Research Article

Gut Microbiota Composition of Indigenous Pigs from North Sulawesi Revealed by 16S rRNA Metagenomics

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Abstract | Indigenous pigs constitute a significant genetic resource for food security and cultural legacy; nevertheless, understanding of their gut microbiome diversity is still inadequate. This study sought to define and analyze the gut microbial makeup of indigenous pigs from several regions of North Sulawesi and to assess its possible consequences for health, adaption, and productivity. A 16S rRNA metagenomic methodology was utilized to characterize gut microbial communities in three populations: Minahasa, Kepulauan, and Bolaang Mongondow. Relative abundance data were examined at the phylum, class, family, and genus levels and represented through histograms, ternary plots, and UPGMA clustering utilizing both weighted and unweighted UniFrac distances. The findings indicated notable disparities in microbial composition across populations, with Gammaproteobacteria, Bacilli, and Clostridia prevailing at the class level, and families including Enterobacteriaceae, Ruminococcaceae, and Lachnospiraceae linked to essential metabolic functions, encompassing carbohydrate fermentation and polysaccharide degradation. UPGMA analysis grouped samples based on their geographical origin, indicating the impact of environmental and nutritional factors on microbial community composition. This study presents the inaugural comprehensive map of gut microbiota composition in indigenous pigs from North Sulawesi, emphasizing its ecological and adaptive importance. Subsequent study ought to integrate functional metagenomics with dietary intervention trials utilizing local feed supplies to maximize gut health and improve the productivity of indigenous pig populations.

Keywords | Gut microbiota, Indigenous, Pigs, North Sulawesi, 16S rRNA, Metagenomics

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INTRODUCTION

Recent advances in metagenomic sequencing technologies have enabled comprehensive exploration of microbial diversity at unprecedented resolution. Metagenomic studies have shown that gut taxonomic composition, functional potential (e.g., carbohydrate-active enzymes and short-chain fatty acid pathways) (Xu et al., 2022; Holman et al., 2022), resistome, and virome are modulated by host genetics (Mi et al., 2024), diet, age, and environment, with direct consequences for disease susceptibility and feed conversion (St-Pierre et al., 2023; Zhang et al., 2025). North Sulawesi local pigs exhibit high genetic diversity based on Cytochrome Oxidase Subunit 1 (CO1) markers (Mege et al., 2025), yet their gut microbial diversity and abundance remain poorly understood.

Figure 1: Workflow of metagenomic analysis of the intestinal microbiome of North Sulawesi local pigs.

Most metagenomic surveys focus on commercial pig breeds in temperate regions, leaving indigenous and ecologically adapted pigs underrepresented in global catalogs (Mege et al., 2022; Pius et al., 2024; Benjamin et al., 2023). These breeds often harbor unique microbial consortia that contribute to ecological adaptation and production traits. Deep shotgun sequencing and metagenome-assembled genome (MAG) analyses have revealed vast numbers of previously undescribed bacterial and viral genomes in pigs, suggesting that local breeds may retain distinct microbial signatures absent from current databases (Yang et al., 2025; Li et al., 2023).

Advances in integrative metagenomics now allow taxonomic profiling (16S/shotgun), functional annotation (HUMAnN, CAZy, KEGG), and strain-level reconstruction (MAGs), enabling stronger cross-population comparisons (Lema et al., 2023). Such multi-level approaches are powerful for identifying ecological drivers of community structure and microbial markers linked to host adaptation or performance (Yang et al., 2024). Here, we used high-throughput metagenomics to characterize and compare gut microbiomes of three geographically and ecologically distinct populations of local pigs in North Sulawesi (Minahasa, Island, and Bolaang Mongondow). Specifically, we compared taxonomic composition and microbial diversity among pigs from three geographical regions. By focusing on uncharacterized local breeds, this work fills a critical knowledge gap and expands the reference catalog of the porcine gut microbiome.

