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Age-Related Dynamics of Fecal Microbiota in the Captive Chimpanzee (*Pan troglodytes*)

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Abstract

Understanding gut microbiome diversity in endangered chimpanzees (*Pan troglodytes*) is crucial for their effective *ex situ* conservation. This study characterized the age-related dynamics of the fecal microbiota in captive juvenile (< 5 years), adolescent (5–10 years), and adult (> 15 years) chimpanzees reared in Shanghai and Hangzhou Zoos using 16S rRNA sequencing and demonstrated significant ontogenetic shifts in the microbiota composition. Alpha diversity peaked in adolescents (significantly higher Ace/Chao1/Observed Species; $p < 0.05$), with the greatest number of unique OTUs (1,139). Community structures significantly differed between the age groups (ANOSIM $R = 0.121$). Furthermore, the captive diets drove the fundamental restructuring of the core phyla. The Firmicutes/Bacteroidetes ratio was considerably lower in captive individuals than in wild conspecifics. The discriminative taxa were also analyzed. Notably, the abundance of *Lactobacillus* increased significantly in adults (5.44% ± 4.43%) compared to younger groups (< 1.26%), whereas that of *Prevotella* decreased with age. These findings demonstrate the regulatory role of age in determining gut microbiome in captivity, which is potentially attributed to physiological transitions and dietary adaptations, which present a basis for microbiome-informed health monitoring and age-specific dietary management for the welfare of captive chimpanzees.

Keywords: chimpanzee, intestinal microbiota, 16S rRNA, Firmicutes, Bacteroidetes

Introduction

Pan troglodytes has been studied in multiple disciplines across ecology, social sciences, biomedical research, and science communication, and it is associated with their high intelligence (Roth 2015; Altschul et al. 2017; Cantwell et al. 2022), complex social behavior (Pascual et al. 2023; van den Heuvel et al. 2023), and unique evolutionary significance. This species is now classified as Endangered by the International Union for Conservation of Nature (IUCN) and is listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). *Ex situ* conservation is considered an effective way to conserve the survival and genetic diversity of this species (Visser et al. 2023; Ye et al. 2023). Zoos, which include wildlife conservation, scientific research, and

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public education, serve as critical centers for the *ex situ* conservation of rare and endangered wildlife (Abeli et al. 2020; Staerk et al. 2024). Since the 1970s, the captive chimpanzees bred in China have yielded a total population of 237 individuals currently distributed among 46 zoos. Shanghai and Hangzhou Zoos are the first two institutions in China to keep chimpanzees in captivity.

The developmental process of chimpanzees includes three distinct ontogenetic stages: juvenile (1–5 years), adolescent (5–10 years), and adult (> 10 years) stages (Goodall 1986; Pusey et al. 2005), each characterized by specific physiological and behavioral transitions that shape the gut microbiome assembly. The juvenile stage encompasses weaning and maternal dependency dissolution, during which the microbiome transitions from the abundance of milk-adapted taxa to a broader fermentative capacity. Adolescence is associated with social independence and sexual maturation, which drive microbiome restructuring via hormonal shifts and dietary exploration. Microbial communities stabilize by adulthood to support full somatic and reproductive functions (Moeller et al. 2016; Reese et al. 2021; Amato et al. 2025). In wild populations, fiber-degrading specialists essential for metabolizing diverse plant substrates are dominant (Nishida and Ochman 2019; Reese et al. 2021; Baniel et al. 2022). Under captive conditions, dietary regimes fundamentally reconfigure the nutritional landscape relative to wild ecosystems, driving divergent microbiome trajectories and cascading effects on host physiology, behavior, and conservation viability. The wild chimpanzees consume about 174 plant species annually, with seasonal shifts in frugivory and folivory promoting microbiome plasticity essential for nutrient extraction and immune resilience (Tutin and Fernandez 1993; Reese et al. 2021). Contrastingly, the captive diets rich in cultivated fruits and vegetables (e.g., apples and leafy greens), which include limited browse (elm/willow), exhibit 40–60% reductions in insoluble fiber and a near-absence of secondary plant metabolites (Campbell et al. 2020; Narat et al. 2020). A study towards the captive apes in Wildlife Reserves Singapore and Longleat Safari and Adventure Park showed that the diet with reduced water-soluble carbohydrates neutral and increased detergent fibre significantly increased 'travelling', 'foraging' and 'social affiliative' behaviors, and decreased 'inactivity' and 'abnormal behavior patterns', such as 'regurgitation and reingestion'. They pointed out that great apes in captivity have been affected by a variety of conditions, including obesity, heart, gastrointestinal and dental diseases, and diabetes, all of which are at least influenced by an inappropriate diet (Cabana et al. 2017). Zoos in Southeast Asia and North America demonstrated the convergence toward a "captive enterotype" dominated by *Bacteroides*, with reduced α-diversity compared to that in wild conspecifics (Clayton et al. 2016). Anthropogenic diets under captive conditions induce gut microbial dysbiosis in primates, and the altered microbiota profoundly influence physiological homeostasis (Campbell et al. 2020; Costantini et al. 2021) and social behavioral repertoires (Pan et al. 2021; Zheng et al. 2021).

