

Variance Analysis of Gut Microbiota and Metabolomics between Wild and Captive Pangolins

Fan Zhou

Nanjing University of Chinese Medicine

Kai Wang

Guangdong Academy of Forestry

Juntong Wu

Nanjing University of Chinese Medicine

Feifei Yang

Nanjing University of Chinese Medicine

Jinyu Yang

Nanjing University of Chinese Medicine

Jiao Chen

Guangdong Academy of Forestry

Qiuyue Tian

Nanjing University of Chinese Medicine

Yan Hua

Guangdong Academy of Forestry

Junfeng Zhang

zhangjunfeng419@njucm.edu.cn

Nanjing University of Chinese Medicine

Research Article

Keywords: Pangolin, Gut microbiota, Metabolomics, Captivity adaptation

Posted Date: October 13th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10901757/v1>

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Additional Declarations: No competing interests reported.

Abstract

This study investigated the underlying gut microbiome and metabolic differences between wild and captive pangolins. 16S rDNA profiling and UPLC-MS/MS metabolomics analysis were conducted on fecal samples from three groups: wild *Manis pentadactyla* ($n = 21$), captive *M. pentadactyla* ($n = 18$), and captive *M. javanica* ($n = 23$). Microbial diversity, community composition, and metabolite profiles were then analyzed. Captive *M. pentadactyla* exhibited higher Simpson diversity and were enriched in cellulose-degrading Firmicutes genera, such as *Romboutsia* and *Terrisporobacter*, whereas wild individuals were enriched in taxa such as *Fusobacterium*. Captive *M. javanica* was characterized by an enrichment of *Lactococcus*. Metabolomics analysis revealed upregulation of metabolites such as spermine and spermidine in captive pangolins, accompanied by downregulation of bioactive natural products, including artemisinin and formononetin. Differential metabolites were associated with host metabolism, diet, microbiota, and environmental factors. These findings demonstrate that captivity restructured the pangolin gut ecosystem and provide a basis for targeted microbiome management to improve the health of captive pangolins.

Introduction

The *Manis pentadactyla* (*M. pentadactyla*) and the *M. javanica*, both belonging to the family Manidae and genus *Manis*, are scaly anteaters primarily distributed in China and Southeast Asia. In recent years, pangolins have become increasingly rare in the wild due to extensive poaching and the global illegal wildlife trade. Both species are currently listed as Critically Endangered on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species and are designated as National First-Class Protected Animals in China [1]. Consequently, artificial rescue and breeding have emerged as crucial conservation measures for these critically endangered species.

As a flagship species in wildlife conservation, the giant panda (*Ailuropoda melanoleuca*) has achieved globally recognized success. Extensive research on its gut microbiota has provided profound insights into its unique dietary adaptations and evolutionary biology, serving as an important reference for the conservation of other rare and endangered animals [2]. Pangolins are highly specialized myrmecophagous mammals. Wild populations primarily feed on various ant and termite species, occasionally consuming insect larvae and other arthropods. In contrast, captive populations face limited dietary options, and artificial diets often fail to meet their nutritional requirements, leading to deficiencies, compromised immunity, and disrupted homeostasis [3]. Therefore, improving dietary composition and establishing scientifically sound nutritional strategies are key to successful captive management and breeding.

Numerous studies have shown that the composition and structure of the gut microbiota are closely associated with host growth, metabolism, phylogeny, ecology, life history, and geographic environment, with diet and phylogeny being the predominant influencing factors [4]. Preliminary studies have indicated that, compared to wild individuals, captive *M. javanica* exhibit higher serum cortisol levels and lower gut

microbial diversity, suggesting that chronic stress associated with captivity may be an important factor shaping their gut microbiota [5]. Comparative metagenomic analyses of wild and captive pangolins have revealed that diet, duration of captivity, and antibiotic exposure can alter gut microbiota structure and abundance. Captive pangolins show enhanced capabilities in degrading chitin and cellulose/hemicellulose, fatty acid metabolism, and short-chain fatty acid synthesis, but a reduced capacity for xenobiotic metabolism. Moreover, captive individuals harbor a higher abundance of antibiotic resistance genes and virulence factors [1, 6]. It was well-established that gut microbiota influences not only the chemical environment of its niche (e.g., the intestine) but also systemic physiology via metabolites, participating in the host's overall functional regulation [7]. Thus, this study investigated wild and captive *M. pentadactyla*, as well as captive *M. javanica*, by integrating gut microbiota and metabolomic analyses. Our aim was to elucidate the nutritional strategies of pangolins under different living conditions and to provide a scientific basis for the development of captive breeding technologies that support their growth, development, and reproduction.

Materials and Methods

Study Subjects and Sample Collection

This study included three groups of pangolins: wild *M. pentadactyla* (*M. pentadactyla* (W), n = 21), captive *M. pentadactyla* (*M. pentadactyla* (C), n = 18), and captive *M. javanica* (*M. javanica* (C), n = 23). All individuals were sourced from wildlife rescue centers and confirmed to be in healthy condition through veterinary examination. Fresh fecal samples were collected aseptically under natural conditions, snap-frozen in liquid nitrogen within 30 min, and stored at – 80°C. All the animal experiment and sample collection procedures were approved by Guangdong Academy of Forestry (007402025-8/10/2025).

Experimental Diets and Animal Husbandry

The experimental diets were formulated based on the reference diets utilized by the Pangolin Conservation Research Center of the National Forestry and Grassland Administration. The specific dietary composition and nutritional levels are detailed in Table S1. In accordance with standard pangolin husbandry regulations, all experimental animals were housed individually throughout the study. Enclosures were cleaned daily and subjected to regular disinfection. The animals were fed once daily at 16:00 with ad libitum access to feed and water. The actual feed intake was recorded at 09:00 the following morning. Throughout the experimental period, the body condition of the animals was continuously monitored, and their fasting body weights were measured at regular intervals.

Gut Microbiota Profiling and Analysis

Following methods described in previous literature [8], 200–500 mg of each fecal sample was used for total RNA extraction utilizing the QIAGEN PowerSoil Pro Kit. The V3–V4 hypervariable region of the

bacterial 16S rRNA gene was amplified by PCR using specific primers. The purified amplicons were subjected to paired-end sequencing on an PacBio platform.