The findings of this study are expected to contribute to a better understanding of indigenous pig gut microbiota and support future nutritional and health management strategies. These insights inform management and conservation strategies and suggest opportunities for targeted interventions, such as dietary modulation, probiotics, or selective breeding, to improve resilience and productivity. More broadly, this study contributes to global efforts to build a comprehensive porcine microbiome reference and to translate metagenomic knowledge into sustainable livestock applications.

MATERIALS AND METHODS

Sample collection

Local pig samples were obtained from three regions of North Sulawesi: Minahasa (A), Island (B), and Bolaang Mongondow (C). Intestinal fluid was collected for metagenomic analysis. The pigs were identified as local breeds based on phenotypic and morphological characteristics, as well as lineage tracing, to ensure the authenticity of North Sulawesi local pigs.

Experimental procedures

Metagenomic analysis followed a standard workflow comprising: Genomic DNA extraction from intestinal fluid, PCR amplification, quantification and pooling of PCR products, purification, library preparation, sequencing, and bioinformatics analysis (Bekele et al., 2025; Liu et al., 2021) (Figure 1).

Genomic DNA extraction and purification

Genomic DNA from intestinal fluid was extracted using the ZymoBIOMICS™ DNA/RNA Miniprep Kit (Zymo Research, USA) following the manufacturer's guidelines. The concentration and purity of DNA were evaluated using a NanoDrop-1000 spectrophotometer and a PicoGreen test.

PCR amplification, library construction, quality control, and sequencing

The V4 region of the bacterial 16S rRNA gene was amplified employing primers 515F(5′-GTGCCAGCMGCCGCGGTAA) and 806R (5′-GGACTACHVGGGTWTCTAAT) with dual indices under defined conditions: Thirty cycles at an annealing temperature of 56 °C. Amplicons were detected using 2% agarose gel electrophoresis, and products of the expected size were selected. Equal quantities of PCR products from each sample were combined, end-repaired, A-tailed, and ligated using Illumina adapters. Libraries underwent quality assessment and sequencing on the Illumina MiSeq technology (Illumina, USA) to produce 250 bp paired-end raw reads (Figure 2).

Libraries were determined utilizing a Qubit fluorometer and real-time PCR, while fragment size distributions were evaluated with a Bioanalyzer. Qualified libraries were aggregated according to effective concentration and sequenced on the Illumina platform to meet the specified data output requirements.

Bioinformatics analysis

Paired-end readings were demultiplexed based on unique barcodes, and the barcode and primer sequences were

removed. readings were amalgamated utilizing FLASH v1.2.7 (http://ccb.jhu.edu/software/FLASH/), which precisely concatenates overlapping paired-end readings. The resultant sequences were designated as raw tags. Quality filtering of raw tags was executed utilizing the QIIME v1.7.0 pipeline [16] (http://qiime.org/scripts/split_libraries_fastq.html) under rigorous criteria to acquire high-quality clean tags. Chimeric sequences were detected using the UCHIME algorithm against the SILVA 138 reference database and then eliminated. The resultant high-quality readings were preserved as functional tags for subsequent analysis (Wei et al., 2022; Chen et al., 2023; Tadee et al., 2025).

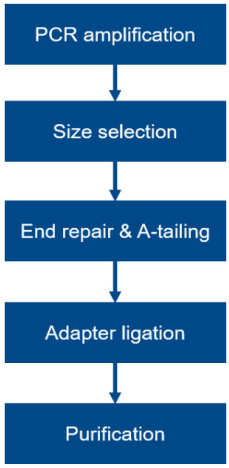

Figure 2: Workflow of library construction.

RESULTS AND DISCUSSION

Results

High-quality sequencing data were obtained from all samples, allowing reliable downstream comparative analysis of gut microbial communities. DNA yield varied across samples, as measured by both PicoGreen and NanoDrop assays. Based on PicoGreen quantification, the highest DNA concentration was obtained from the Bolaang Mongondow sample, whereas the lowest was observed in the Island sample. In contrast, NanoDrop measurements indicated the highest DNA concentration in the Island sample and the lowest in Bolaang Mongondow. Despite these differences, the extracted DNA met the quality and quantity requirements for downstream metagenomic sequencing (Supplementary Table 1).