Although the chimpanzee husbandry protocol followed across Chinese institutions adheres to the EAZA Best Practice Guidelines for Great Apes Taxon Advisory Group Chimpanzees (*Pan troglodytes*) (Carlsen et al. 2022) and the Chimpanzee (*Pan troglodytes*) Care Manual (AZA Ape TAG 2010), regional supply chains limit provisioned diets, leading to inter-facility heterogeneity in gut microbiome architecture. The Yangtze River Delta region represents the earliest and largest habitat for captive chimpanzee populations in China, with Shanghai and Hangzhou Zoos housing the highest number of individuals. However, information on the microbiota of captive chimpanzees in this region is limited. In this study, we characterized the fecal microbiota composition across juvenile, adolescent, and adult developmental stages via 16S rRNA sequencing using samples from captive populations in this region. This analysis can potentially elucidate the age stage-related microbial signatures in East Asian *ex situ* conservation and provide a theoretical basis for optimizing regionally tailored management strategies.

Experimental

Materials and Methods

Animal rearing and fecal sample collection. Chimpanzee husbandry protocol adheres to the EAZA Best Practice Guidelines (Carlsen et al. 2022) and the Chimpanzee (*Pan troglodytes*) Care Manual (AZA Ape TAG 2010). The experimental procedures included in this study were reviewed and approved by the An-

imal Use and Care Committee of the Shanghai Zoo. Twenty-one captive chimpanzees (aged 1–36 years) were raised at Shanghai Zoo (31°11′N, 121°21′ E) and Hangzhou Zoo (30°12′N, 120°08′E) in China. All participating chimpanzees were healthy, disease-free, and had received no medical care for at least 2 months before sampling. Chimpanzees were divided into three age groups (Goodall 1986; Pusey et al. 2005): each of the groups A, B, and C included seven samples from

The detail information of grouping.

Age group	Group	Sample size
2 < years old < 5	juvenile group A	7
5 < years old < 10	adolescent group B	7
> 15 years old	adult group C	7

juvenile (< 5 years old), adolescent (5–10 years old), and adult (> 15 years old) chimpanzees. Table I lists the detailed grouping information. Standardized diet was supplied based on the standard maintenance procedures for chimpanzees set by the Shanghai Zoo and Hangzhou Zoo. Water was provided as libitum. Fresh fecal samples were collected from each individual at 8:00 AM every third day. Three consecutive sampling events were performed per chimpanzee. To minimize variation, a composite sample was created for each animal by combining equal proportions (1:1:1 ratio by weight) of the three collected samples, resulting in a final representative sample of 3 grams. Fecal samples were stored at −80°C until further DNA extraction.

DNA extraction and PCR amplification. Total genomic DNA was extracted from fecal samples using the CTAB/SDS method. The DNA concentration and purity were analyzed using a 1% agarose gel. Subsequently, DNA was diluted to 1 ng/μl using sterile water, and all DNA samples were stored at −20°C for further PCR amplification.

16S rRNA gene amplification sequencing. For PCR reactions, a mix (30μl) was prepared using 15μl of Phusion™ High-Fidelity PCR Master Mix (New England Biolabs, USA), 0.2μM of forward and reverse primers, and about 10 ng template DNA. The thermal cycling process included an initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10s, annealing at 50°C for 30s, elongation at 72°C for 60s, and a final step at 72°C for 5 min. The PCR products were purified from a 2% agarose gel using a Gel Extraction Kit. Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, USA) following the manufacturer's recommendations. After the assessment was performed using a Qubit® 2.0 Fluorometer (Invitrogen™; Thermo Fisher Scientific Inc., USA) and Agilent Bioanalyzer 2100 system (Agilent Technologies, Inc., USA), the library was sequenced on an Illumina®NovaSeq™ 6000 platform (Illumina, Inc., USA), and 250 bp paired-end reads were generated. The 341F (5′CCTAYGGGRBGCASCAG3′) and 806R (5′GGACTACNNGGGTATCTAAT3′) primers were used to amplify the V3–V4 region of the 16S rRNA gene. The sequencing data has been deposited in NCBI (PRJNA1290099).