To obtain high-quality clean data, raw sequencing reads were demultiplexed and quality-filtered using fastp. Subsequently, the high-quality sequences were clustered into operational taxonomic units (OTUs) at a 97% sequence similarity threshold utilizing. Alpha diversity, beta diversity, and differential taxon abundance analyses were performed using R software. Finally, PICRUSt2 was employed to predict microbial functional profiles, and the results were mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Untargeted Fecal Metabolomic Analysis

Fecal metabolomics was performed based on previously described methods[9]. Briefly, approximately 100 mg of feces was homogenized in pre-cooled extraction solvent (methanol: water = 4 : 1, containing L-2-chlorophenylalanine as internal standard for quality control). Following centrifugation at 4°C (12,000–13,000 rpm, 20 min), the supernatant was collected, mixed with an equal volume of acetonitrile for protein precipitation, centrifuged again, filtered through a 0.22 µm membrane, and analyzed by UPLC-MS/MS. Peak areas were extracted and retention times aligned. Quality control (QC) samples, prepared by pooling equal volumes from all samples, were used to verify analytical stability (total ion current (TIC) overlap rate ≥ 80%). SIMCA software was used for principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA). Differential metabolites were screened using thresholds of variable importance in projection (VIP) ≥ 1, fold change ≥ 1.5 or ≤ 0.67, and $P < 0.05$. Metabolites were identified by matching against the HMDB, METLIN, and other databases. Metabolic pathway enrichment was predicted using Metabo Analyst.

Metabolic Source Analysis

A systematic tracing approach was employed to determine the origins of differential metabolites. First, based on untargeted metabolomics identifications, chemical properties and biosynthetic backgrounds of metabolites were verified by querying HMDB, KEGG, PubChem, and relevant literature. Subsequently, according to known metabolic pathways and biological source evidence, metabolites were classified into four categories: host-derived metabolites, diet- or plant-related components, microbial metabolites, and exogenous exposure compounds. A Sankey diagram was generated using the Sangerbox cloud platform to visually illustrate the compositional contributions of metabolites from different sources, thereby systematically clarifying the multi-origin characteristics of metabolic changes in captive pangolins.

Statistical Analysis

Data were presented as means ± SEM The plotting and statistical analysis were conducted in GraphPad Prism 9 and R software (version 3.6.2). The Mann-Whitney U test was applied for non-normally

distributed data, while, Student's t-test was used for normally distributed data. P -value < 0.05 was considered statistically significant.

Results

Analysis of Gut Microbiota Diversity in Pangolins

Alpha diversity was assessed using Chao1, observed OTUs, Shannon, and Simpson indices. Compared with captive *M. pentadactyla*, captive *M. javanica* exhibited significantly lower Chao1 and observed OTUs (Fig. 1A, B; $P < 0.05$), indicating markedly lower gut microbial richness in *M. javanica*. No statistically significant differences were found in Shannon index among the three groups (Fig. 1C; $P > 0.05$). However, the Simpson index was significantly higher in captive *M. pentadactyla* than in wild conspecifics (Fig. 1D; $P < 0.05$), suggesting that captivity reduced microbial evenness/diversity in this species. Consistently, analysis of the captive-only subset (Figure S1) further confirmed that captive *M. javanica* had significantly lower Chao1 and observed OTUs than captive *M. pentadactyla* ($P < 0.01$), while Shannon and Simpson indices showed no differences, reinforcing the species-specific richness difference.

For beta diversity, principal coordinate analysis (PCoA) based on Bray–Curtis distance was performed. In the overall comparison (Fig. 1E), samples from the three groups—wild *M. pentadactyla*, captive *M. pentadactyla*, and captive *M. javanica*—formed distinct clusters. The first two principal coordinates explained 33.9% and 14.57% of the total variation, respectively. Wild and captive *M. pentadactyla* were partially separated, indicating that captivity also shaped the community composition. More importantly, captive *M. javanica* and captive *M. pentadactyla* were clearly segregated, suggesting strong species-level differences in microbial community structure. This separation was further supported by the two-group PCoA of captive individuals (Figure S1), where PC1 and PC2 accounted for 25.51% and 16.74% of the variation, respectively, and the two species formed well-separated ellipses. Taken together, these results demonstrate that both host species and captivity significantly influence the diversity and community composition of gut microbiota.

Analysis of Gut Microbiota Composition in Pangolins

Phylogenetic analysis of the pangolin gut microbiota revealed that the 100 most widespread genera belonged to nine phyla. Among these, the phylum Firmicutes comprised 55 genera, including *Romboutsia*, *Blautia*, and *Terrisporobacter*, etc. while the phylum Proteobacteria contained 24 genera, such as *Escherichia-Shigella* and *Morganella* (Fig. 2A). Linear Discriminant Analysis (LDA) indicated that wild *M. pentadactyla* were enriched with seven bacterial taxa, including Fusobacteriota, *Fusobacterium*, and *Epulopiscium*. In contrast, captive *M. pentadactyla* were enriched with 10 bacterial taxa, such as Firmicutes, *Romboutsia*, *Clostridium sensu stricto 1*, *Terrisporobacter*, and *Cellulosilyticum*. Captive *M. javanica* was enriched with three bacterial taxa, including *Lactococcus* (Fig. 2B). Compared with wild *M. pentadactyla*, captive individuals showed significantly increased abundance of Firmicutes and significantly decreased abundance of Proteobacteria.

Further analysis showed that compared to wild *M. pentadactyla*, captive *M. pentadactyla* exhibited significantly increased abundances of eight genera, including *Clostridium sensu stricto* 13, *Paraclostridium*, *Paeniclostridium*, *Turicibacter*, *Romboutsia*, *Terrisporobacter*, *Epulopiscium*, and *Cellulosilyticum* ($P < 0.05$). Conversely, the abundances of four genera (*Escherichia-Shigella*, *Morganella*, *Blautia*, and *Roseburia*) were significantly decreased ($P < 0.05$). When compared to captive *M. pentadactyla*, captive *M. javanica* showed significantly higher abundances of *Escherichia-Shigella* and *Lactococcus* ($P < 0.05$), while the abundances of *Paeniclostridium*, *Turicibacter*, *Lachnoclostridium*, and *Epulopiscium* were significantly lower ($P < 0.05$) (Fig. 2C). The data suggested that the captive environment has a certain influence on gut microbiota.

Differential Analysis of Fecal Metabolite Profiles Between Wild and Captive *M. pentadactyla*

Metabolites are direct functional products of gut microbiota activity. Untargeted metabolomics analysis revealed significant differences in fecal metabolites between wild and captive *M. pentadactyla* (Fig. 3A), a finding further validated by model stability assessment (Fig. 3B). Differential analysis of fecal metabolites showed that, in negative ion mode, captive *M. pentadactyla* exhibited significant upregulation of 20 metabolites—including L-cysteinesulfinic acid, Gamma-caprolactone, 3-methyladipic acid, punicic acid, and formononetin—compared to wild individuals. Conversely, 20 metabolites, such as 1-naphthyl acetic acid, Arachidic acid, and Anacardic acid, were significantly downregulated (Fig. 3C). In positive ion mode, captive *M. pentadactyla* displayed significant upregulation of 20 metabolites, including spermine, spermidine, and 1-methylguanine, whereas 20 metabolites, such as ferutinin, artemisinin, vitamin A, and erucic acid, were significantly downregulated compared to wild individuals (Fig. 3D). These results suggest that captivity induces substantial changes in the intestinal metabolism of *M. pentadactyla*, consistent with the observed alterations in their gut microbiota composition.

