Ruminococcaeae UCG 010*, *Rikenellaceae RC9 gut group*, and *Bacteroides* (Fig. 3c). Furthermore, the PCoA analysis, using unweighted Unifrac distance, displayed a mixed pattern (Fig. 3d; Fig. S2, Supporting Information), with no significant difference in the gut microbial community between OMUs (Adonis: 999 permutations, $F = 1.099$, $P = 0.111$) or between sexes (Adonis: 999 permutations, $P > 0.05$), indicating a high similarity in the gut microbiomes among social units of wild Yunnan snub-nosed monkeys. One possible explanation might be the transfer and immigration of individuals among the units. For example, during the previous 2 years, we observed 11 times for the adult female's transfer between OMUs, and 6 times for the adult male's immigration from the AMU in this study (Fig. 4). In humans and chimpanzees, immigration can result in changes in the gut microbiome, such as acquiring the microbiome of the local population and also the introduction of new microbes into the local population due to social contact and similar diet (Degnan *et al.* 2012; Vangay *et al.* 2018). Another reason could be cohabitation. The individuals in this study lived in the same national nature reserve and had the same diet, with grooming being one of the main social behaviors (Fig. 1c). Cohabitation (same living habitat and diet) and grooming leading to a similarity in the gut microbiomes among social groups has been observed in other nonhuman primates,



Figure 2 The photos of each social unit in this study. The copyright of these photos belonged to Dr. Dayong Li (one of the coauthors in this study).

including chimpanzees, baboons, and black howler monkeys (Tung *et al.* 2015; Moeller *et al.* 2016; Amato *et al.* 2017). Therefore, we speculated that these factors might explain the high similarity in the gut microbiomes among these OMUs. We then investigated the changes in gut microbial community based on sex and social units.

Adult males between OMUs harbor the significantly higher similarity in the gut microbiome community than that of adult females between OMUs

Interestingly, we found that the mean unweighted Unifrac distance between adult males from different