Bioinformatics and statistical analysis. Paired-end reads generated from the original DNA fragments were merged using FLASH (Magoč and Salzberg 2011), designed to merge paired-end reads when at least some of the reads overlapped with reads generated from the opposite end of the same DNA fragment. Paired-end reads were assigned to each sample based on unique barcodes. Sequence analysis was performed using the UPARSE software package with UPARSE-OUT (Operational Taxonomic Units) and UPARSE-OUT ref algorithms (Edgar 2013). In-house Perl scripts were used to analyze alpha (within samples) and beta (among samples) diversities. Sequences exhibiting ≥ 97% similarity were assigned to the same OTUs. We selected representative sequences for each OTU and used the RDP classifier to annotate the taxonomic information for each representative sequence. To assess the alpha diversity, we rarified the OTU table and assessed the species richness and diversity of chimpanzees based on the following indices: Chao1 index and Observed Species (to assess species richness), Shannon and Simpson indices (to assess the diversity of species). The dilution curve of the corresponding index was generated to assess the saturation of the overall detection of the experimental microbial community. Based on a Venn diagram, we compared the species composition between different samples. The species composition was compared between different samples or groups using dimensionality reduction analysis with NMDS. Significant differences in the microbial community structure between groups were analyzed using ANOSIM. Linear discriminant analysis (LDA) coupled with effect size (LEfSe) was used to evaluate differentially abundant taxa, focusing on both statistical significance and bio-

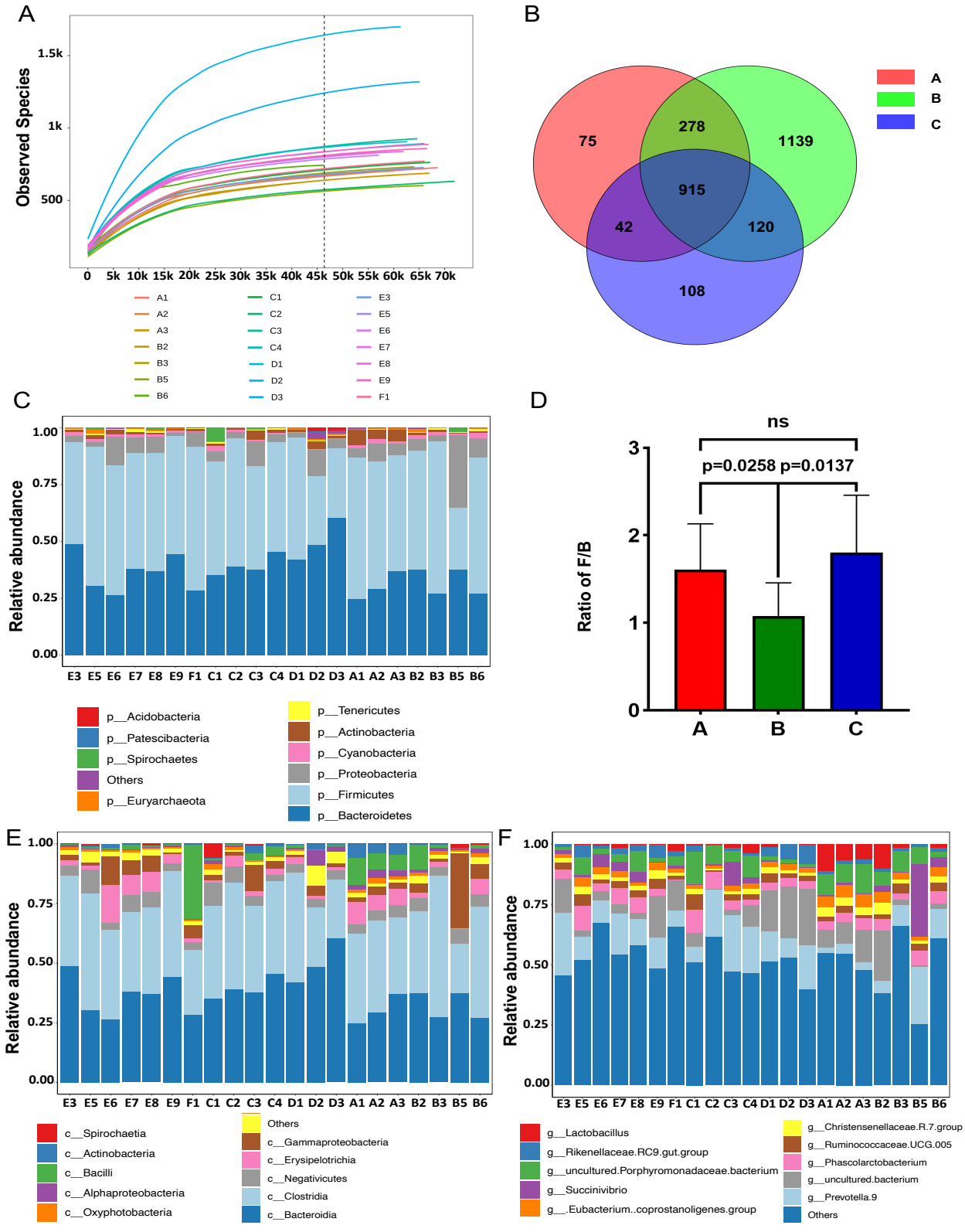


Fig. 1. Gut Microbiota Composition of *Pan Troglodytes* feces at different Ages.