Differential Analysis of Fecal Metabolites Between Captive *M. pentadactyla* and *M. javanica*

Principal component analysis revealed significant differences in fecal metabolites between captive *M. pentadactyla* and *M. javanica* (Fig. 4A), which was further confirmed by model stability validation (Fig. 4B). Compared to captive *M. javanica*, in negative ion mode, captive *M. pentadactyla* exhibited significant upregulation of metabolites such as L-cysteinesulfinic acid, gamma-caprolactone, 3-methyladipic acid, 6-aminonicotinamide, gamma-Glu-Leu, undecanedioic acid, formononetin, corchorifatty acid F, and N-formylglycine. Conversely, metabolites including docosanoic acid, sodium cholate, anacardic acid, and gibberellin A4 were significantly downregulated (Fig. 4C). In positive ion mode, metabolites such as spermine, spermidine, 1-methylguanine, and stercobilin were significantly upregulated in captive *M. pentadactyla*, while metabolites including 4-deacetylneosalniol, spiculisporic acid, cafestol, vitamin A, and artemisinin were significantly downregulated (Fig. 4D). These results suggest that differences in fecal metabolites may reflect species-specific or dietary variations between *M. pentadactyla* and *M. javanica*.

Impacts of Captivity on Gut Metabolites in *M. pentadactyla* and *M. javanica*

A total of 763 differential metabolites were identified between wild and captive pangolins, while 383 differential metabolites were observed between captive *M. pentadactyla* and *M. javanica*. Through intersection analysis, 83 shared differential metabolites were identified to elucidate the effects of captivity on the gut metabolome of pangolins. Based on abundance, these metabolites were categorized into three groups.

Among the 27 high-abundance differential metabolites, captivity led to increased abundance of 18 metabolites, including corchorifatty acid F, isobutyryl carnitine, thromboxane B2, hydroxyproline, cyclohexaneacetic acid, and azelaic acid, whereas the abundance of 9 metabolites decreased, including cholesterol, DL- α -methoxyphenylacetic acid, 20-carboxy-leukotriene B4, L-tryptophan, galacturonic acid, desoxycortone, prostaglandin K1, and artemisinin (Fig. 5A). Of the 28 medium-abundance differential metabolites, captivity resulted in elevated abundance of 23 metabolites, such as oxymorphone, tryptoline, undecanedioic acid, Gly-Tyr-Ala, punicic acid, and tetrahydroaldosterone, while five metabolites exhibited reduced abundance, including bilirubin, o-veratraldehyde, 5-phenylvaleric acid, dihydrosphingosine, and biliverdin (Fig. 5B). Among the 28 low-abundance differential metabolites, captivity induced increased abundance of 23 metabolites, including L-histidine, benzathine, 5-hydroxy-L-lysine, S-(methyl)glutathione, 2-aminobenzenesulfonic acid, 3-chloro-L-tyrosine, senecionine, cycloheximide, and L-anserine, whereas the abundance of three metabolites, including 1-stearoylglycerol and 3-methylglutaric acid, decreased (Fig. 5C). These differential metabolites provide novel insights for further investigating the metabolic mechanisms underlying environmental adaptation in captive pangolins.

Source Analysis of Intestinal Metabolites in Pangolins

Based on the 83 shared fecal differential metabolites identified among wild *M. pentadactyla*, captive *M. pentadactyla*, and captive *M. javanica*, traceability analysis revealed that these metabolites originated primarily from six pathways: host metabolism, diet, microorganisms, diet–microbial interactions, microbial–host interactions, and environmental factors (Fig. 6).

These results indicate that the metabolic patterns of pangolins under captive conditions are influenced by multiple factors, including dietary composition, intestinal microbiota, and environmental exposure.

Correlation Analysis Between Gut Microbiota and Metabolites in Pangolins

Using the top 50 intestinal bacterial genera based on relative abundance and the 83 sharing differential metabolites, a gut microbiota-metabolite network was constructed. The results revealed 211 significantly negative correlations ($r < -0.25$, $P < 0.05$) and 146 significantly positive correlations ($r > 0.25$, $P < 0.05$) between bacterial genera and metabolites (Fig. 7).

Among the four differential bacterial genera between wild and captive *M. pentadactyla*: *Terrisporobacter* showed significant positive correlations with artemisinin ($r = 0.349$) and bilirubin ($r = 0.504$), while

significant negative correlations with isobutyryl carnitine ($r = -0.326$) and punicic acid ($r = -0.325$). *Cellulosilyticum* was significantly positively correlated with bilirubin ($r = 0.418$) and significantly negatively correlated with methyl nicotinate ($r = -0.328$). *Escherichia-Shigella* exhibited significant positive correlations with thromboxane B2 ($r = 0.323$) and oxymorphone ($r = 0.341$), and significant negative correlations with 3-methylglutaric acid ($r = -0.396$), bilirubin ($r = -0.365$), and artemisinin ($r = -0.341$). *Romboutsia* showed a significant positive correlation with bilirubin ($r = 0.402$) and significant negative correlations with 2-aminobenzenesulfonic acid ($r = -0.329$), methyl nicotinate ($r = -0.372$), and isobutyryl carnitine ($r = -0.360$). These findings suggest that dietary differences between wild and captive conditions shape distinct gut microbiota compositions and associated metabolic functions.

Among the four differential gut bacterial genera between captive *M. pentadactyla* and *M. javanica*, *Epulopiscium* showed significant positive correlations with artemisinin ($r = 0.457$) and cholesterol ($r = 0.328$), and significant negative correlations with corchorifatty acid F ($r = -0.576$) and punicic acid ($r = -0.558$). *Lachnoclostridium* was significantly positively correlated with 20-carboxy-leukotriene B4 ($r = 0.322$) and cyclohexaneacetic acid ($r = 0.327$), and significantly negatively correlated with bilirubin ($r = -0.361$) and DL- α -methoxyphenylacetic acid ($r = -0.333$). *Paeniclostridium* exhibited significant negative correlations with isobutyryl carnitine ($r = -0.347$), mirtazapine-d3 ($r = -0.343$), and methyl nicotinate ($r = -0.333$). *Lactococcus* demonstrated significant positive correlations with mirtazapine-d3 ($r = 0.408$), benzathine ($r = 0.441$), and Gly-Tyr-Ala ($r = 0.350$), and a significant negative correlation with 20-carboxy-leukotriene B4 ($r = -0.375$). These results suggest that under the same captive conditions, differences in gut microbiota and metabolic functions between *M. pentadactyla* and *M. javanica* may arise from species-specific adaptive evolution.