Gut microbial composition of local pigs was characterized by clustering high-quality sequences into OTUs at 97% similarity, followed by taxonomic annotation (Figure 3).

OTU-based taxonomic analysis demonstrated differences in the composition and relative abundance of gut microbial communities among North Sulawesi local pigs, as reflected by distinct clustering patterns in the heatmap (Figures 4 and 5).

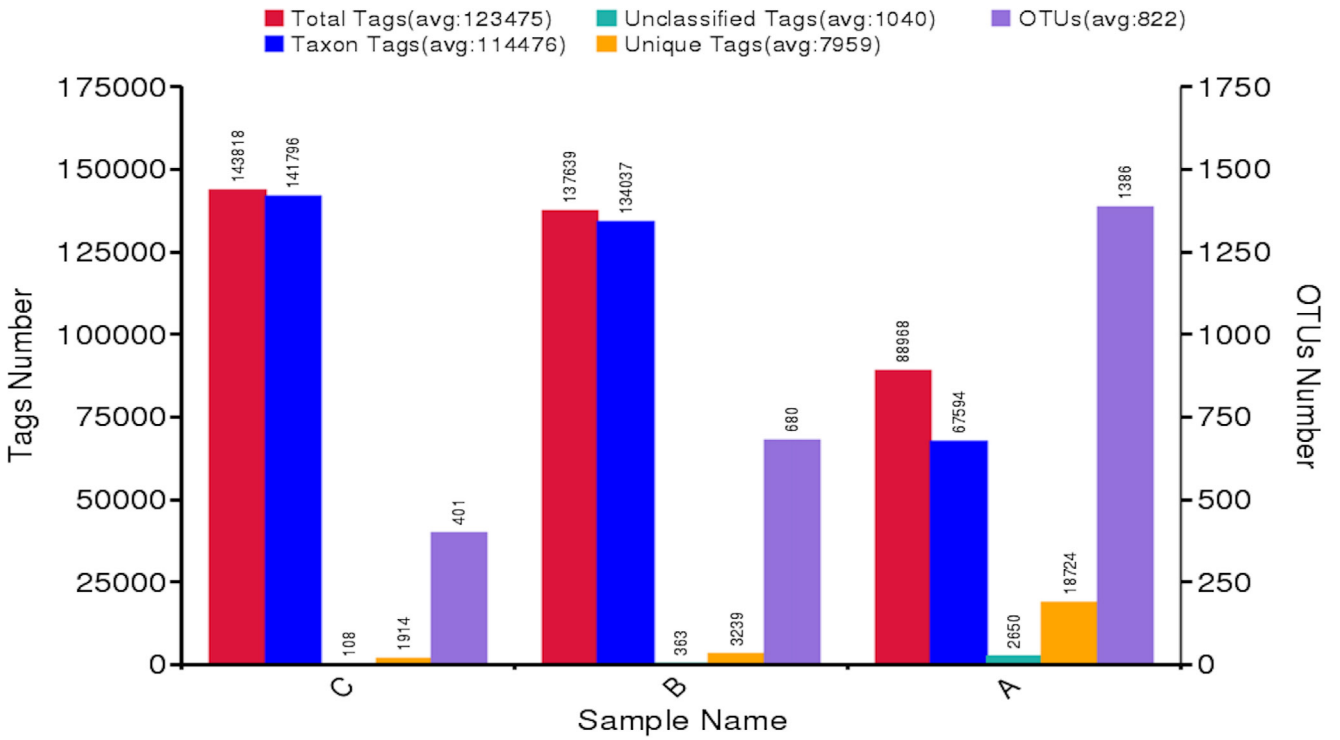

Figure 3: Summary of effective tags and OTU numbers from each sample: A = Minahasa local pig, B = Island local pig, C = Bolaang Mongondow local pig. OTU-based taxonomic analysis demonstrated differences in the composition and relative abundance of gut microbial communities among North Sulawesi local pigs, as reflected by distinct clustering patterns in the heatmap (Figures 4 and 5).