A) The rarefaction curve of the microbiota of all samples. Each curve representing a different sample. The curves E3, E5, E6, E7, E8, E9, and F1 indicate the juvenile group; the curves C1, C2, C3, C4, D1, D2, and D3 indicate the adolescent group; the curves A1, A2, A3, B2, B3, B5, and B6 indicate the adult group. The x axis showed qualitative sequence of random number; The y axis showed OTU number. B) Venn plots show the OTU distribution based Venn plots between juvenile (A) adolescent (B) and adult (C) groups. C) The microbial community composition of *Pan Troglodytes* at the level of phylum in groups A, B, and C. D) The ratio of Firmicutes/Bacteroides in group A, B and C. E–F) The microbial community composition of *Pan Troglodytes* at the level of class E) and genus F) in group A B and C.

logical relevance.

Results

Fecal bacterial compositions in juvenile, adolescent, and adult chimpanzees. After processing the raw data, a total of 1,986,038 (47,188–74,191/each) high-quality reads were generated through the 16S rRNA sequencing of 21 fecal samples (seven juveniles, seven adolescents, and seven adults). The valid tags, rates, and quality scores Q20% and Q30% are presented in Table SI. While analyzing the diversity of species composition in the samples, the UCLUST in QIIME v1.8.0 (Caporaso et al. 2010) was used to cluster the clean reads of all samples. Next, the clean reads were first subjected to dechimerism processing, then the repeated sequences were clustered into Operational Taxonomic Units (OTUs) based on 97% homology. Representative sequences of OTUs were annotated with species using the annotation database Silva (Quast et al. 2013) (Table SII). Fig. 1A shows each curve representing a different sample. The sequencing depth first increased as the number of features increased, followed by a plateau, indicating that a small number of new features were detected with the increase in the sequencing depth. Therefore, this trend suggested that the sequenced samples were sufficient and reasonable. A total of 1,310, 2,452, and 1,185 features were detected in the juvenile, adolescent, and adult groups, respectively. The Venn diagram (Fig. 1B) revealed that the three tested groups shared 915 common features, suggesting a common effect on chimpanzee gastrointestinal microbiota at the three different developmental stages. There were 75, 1,139, and 108 unique features in the juvenile (Group A), adolescent (Group B), and adult (Group C) groups, respectively. The large number of unique features in adolescents indicates that the chimpanzee gut microbiota played an essential role at this developmental stage.

A total of 31 bacterial phyla were identified, including Firmicutes, Bacteroidetes, Proteobacteria, Cyanobacteria, Actinobacteria, Tenericutes, Euryarchaeota, Spirochaetes, Patesobacteria, and Acidobacteria, which were the top 10 phyla in all samples (Fig. 1C). Firmicutes and Bacteroidetes presented the most abundant phyla. Firmicutes accounted for 54.39% ± 6.00%, 44.92% ± 9.77%, and 53.57% ± 11.89% in the juvenile, adolescent, and adult groups, respectively, whereas Bacteroidetes accounted for 36.12% ± 7.76%, 43.92% ± 7.90%, and 31.42% ± 5.23%, respectively, in these groups. The ratio of Firmicutes/Bacteroidetes (F/B) was 1.51 in the juvenile group, which decreased to 1.02 in the adolescent group, and increased to 1.70 in the adult group (Fig. 1D).

Seventy-nine classes were identified, which included 33 classes identified in all three groups. Among these, Spirochaetia, Actinobacteria, Bacilli, Alphaproteobacteria, Oxyphotobacteria, Gammaproteobacteria, Erysipelotrichia, Negativicutes, Clostridia, and Bacteroidia presented the top 10 classes (Fig. 1E), with Clostridia and Bacteroidia exhibiting the highest abundance. Clostridia accounted for 37.83% ± 6.47%, 36.33% ± 7.93%, and 38.46% ± 11.27% in the juvenile, adolescent, and adult groups, respectively, whereas Bacteroidia accounted for 36.12% ± 7.76%, 43.92% ± 7.90%, and 31.41% ± 5.23%, respectively, in these groups. The proportion of Bacteroidia first increased transiently, and subsequently, decreased with age. Notably, the higher abundance of Bacilli was detected in the adult group than in the juvenile and adolescent groups. Interestingly, Bacteroidia and Bacilli belong to Bacteroidetes and Firmicutes, respectively; hence, these distribution patterns were consistent with the phylum-level data.