Discussion

As the most heavily trafficked mammals globally, pangolins face an acute conservation crisis, making successful captive breeding and population restoration an urgent priority [1]. However, the long-term viability of captive populations remains severely constrained by a profound knowledge gap regarding their digestive physiology and species-specific nutritional requirements [10, 11]. Current captive management practices rely heavily on empirical dietary formulations that often fail to replicate the complex nutritional ecology of their wild, myrmecophagous diet. Consequently, captive pangolins suffer from recurrent health issues, including metabolic disorders, immunological compromise, and gastrointestinal dysbiosis [5]. While recent studies have begun to characterize the gut microbiome of captive pangolins, most have focused on taxonomic shifts without integrating functional metabolic outcomes. Furthermore, comparative investigations across species and rearing conditions remain scarce [1, 6, 12]. This paucity of integrated microbe–metabolite data hinders our ability to decipher the mechanistic links between captivity-induced dysbiosis and physiological maladaptation [1]. Because the gut microbiota functions as a critical “metabolic organ” bridging diet, immunity, and host energy homeostasis [9, 10, 13], it offers a uniquely sensitive window into these challenges. By systematically comparing the gut microbiota and fecal metabolome of wild *M. pentadactyla*, captive *M. pentadactyla*, and captive *M. javanica*, this study aims to delineate the captivity-driven restructuring of the gut

ecosystem and provide mechanistic insights into the metabolic vulnerabilities underpinning the poor captive performance of these threatened species.

Analysis of gut microbiota composition revealed that wild *M. pentadactyla* exhibited significant enrichment of *Fusobacteriota* and *Fusobacterium*. Specific species within *Fusobacterium*, such as *Fusobacterium mortiferum*, produce metabolites like 5-aminovaleric acid (5-AVA) that directly regulate host metabolic pathways[10], confirming the functional activity of this genus under high-nutrient dietary conditions. *Fusobacterium* is associated with diets rich in protein and animal fats [10], likely reflecting the high protein and lipid content of fresh ants and termites consumed in the wild population [10]. In contrast, captive *M. pentadactyla* showed enrichment of several Firmicutes genera, including *Romboutsia*, *Terrisporobacter*, and *Cellulosilyticum*. Existing studies have demonstrated that these genera are involved in the degradation of complex plant polysaccharides such as cellulose and hemicellulose, as well as in the synthesis of short-chain fatty acids [1, 13], which may be linked to the inclusion of fiber-rich components like apple and dried hawthorn in artificial diets. Captive *M. javanica* was notably enriched in *Lactococcus* and *Escherichia-Shigella*. Previous research suggests that *Lactococcus*, as a common lactic acid bacterium, may reflect a unique fermentation pattern of carbohydrates or specific protein components in the artificial diet [14]. Therefore, captivity may shift the gut microbiota of pangolins from a wild-type community specialized in metabolizing animal-derived proteins and lipids toward one enriched with bacteria adapted to utilizing plant-based fiber components in artificial diets. This adaptive shift in captivity may also be accompanied by a reduction in certain beneficial bacteria, such as the butyrate-producing genus *Roseburia*, potentially weakening intestinal barrier function [15].

Fecal metabolomics further corroborated captivity-associated alterations in intestinal metabolic patterns, with numerous differential metabolites implicated in multiple signaling pathways including amino acid metabolism, lipid metabolism, bile acid metabolism, and plant secondary metabolites. For instance, the upregulation of polyamines such as spermine and spermidine in the intestines of captive pangolins may reflect active intestinal epithelial repair and mucosal immune cell differentiation [16]. Notably, compared to wild *M. pentadactyla*, natural products with anti-inflammatory and antioxidant potential, such as artemisinin, formononetin, and anacardic acid, were significantly downregulated in captive pangolins. Artemisinin is a well-known antimalarial drug, and these metabolites are likely directly or indirectly derived from ants, termites, or associated plant materials consumed by wild pangolins [11]. However, the artificial diet provided to captive pangolins lacks these plant-derived bioactive compounds, potentially increasing their susceptibility to parasitic infections, oxidative stress, and immune dysregulation [17].

Through traceability analysis of the 83 common differential metabolites, it was found that most metabolites originated from the diet. For example, glycolithocholic acid is derived from ants, and cholesterol is derived from ants and apples. Captive pangolins exhibited elevated levels of isobutyryl carnitine and significantly reduced levels of L-tryptophan in their intestines [18, 19]. Studies have shown that the accumulation of isobutyryl carnitine indicates a shift in energy metabolism toward inefficient protein catabolism, accompanied by mitochondrial stress and metabolic suppression of immune cells,

particularly T cells [20]. The depletion of L-tryptophan reflects the activation of the immunosuppressive kynurenine pathway and a weakening of the intestinal barrier function [21, 22]. This suggests that captive pangolins may be in a state of stress-induced physiological imbalance, characterized by reduced energy production efficiency, suppressed immune defense, impaired intestinal barrier integrity, and potential neurobehavioral dysregulation via the tryptophan-serotonin axis. These factors may collectively contribute to the risk of a vicious cycle of “metabolic-immune exhaustion” [5, 23]. Consequently, future efforts in diet formulation could benefit from exploring the inclusion of functional components, such as plant secondary metabolites and specific fatty acids [24, 25]. Such targeted adjustments may help provide more sustainable nutritional support for the captive conservation and population restoration of pangolins.

Analysis of the microbiota-metabolite association network revealed that *Terrisporobacter*, which was enriched in captive individuals, showed a positive correlation with artemisinin and a negative correlation with isobutyryl carnitine. Isobutyryl carnitine is considered a marker of lower metabolic stress in the host [12], suggesting that *Terrisporobacter* may thrive in intestinal environments with reduced catabolic pressure [26, 27]. Furthermore, in wild *M. pentadactyla*, the abundance of the opportunistic pathogen *Escherichia-Shigella* was significantly positively correlated with the inflammatory molecule thromboxane B2. This relationship may reflect controlled infection stimuli and active immune defense mechanisms in the wild [26, 27]. These findings indicate that targeted modulation of the gut microbiota represents a potential strategy for improving metabolic health in pangolins.

In summary, this study preliminarily reveals the profound impacts of captivity on the gut microbiota and metabolism of pangolins. Specifically, wild-enriched bacterial genera associated with animal protein and lipid metabolism, such as *Fusobacterium*, are replaced by Firmicutes genera involved in plant cellulose degradation, including *Romboutsia*. Concurrently, intestinal endogenous anti-inflammatory substances (e.g., artemisinin) are depleted, while markers of energy metabolism stress (e.g., isobutyryl carnitine) accumulate [28, 29]. These changes collectively indicate that captivity may force a shift in pangolin intestinal function from a carnivorous-adapted metabolic pattern aligned with wild dietary habits to a “herbivorous-adapted” metabolic pattern centered on plant fiber utilization [30, 31]. However, this adaptive transition appears to be associated with a reduction in natural anti-inflammatory substances and altered energy metabolism, which may predispose captive individuals to metabolic and immune challenges. Consequently, exploring an ecological niche simulation strategy for nutritional intervention could be a beneficial approach. Potential strategies might include: (1) incorporating plant-derived anti-inflammatory components (e.g., flavonoids and polyphenols) to partially mimic the natural dietary defenses; (2) optimizing feed formulations with prebiotics (e.g., fructooligosaccharides) to support beneficial, short-chain fatty acid-producing bacteria; and (3) gradually developing probiotic supplements tailored to their natural ant-eating habits. By implementing these targeted, biologically informed interventions, it may be possible to alleviate metabolic stress and support immune resilience [32]. Ultimately, these strategies could contribute to improving the survival and reproductive success of captive pangolins, thereby helping to establish a robust foundation for ex situ conservation and eventual

population restoration. We believe that these measures may provide a sustainable and precise management system for the captive conservation and population restoration of pangolins.