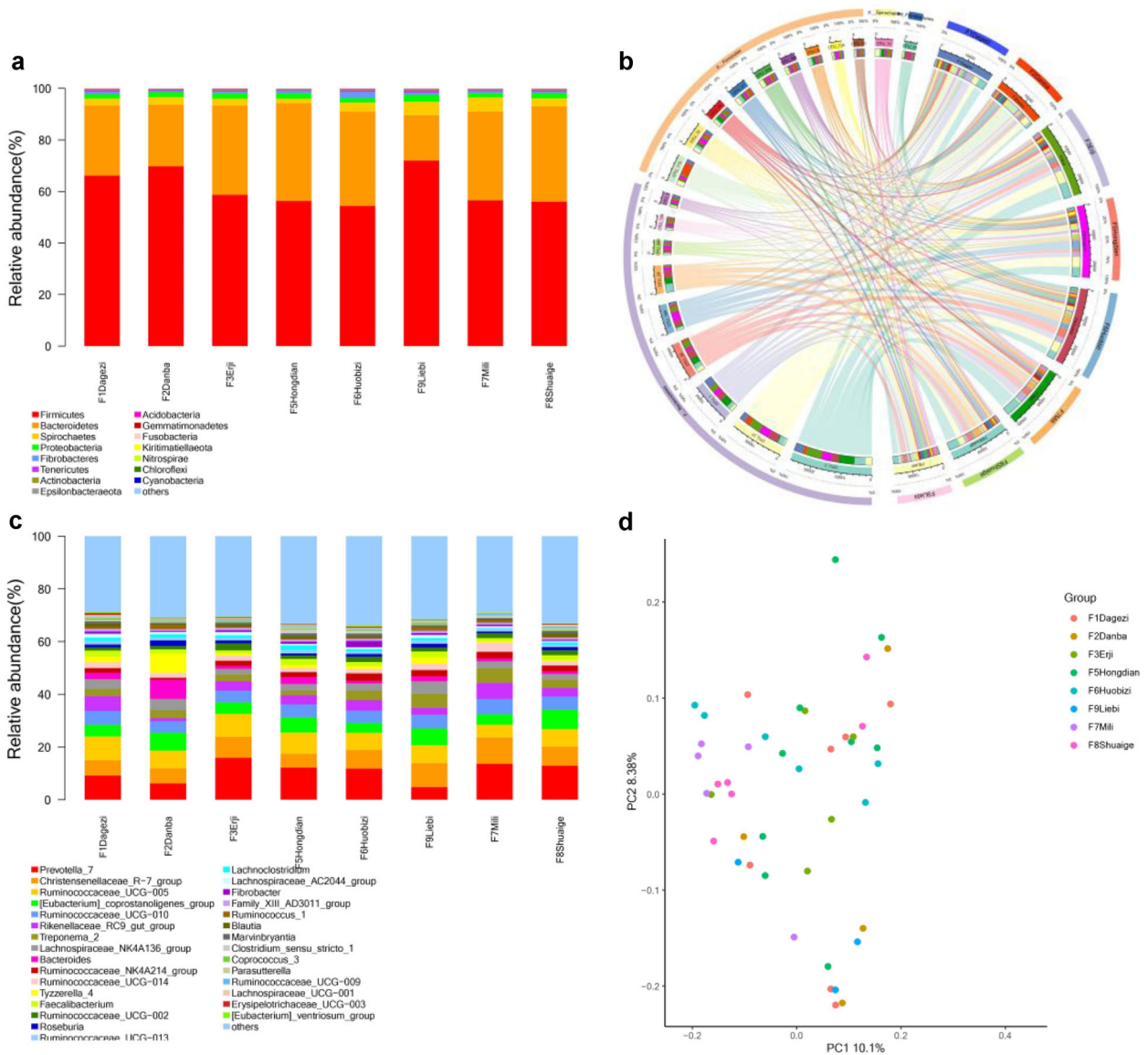


Figure 3 The gut microbial community in the Yunnan snub-nosed monkeys (*Rhinopithecus bieti*). (a) Relative abundance (%) of phyla. (b) Circos analysis displaying the dominant OTUs and their relative abundance in each OMU. (c). Relative abundance (%) of main genera. (d) PCoA clustering analysis using unweighted Unifrac distance shows the mixed pattern among these OMUs.

OMUs was significantly lower than that between adult females from different OMUs (Fig. 5a; paired Wilcoxon test, $P = 0.007$). There was no significant difference in the mean unweighted Unifrac distance between females within the same OMU or between females from different OMUs (Fig. 5a). This finding indicated a higher similarity in the adult male gut microbiome among OMUs compared to that of the adult female gut microbiome among OMUs. However, the mean unweighted Unifrac

distance between the adult male and their mate(s) was not significantly different compared to that between the adult male and unrelated females from other OMUs or between adult females and unrelated males from other OMUs (Fig. 5a; $\chi^2 = 1.400$, $df = 2$, $P = 0.497$). The mean unweighted Unifrac distance between adults and their offspring were not significantly different compared to that between adults and unrelated offspring from other OMUs (Fig. S3, Supporting Information; $\chi^2 = 3.514$, df