Among the 511 identified genera, 215 genera were identified in all three groups. *Lactobacillus*, *Rikenellaceae* RC9 gut group, uncultured *Porphyromonadaceae* bacterium, *Succinivibrio*, *Eubacterium coprostanoligenes* group, *Christensenellaceae* R.7 group, *Ruminococcaceae* UCG-005, *Phascolarctobacterium*, uncultured bacterium, and *Prevotella 9* presented the top 10 taxa (Fig. 1F). *Lactobacillus* accounted for 0.99% ± 1.01% and 1.14% ± 1.26% in the juvenile and adolescent groups, respectively, whereas its abundance significantly increased to 5.44% ± 4.43% in the adult group. Similarly, the relative abundance of the *E. coprostanoligenes* group was significantly increased during the development of chimpanzees; it accounted for 2.43% ± 0.90% and 2.06% ± 0.59% in the juvenile and adolescent groups, respectively, and 3.93% ± 1.47% in the adult group. The *Prevotella 9* accounted for 13.30% ± 6.43%, 15.36% ± 6.50%, and 8.57% ± 7.57% in the juvenile, adolescent, and adult groups, respectively, in-

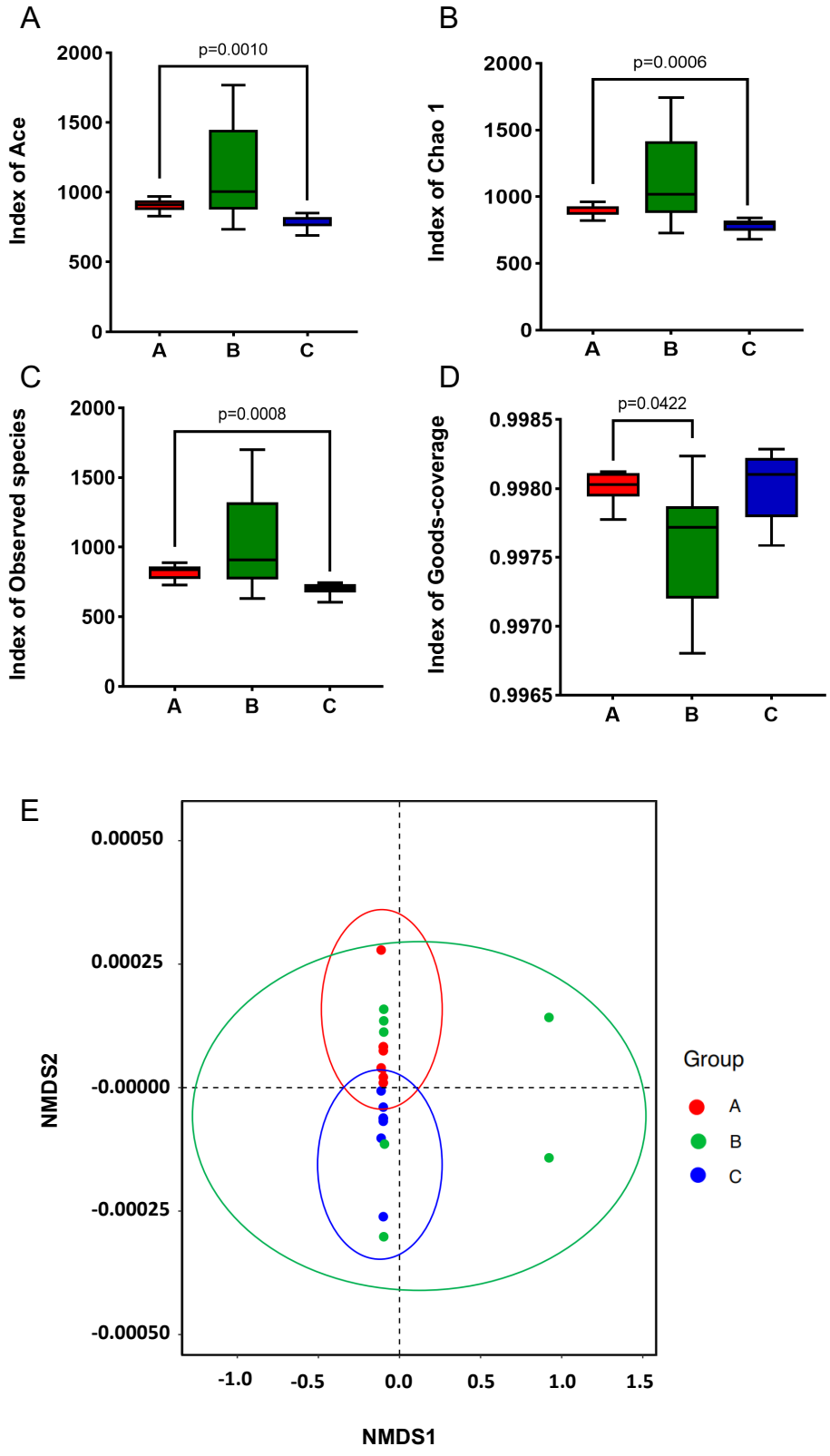


Fig. 2. Diversity analysis of fecal microbiota of *Pan Troglodytes* in different ages.
A–D) Box plot of the α-diversity index of Ace (A) Chao1 (B) Observed species (C) and Goods-covrage (D). E) The Non-Metric Multi-Dimensional Scaling (NMDS) illustrating that the microbial community differences between chimpanzees in groups A, B and C was statistically significant at the Stress level of 1×10^{-4}.

dicating a moderate but significant reduction in the abundance. The dynamic trends of the three genera were consistent at both the class and phylum levels.

Diversity of fecal microbiota in the juvenile, adolescent, and adult chimpanzee groups. Next, the sequences were used in a serial estimation of and diversity to evaluate the fecal bacterial diversity in the juvenile, adolescent, and adult chimpanzees. Significant differences in the Ace (A:905.87 ± 45.52 vs. B:1128.06 ± 357.01 vs. C:792.09 ± 5 3.08; $p = 0.024$) (Fig. 2A), Chao 1 (A:903.96 ± 45.99 vs. B:1129.37 ± 343.91 vs. C:779.68 ± 54.89; $p = 0.015$) (Fig. 2B), and Observed species (A:819.57 ± 54.85 vs. B:1021.00 ± 366.85 vs. C:697.71 ± 47.24; $p = 0.036$) (Fig. 2C) indices were detected between the juvenile and adult groups. The goods-coverage assay revealed a significant increase in the features in the adolescent group than in the juvenile group (A:1 ± 0 vs. B:1 ± 0, $p = 0.0422$) (Fig. 2D). The above data