Conclusion

This integrated multi-omics study systematically delineates the captivity-induced restructuring of the gut ecosystem in Chinese (*Manis pentadactyla*) and Malayan (*M. javanica*) pangolins. Captive *M. pentadactyla* exhibited increased Simpson diversity and significant enrichment of cellulose-degrading Firmicutes genera (*Romboutsia*, *Terrisporobacter*, *Cellulosilyticum*), while wild conspecifics were dominated by *Fusobacterium* associated with animal protein/lipid metabolism. Captive *M. javanica* was characterized by enrichment of *Lactococcus*. Metabolomic profiling revealed that captive pangolins accumulated stress-related metabolites (spermine, spermidine, isobutyryl carnitine) alongside marked depletion of bioactive natural products with anti-inflammatory and antioxidant properties (artemisinin, formononetin, anacardic acid). Source-tracing analysis attributed most differential metabolites to dietary, microbial, and host-derived pathways, with correlation networks linking specific bacterial genera to key metabolic alterations. Collectively, these findings demonstrate that captivity shifts pangolin intestinal function from a carnivorous-adapted metabolic pattern toward a plant-fiber-based metabolism, but this adaptive transition is accompanied by metabolic stress, compromised immune resilience, and impaired intestinal barrier integrity. These results provide a mechanistic framework for understanding captivity-induced physiological maladaptation in pangolins and highlight that optimizing artificial diets—through supplementation with plant-derived anti-inflammatory compounds, prebiotics, and specific fatty acids—represents a promising strategy to alleviate metabolic burden, restore gut homeostasis, and enhance immune function. Such targeted nutritional interventions hold significant potential for improving the health, welfare, and reproductive success of captive pangolins, thereby contributing to more effective ex situ conservation and population recovery of these critically endangered species.

Declarations

Funding

This work was supported by the National Key R&D Program of China (No. 2025YFC3507700), Guangdong Basic and Applied Basic Research Foundation (2024A1515012617).

Ethics approval and consent to participate: All the animal experiment and sample collection procedures were approved by Guangdong Academy of Forestry Institutional Animal Care and Use Committee (007402025-8/10/2025).

Consent for publication Not applicable.

The Authors declare no competing interests.

Disclaimer The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Contributor Information

Y.H and J.Z designed the study. F. Z and K. W were responsible for the microbial and metabolomic testing K. W and Y.H collected and restored the fecal samples, F. Z performed the experimental methodology and generated the visualizations. F. Z, J. W and F. Y analyzed the data and prepared the manuscript. F. Z, J. Y, J. C and Q. T prepared the information and deposited the raw data in the public website. J. Z, Y.H and K. W revised the manuscript. All authors read and approved the submitted manuscript.

Data availability

Sequencing data was deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA040169) that are publicly accessible at <https://ngdc.cncb.ac.cn/gsa>. can be found in online repositories.

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Figures

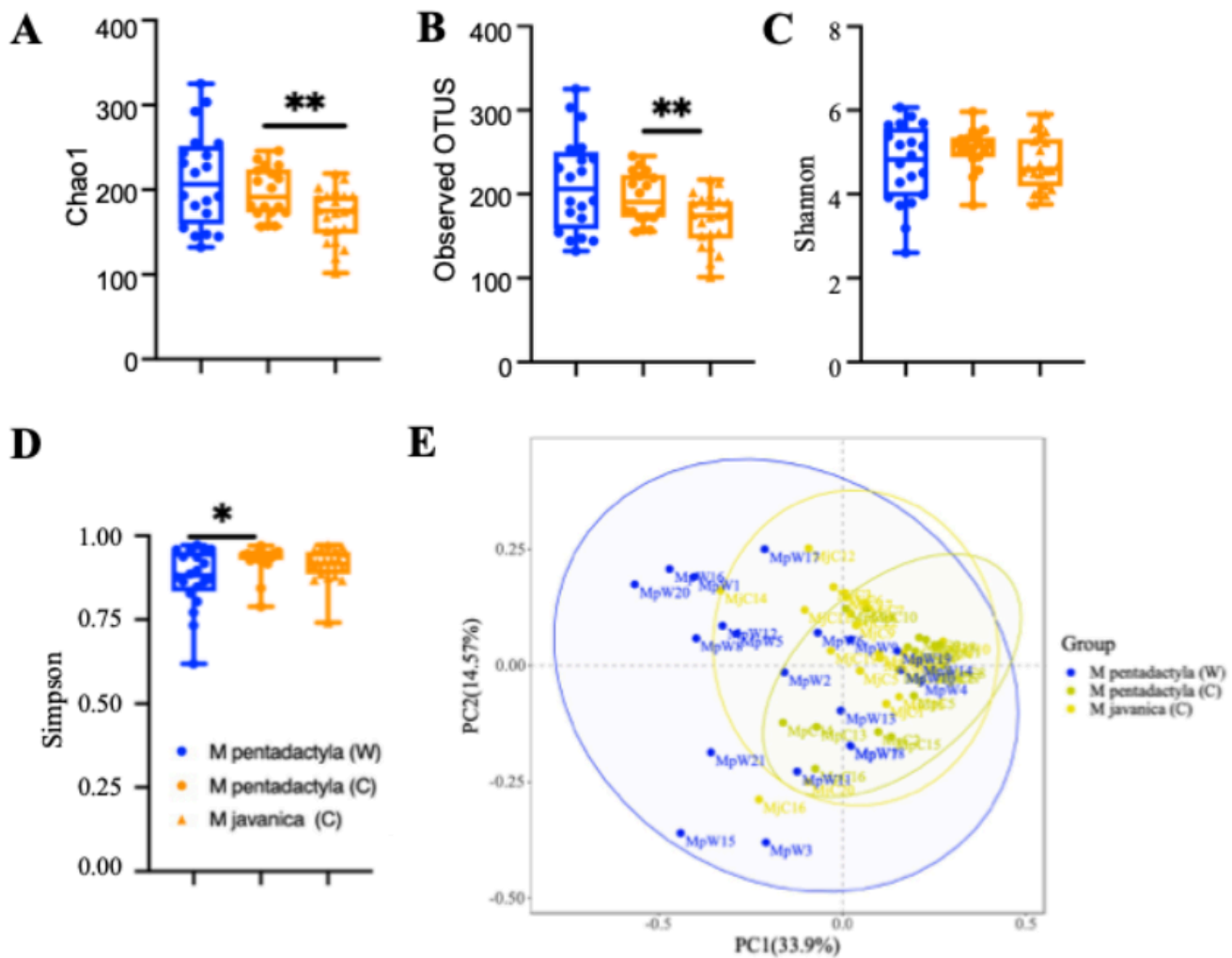


Figure 1

Diversity analysis of gut microbiota in pangolins

(A) Chao1 richness index. (B) Observed OTUs richness index. (C) Shannon diversity index. (D) Simpson diversity index. (E) Principal coordinate analysis (PCoA) based on Bray-Curtis distances, with the first two principal coordinates explaining 33.9% and 14.57% of the total variation, respectively. Each point represents an individual sample, and ellipses indicate 95% confidence intervals for each group. OTU, operational taxonomic unit; PCoA, principal coordinate analysis. * $P < 0.05$, ** $P < 0.01$