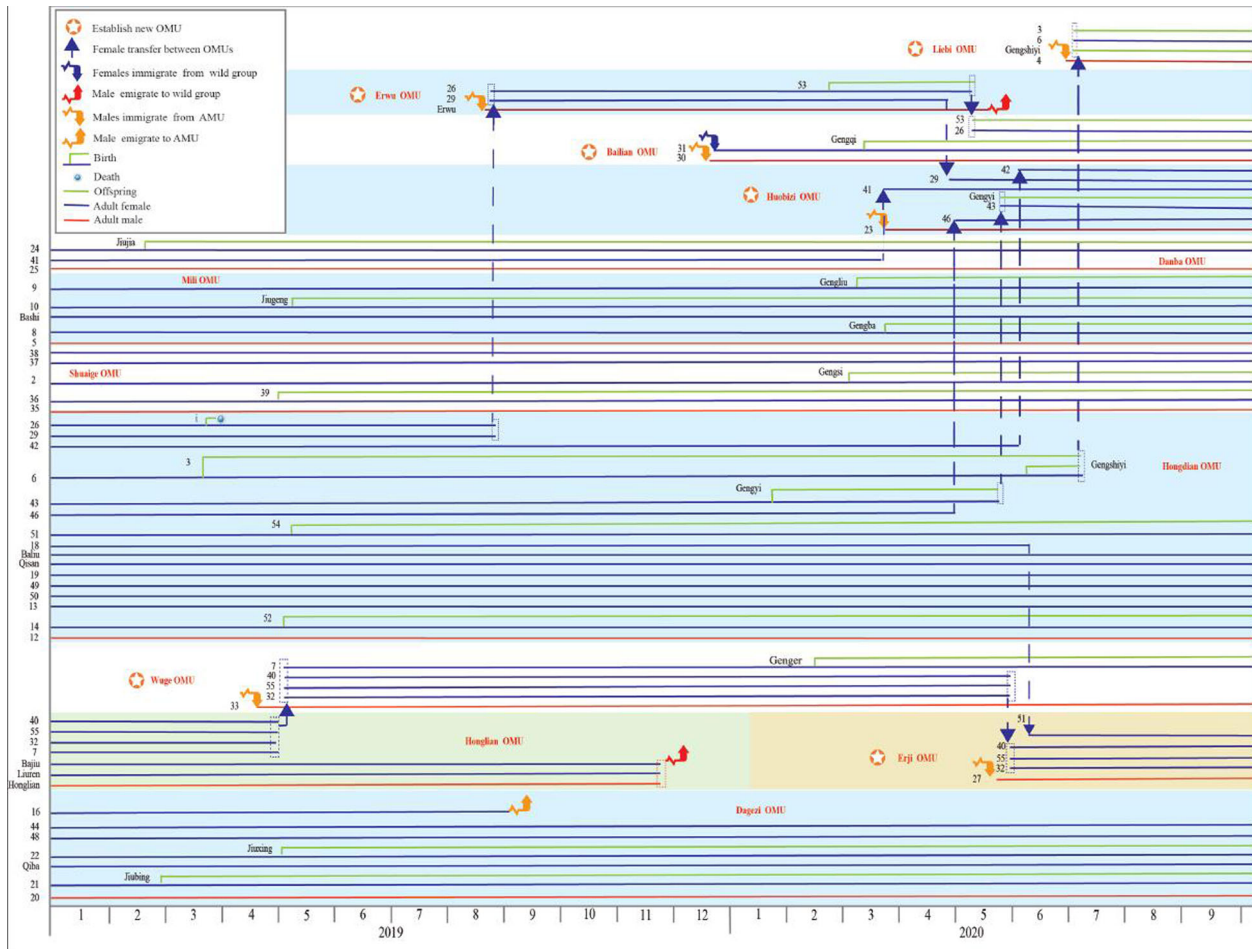


Figure 4 Individuals transfer between one-male units (AMUs) in a breeding band from 2019 to 2020: Different colored boxes represent different OMUs; the letters and numbers in front of the lines represent different individuals.

= 3, $P = 0.319$). There are several potential reasons for these findings. Based on long-term wild-field observation, we found that the frequency of defensive behavior (directly physical contact, e.g., fighting) between adult males from different OMUs was significantly higher than that of physical contact behavior between adult females from different OMUs (interaction index: adult male = about 2; adult female = 0) (Fig. 5b, t -test $P < 0.001$). High-frequency social contact would increase the chance of gut microbiome transmission (Ezenwa *et al.* 2012; Archie & Tung 2015; Brito *et al.* 2019), which might further increase gut microbiome similarity between adult males among the different OMUs in this study.

Moreover, some of the adult males in these OMUs had previously lived together, which might result in

the similarity of their gut microbiomes. In Yunnan snub-nosed monkeys, AMUs contain juvenile, subadult, and adult bachelor males (Kirkpatrick *et al.* 1998; Li *et al.* 2014). For example, based on our 11 years of wild-field observation, males 5, 12, and 30 had lived in the same AMU in 2010 prior to setting up their own OMUs in 2016, 2014, and late 2010, respectively, through invasion (Fig. 6). Males 4, 23, 30, and 33 lived together in 2019 and then set up their own OMUs in 2020 through invasion. Primate AMUs, especially those of snub-nosed monkeys and baboons, can include several adult and subadult males associated closely with the band (Kirkpatrick *et al.* 1998; Grueter 2013; Li *et al.* 2014; Grueter *et al.* 2017, 2020; Qi *et al.* 2017). Both the males in AMUs and solitary males periodically invade an OMU, resulting in the replacement of the former leader