suggest that the richness and diversity of the fecal microbiota were gradually increased during adolescence, followed by a decline to relatively steady levels detected in the adult group. The NMDS analysis based on Bray–Curtis dissimilarity demonstrated the β-diversity of the microbial community. The result was directionally distributed on the NMDS plot. Furthermore, the clusters of plots in the juvenile, adolescent, and adult groups were well-separated (stress = 1×10^{-4}), suggesting a difference between the three groups (Fig. 2E). The ANOSIM (Bray–Curtis algorithm) results indicated a significant difference among the three groups ($R = 0.121$, $p = 0.045$) (Table II).

Table II
The ANOSIM analysis was used to test the significance of differences in community structure between groups.

Groups	*R*	*p*-value
A vs. B	0.0068027	0.383
A vs. C	0.1574344	0.082
B vs. C	0.1977648	0.064
A vs. B vs. C	0.1209373	0.045
C vs. B vs. A	0.1209373	0.045

Analysis of the discriminative keystone taxa between the juvenile, adolescent, and adult chimpanzee groups. A linear discriminant analysis was used to identify the discriminative keystone taxa between the juvenile, adolescent, and adult groups ($p < 0.05$, |log10 LDA score| ≥ 3.0). As a result, a total of 8 taxa were detected in the three groups. The juvenile group was distinguished by the most prevalent family *Streptococcaceae* (3.59, $p = 0.013$) (two taxa). The adolescent group was characterized by the highest abundance of the phylum Bacteroidetes (4.11, $p = 0.031$) (three taxa) and genus *Ruminococcaceae* UCG-014 (3.12, $p = 0.038$) (one taxon). The adult group was characterized by the genus *E. coprostanoligenes* (3.20, $p = 0.049$) (one taxon) and class Alphaproteobacteria (3.15, $p = 0.020$) (one taxon) (Fig. 3A). The cladogram in Fig. 3B suggests the microbiotal structure of the three groups.

Discussion

This comprehensive study demonstrates the comparative fecal microbiota structure in captive chimpanzees at different developmental stages. We observed dynamic changes in the abundance of the gut microbial community at the phylum, class, and genus levels in the juvenile, adolescent, and adult stages. The chimpanzees at all stages were maintained using the same husbandry protocol and the standard recipes. However, the unique features reflected in the results strongly suggest that age critically regulates the gut microbiota structure in captive chimpanzees.