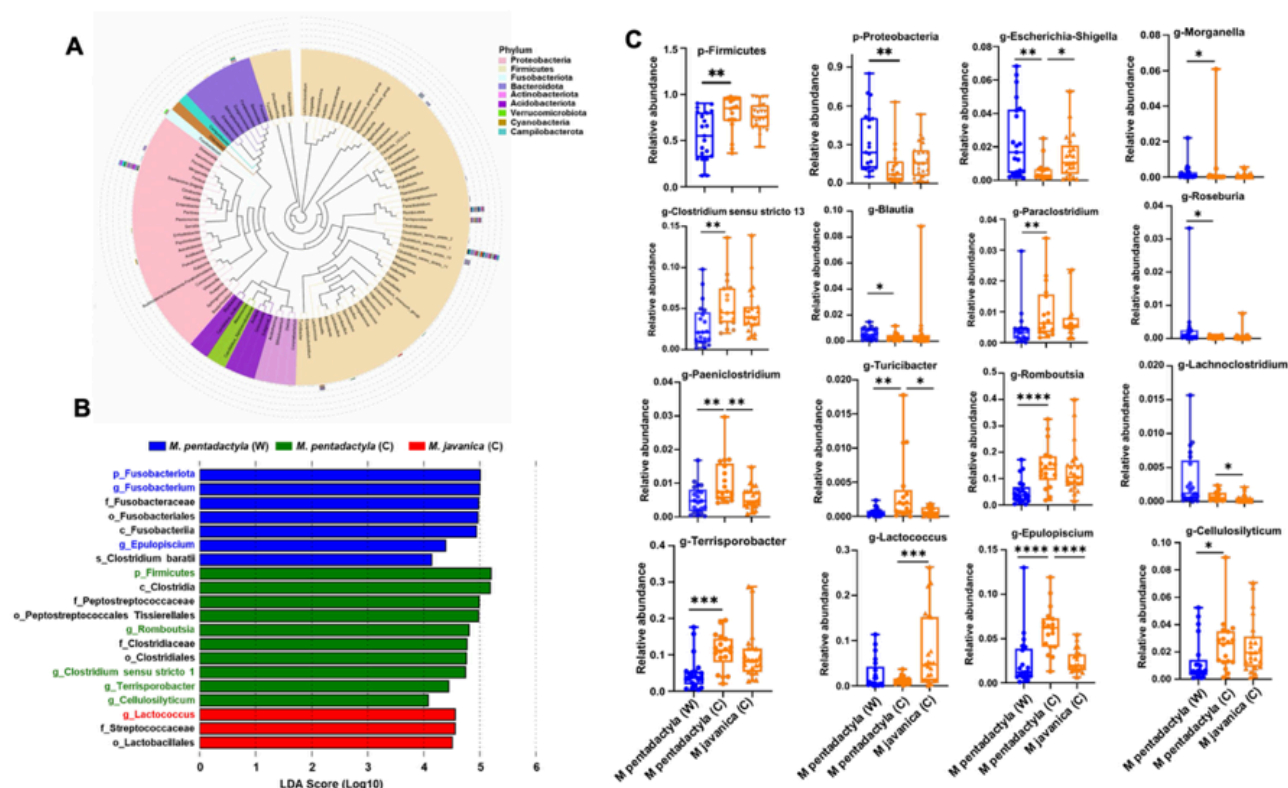


Figure 2

Differential analysis of gut microbiota between *M. pentadactyla* and *M. javanica*

(A) Phylogenetic distribution of the 100 most abundant bacterial genera (based on relative abundance) across all samples. (B) Analysis of gut marker microbiota in wild *M. pentadactyla*, captive *M. pentadactyla*, and captive *M. javanica*. (C) Analysis of representative differential bacterial phyla and genera in the gut among the three pangolin populations. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

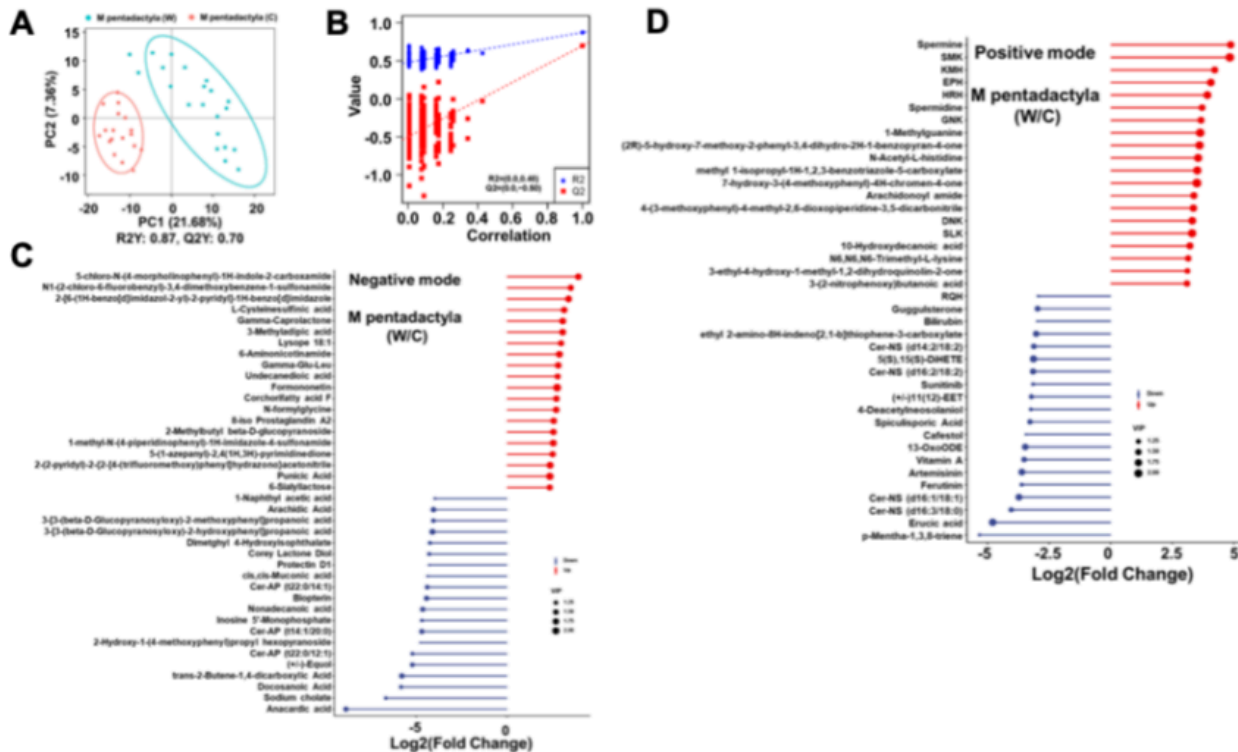


Figure 3

Differential analysis of fecal metabolites between wild and captive *M. pentadactyla*

(A) PLS-DA analysis of fecal metabolites in wild and captive *M. pentadactyla*. (B) Validation analysis of differences in fecal metabolites between wild and captive *M. pentadactyla*. (C) Differential metabolite molecules in the feces of wild and captive *M. pentadactyla* under anion mode. (D) Differential metabolite molecules in the feces of wild and captive *M. pentadactyla* under cation mode.