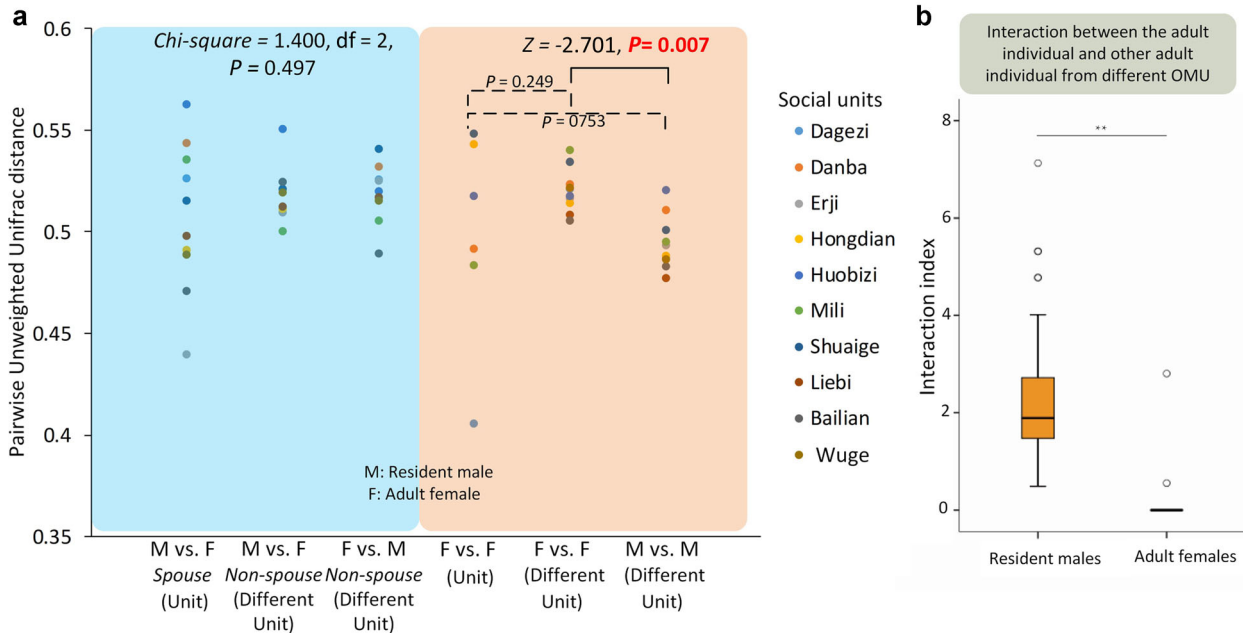


Figure 5 The connection between the social unit defense and gut microbiome community. (a) The mean pairwise unweighted Unifrac distance between adult individuals among the OMUs. k-related samples analysis (Chi-square) was used to assess for significant difference among these 3 types of mean distance matrixes. If significant, the paired Wilcoxon test was performed. (b) The difference (non-parametric *t* test) in the interaction of adult individuals among these OMUs. The interaction index (based on the frequencies in defense behavior) between the adult males among these OMUs was significantly higher than that between the adult females among these OMUs. **, $P < 0.01$.

or mating with resident females (Kirkpatrick *et al.* 1998; Guo *et al.* 2010; Li *et al.* 2014; Xiang *et al.* 2014; Grueter *et al.* 2020). In addition, during the previous 2 years (2019–2020), we observed 11 times for the adult female's transfer between OMUs, and 6 times for the adult male's immigration from the AMU in this study (Fig. 4), which might explain no significant difference in the gut microbial community among the females of different OMUs. Thus, we speculated the defense and invasion of an OMU with aggressive interaction behavior could lead to the high similarity in the adult male gut microbiome among different OMUs under the premise of previous cohabitation within the same AMU. Therefore, these periodic invasions, along with the mating behavior (if successful), and expulsion, could further explain the non-significant difference in the gut microbiome community between the adult male versus their mates and the adult male versus unrelated females from other OMUs or between the parent versus offspring and the parent versus unrelated offspring.

Grooming partially increased the similarity in the gut microbiome community within the OMU

We further investigated the relationship between grooming and the gut microbial community within OMUs and found a partially negative relationship (Fig. 7; Fig. S4f, Supporting Information). For instance, the frequency of grooming was significantly negatively associated with unweighted Unifrac distance of the gut microbiome in fecal samples from the social unit Dagezi (Fig. 7), while other OMUs (Mili and Hongdian) displayed a slightly negative (but non-significant) relationship (Fig. 7). These findings indicated that grooming partially increased the similarity in the gut microbiome community within the OMU. Several studies in nonhuman primates have shown a similar relationship (Tung *et al.* 2015; Moeller *et al.* 2016; Amato *et al.* 2017). It was novel to observe that this relationship also occurred within the small social unit of primates with high social organization.