LEfSe analysis identified the family *Streptococcaceae* (two taxa) as the characteristic flora in the juvenile group; *Streptococcus pneumoniae* (Li et al. 2022) and *Streptococcus varani* (Bakour et al. 2016) were detected, however, they were not abundant at the species level. The presence of *S. pneumoniae* and *S. varani* in the gut microbiota indicates the presence of an immature intestinal microbiota in juvenile individuals. In the adolescent group, as the intestinal microbiota continued to mature, the phylum Bacteroidetes (three taxa) and the genus *Ruminococcaceae* UCG-014, belonging to the phylum of Firmicutes, became the most distinct flora. *E. coprostanoligenes* was one of the most characteristic flora in the adult group. The genus *Eubacterium* represents a pivotal butyrogenic taxon that ferments dietary fibers into butyrate via glycolytic pathways and is associated with colonic butyrate production in healthy individuals (Louis and Flint 2009; Rivière et al. 2016; Litty and Müller 2021). Its increased abundance in the gut of adult chimpanzees is a collective outcome of dietary adaptation, physiological demands, and mi-

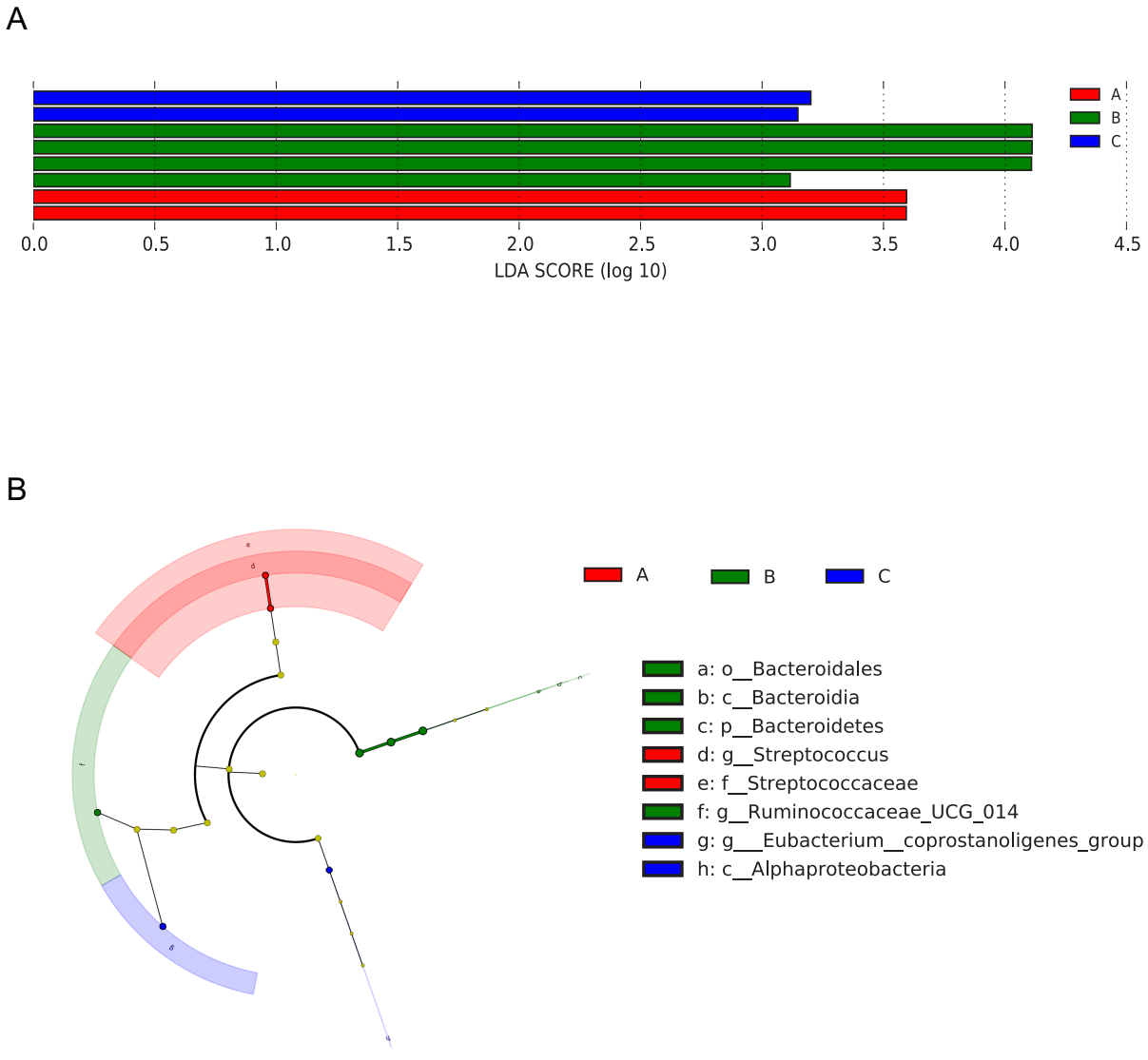


Fig.3. The keystone taxa of *Pan Troglodytes* feces *in* different ages.
A) Histogram of the linear discriminant analysis (LDA) scores computed for features differentially abundant. Red green and blue bars represented bacterial conmmunities of juvenile adolescent and adult groups respectively. The criteria was set as $p < 0.05$, |log10 LDA score| ≥ 3.0. B) The cladogram analysis. Colors indicated represent the core bacterial populations in respective groups.