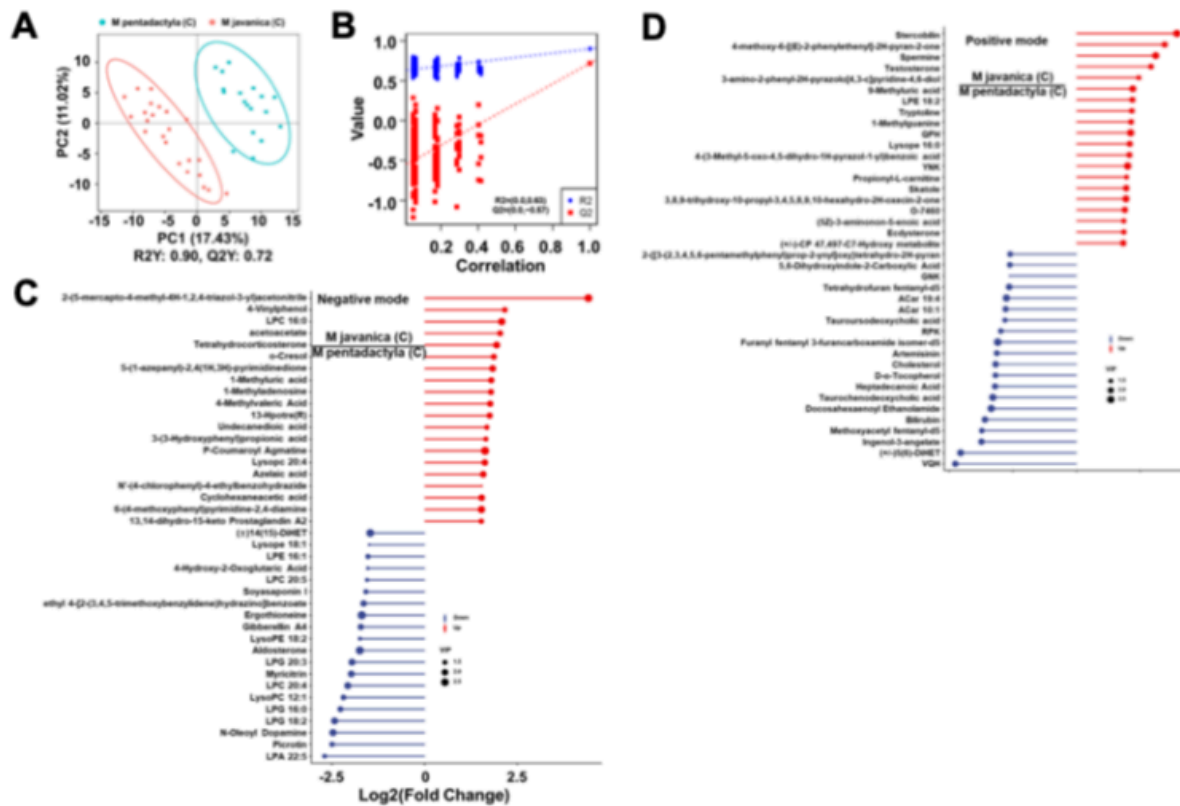


Figure 4

Differential analysis of fecal metabolites between captive *M. pentadactyla* and captive *M. javanica*

(A) PLS-DA analysis of fecal metabolites in captive *M. pentadactyla* and captive *M. javanica*. (B) Validation analysis of differences in fecal metabolites between captive *M. pentadactyla* and captive *M. javanica*. (C) Differential metabolite molecules in the feces of captive *M. pentadactyla* and captive *M. javanica* under negative ion mode. (D) Differential metabolite molecules in the feces of captive *M. pentadactyla* and captive *M. javanica* under positive ion mode.