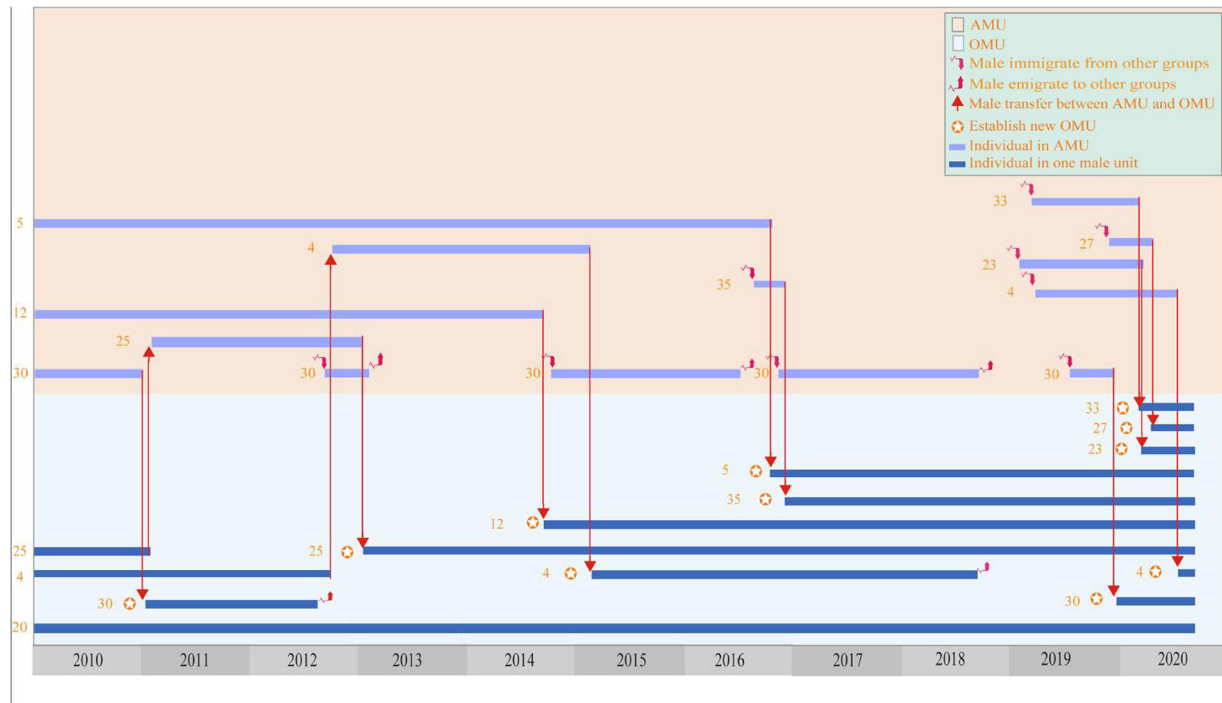


Figure 6 Resident male transfer between OMU and AMU from 2010 to 2020: The individuals of the collected fecal sample are represented by a number corresponding to the fecal sample number. If an individual is given a non-number name, such as Gengshiyi from the Liebi OMU, no fecal sample was collected from that individual.

CONCLUSION

Here, based on long-term wild-field observation (e.g. social unit dynamics, transfer, and behavior), we revealed the potential connection between the defense and invasion of social units and the gut microbiome community in wild Yunnan snub-nosed monkeys. We speculated that the resident adult males in different OMUs harboring a gut microbiome community that was significantly more similar than that of adult females in different OMUs might be related to the sexual differences in social interactions. Grooming within an OMU might increase the similarity in the gut microbial community among individuals of that OMU. Therefore, this study suggested that multilevel societies might have an effect on the gut microbiome in this wild nonhuman primate species. In addition, other potential factors (e.g. sex-specific physiology) would also influence the gut microbial community. However, the associated experiments are hard to conduct in the wild primates.

The gut microbiome might be used as a proxy for social structure. In these multilevel societies, the male may have 3 stages during their grow-up: OMU (birth)-AMU-OMU (setting up); and some of the female may have 2 stages: OMU (birth)-OMU (dif-

ferent); and some of the female will stay in their original OMU. Thus, the maintaining of multilevel societies might be through both avoiding inbreeding (male immigration) and even the high gut microbiome diversity (relatively high dissimilarity in the adult females among the different OMUs compared to that in the adult males among the different OMUs).