crobial niche competition. *E. coprostanoligenes*, one of the most important species, plays an essential role in cholesterol metabolism, cardiovascular protection (Ren et al. 1996; Le et al. 2022; Rouskas et al. 2025), and the regulation of reproductive health (Fu et al. 2024). Overall, these results suggest that differential microbial biomarkers undergo dynamic changes with temporal progression throughout gut development and maturation in captive chimpanzees.

Previously, the gut microbial communities within chimpanzees were reported to show a decrease in the α diversity (Shanon index) from infant to elderly (Degnan et al. 2012). Similarly, we found a declining trend in the gut microbial communities in captive chimpanzees. Additionally, we demonstrated that α diversity indices, such as Chao1, ACE, Observed species, decreased from the juvenile to the adult. Moreover, a transient increase of the α diversity index was found in the adolescent stage; however, no significant change was detected. This transient climax of the gut microbi-

ome may be attributed to intensified host physiological demands, particularly somatic growth, immune maturation, and metabolic adaptation during adolescence. However, further analysis to elucidate the interplay of the gut microbial community structure throughout the development of chimpanzees will be required in our future research.

We, as well as other researchers (Szekely et al. 2010; Degnan et al. 2012; Moeller and Ochman 2013), have reported Firmicutes and Bacteroidetes to be the two dominant phyla. The Firmicutes phylum enables the host to extract substantial energy from dietary substrates (Sun et al. 2023; Dias et al. 2025), whereas Bacteroidetes exhibits comparatively limited efficiency in converting nutrients into available energy (Wexler and Goodman 2017; Pan et al. 2023). Therefore, the F/B ratio is widely accepted to regulate body weight crucially (Koliada, Syzenko et al. 2017; Aragón-Vela et al. 2021; Mohamed Qadir and Assafi 2021; Komodromou et al. 2024). In an extensive ape study, the F/B ratio was approximately 3.34 in wild adult chimpanzees (Degnan et al. 2012). In this study, the F/B ratio was lower than that of the wild species. Interestingly, a relatively low F/B ratio in captive chimpanzees compared to that in wild species was also reported previously (Narat et al. 2020). We hypothesized that captive chimpanzees require less biological energy to sustain vital functions than their wild counterparts owing to the reduced ecological demands, which is substantiated by the reduced body weights (Table SIII) observed in captive individuals than in the corresponding wild conspecifics at comparable age (Pusey et al. 2005). Interestingly, we found that the abundance of *Lactobacillus* increased, whereas that of *Prevotella 9* was decreased. It is possible the captive dietary regimen mainly induced this microbial shift. *Prevotella* spp. harbor carbohydrate-active enzyme (CAZyme) systems specialized for degrading complex plant polysaccharides, such as xylan and cellulose, which are abundant in fibrous wild diets (e.g., leaves, fruits, and bark) (El Kaoutari et al. 2013; Kovatcheva-Datchary et al. 2015; Accetto and Avguštin 2019; Aakko et al. 2020; Wardman et al. 2022). Under captive conditions, a reduction in dietary fiber diminishes the ecological competitiveness of *Prevotella*. Concurrently, *Lactobacillus* spp. or *Eubacterium* proliferate owing to their rapid metabolic turnover in the captive oligotrophic environment, including the anthropogenic diets with simplified carbohydrates (Watson et al. 2013).

Collectively, this study, for the first time, delineates the ontogenetic trajectory of the gut microbiome assembly in captive chimpanzees (*P. troglodytes*) at the Shanghai Zoo and Hangzhou Zoo. Our study, revealing the longitudinal profiles of core taxa across developmental stages, provides: i) a preliminary framework for noninvasive health monitoring using fecal microbial signatures, where taxon-specific shifts (e.g., *E. coprostanoligenes* decline) can potentially indicate metabolic dysregulation before clinical manifestation; ii) evidence-based dietary guidelines for optimizing captive management to maintain *Prevotella*-associated detoxification capacity while accommodating age-dependent nutritional needs. In the subsequent investigations, multi-omics analyses (metagenomics/metabolomics) with longitudinal records of gastrointestinal hormone profiles, immune markers, and stereotypic behavior frequency will be integrated to unravel the microbiome-host physiological crosstalk.

Acknowledgments

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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