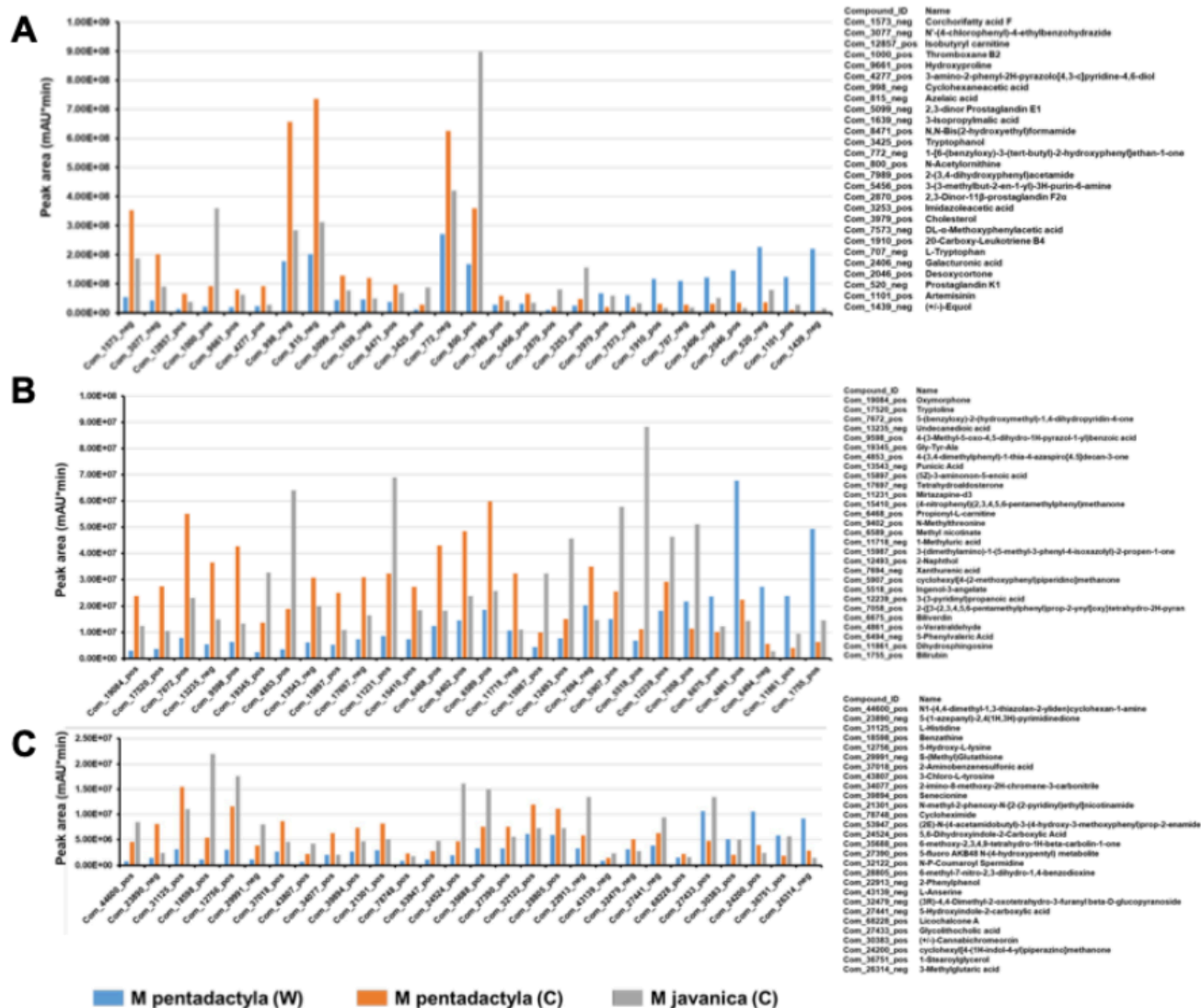


Figure 5

Effects of captivity on fecal metabolites in *M. pentadactyla* and *M. javanica*

Based on the differential fecal metabolites between wild and captive *M. pentadactyla*, as well as between captive *M. pentadactyla* and captive *M. javanica*, 83 common differential metabolites were identified. These metabolites were classified into high-abundance, medium-abundance, and low-abundance groups according to their total abundance. (A) Among the 27 high-abundance differential metabolites, 18 were elevated and 9 were reduced in the feces of captive pangolins. (B) Among the 28 medium-abundance differential metabolites, 22 were elevated and 6 were reduced in the feces of captive pangolins. (C) Among the 28 low-abundance differential metabolites, 23 were elevated and 5 were reduced in the feces of captive pangolins.

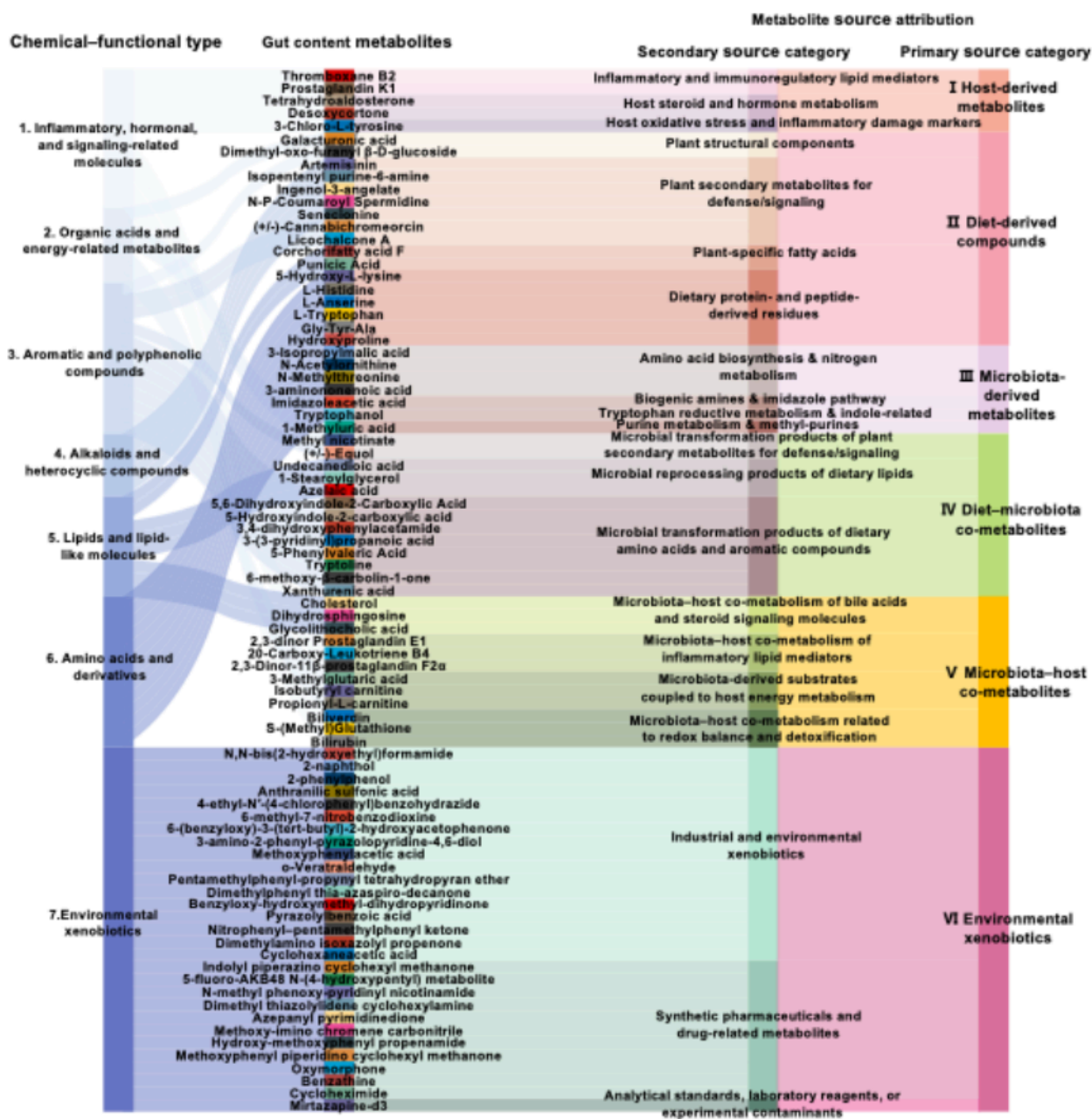


Figure 6

Metabolite traceability analysis of fecal differences in pangolins

This four-layer diagram shows the attribution of gut metabolites to their potential origins. Based on primary source, metabolites are classified into four groups: (I) host-derived (steroid/hormone metabolism, oxidative stress markers), (II) diet-derived (plant components, fatty acids, protein/peptide residues), (III) microbiota-derived (biogenic amines, tryptophan metabolites, purines, microbial transformations), and (IV) diet-microbiota co-metabolites plus environmental xenobiotics (bile acid/amino acid co-metabolites, redox compounds, contaminants, pharmaceuticals). Flow widths reflect

coefficient (r) is greater than 0.25 and $P < 0.05$; ** indicates that the absolute value of the correlation coefficient (r) is greater than 0.3 and $P < 0.01$.

Supplementary Files

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