CONFLICT OF INTEREST

The authors declare no competing interests.

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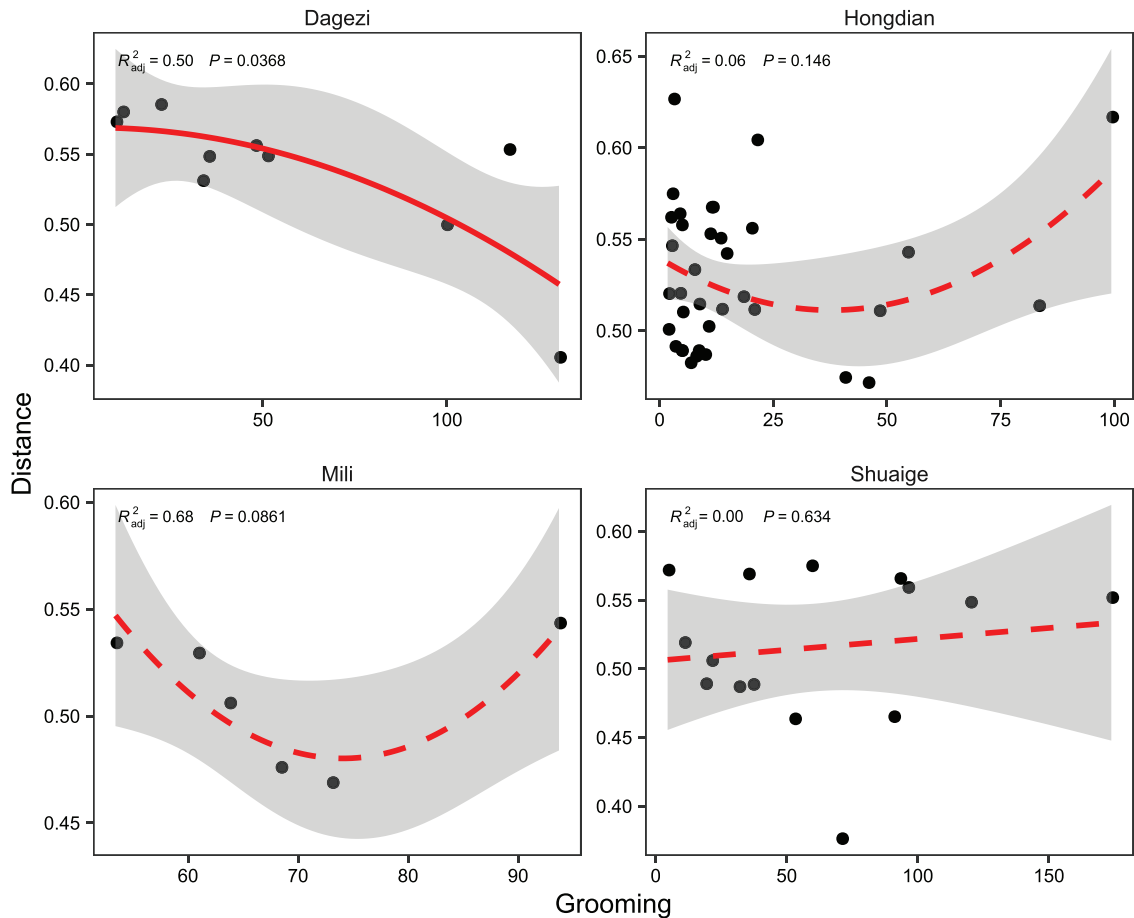


Figure 7 The relationships between the pairwise unweighted Unifrac distance and the frequencies of pairwise grooming were tested via linear and quadratic models. The better model was selected according to the lower value of Akaike's information criterion (Yamaoka *et al.* 1978). Only 4 OMUs were used in this analysis due to the amount of data required.

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SUPPLEMENTARY MATERIALS

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 The dominant OTUs and their taxonomic information in the Yunnan snub-nosed monkeys in this study.

Figure S2 The PCoA analysis using unweighted Unifrac distance (based on OTUs table).

Figure S3 The mean pairwise unweighted Unifrac distance between parents and offspring (O) or non-offspring (N-O) among the OMUs.

Figure S4 The relationships between the pairwise unweighted Unifrac distance and the frequencies of pairwise grooming were tested via linear and quadratic models across all OMUs.

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