Heatmap analysis revealed distinct clustering patterns among the three local pig populations, reflecting differences in dominant gut microbial taxa. While several core microbial groups were shared across populations, region-specific taxa contributed to variations in community structure among Minahasa, Island, and Bolaang Mongondow pigs.

Phylogenetic and KRONA-based analyses revealed both shared and population-specific microbial lineages among the three local pig populations. While several taxa were consistently present across all regions, distinct location-associated taxa dominated specific phylogenetic groups, contributing to variations in community structure among Minahasa, Island, and Bolaang Mongondow pigs (Figure 6).

Consensus Lineage	C	B	A	#OTU ID
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	5349	3890	47096	OTU_1
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus_delbrueckii	14738	8285	6	OTU_2
k__Bacteria;p__Firmicutes;c__Negativicutes;o__Veillonellales-Selenomonadales;f__Veillonellaceae;g__Megasphaera;s__Megasphaera_elsdenii	14499	5685		OTU_3
k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcaceae;g__Romboutsia;s__Romboutsia_ilealis	2765	8268	418	OTU_4
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus_fermentum	4086	3174		OTU_5
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1;s__Clostridium_perfringens	983	2529	7	OTU_6
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1	1038	2462	31	OTU_7
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	8095	6145	194	OTU_8
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus	1418	1561	146	OTU_9
k__Bacteria;p__Fusobacteriota;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae;g__Fusobacterium			3256	OTU_10
k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium_thermophilum	825	597	6	OTU_11
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales;f__Acetobacteraceae;g__Acetobacter;s__	569	837		OTU_12
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae;g__Weissella;s__Weissella_ghanensis	702	951		OTU_13
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae;g__Weissella;s__Weissella_cibaria	909	999	35	OTU_14
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Enterobacteriaceae;g__Escherichia-Shigella;s__Escherichia_coli	490	829	15	OTU_15
k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Agathobacter;s__Eubacterium_rectale	1	955	449	OTU_16
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus;s__Bacillus_thuringiensis	755	623	237	OTU_17
k__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Turicibacter;s__	160	1165	3	OTU_18
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae	572	625		OTU_19
k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Corynebacterium;s__Corynebacterium_variabile	1058	1385		OTU_20
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae	294	352		OTU_21
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1;s__Clostridium_beijerinckii	309	318	7	OTU_22
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;g__Enterococcus;s__Enterococcus_italicus	506	566	3	OTU_23
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus_plantarum	520	94	2	OTU_24
k__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Faecalibacterium;s__Faecalibacterium_prausnitzii	2	1272	320	OTU_25
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Burkholderiaceae;g__Ralstonia;s__Ralstonia_pickettii	5	60	625	OTU_26
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter;s__Acinetobacter_baumannii	498		1	OTU_27
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Acetobacterales;f__Acetobacteraceae;g__Acetobacter;s__Acetobacter_pasteurianus	192	555	3	OTU_28
k__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium	284	238		OTU_29
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus;s__Lactococcus_lactis	230	250	121	OTU_33
k__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcaceae;g__Terrisporobacter;s__	277	443	17	OTU_34
k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides_sartorii			904	OTU_35
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae;g__Fructobacillus;s__	160	252	1	OTU_38
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus	299	394		OTU_39
k__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Atopobiaceae;g__Olsenella;s__	265	200		OTU_40
k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Blautia	2	84	533	OTU_41
k__Bacteria;p__Firmicutes;c__Negativicutes;o__Veillonellales-Selenomonadales;f__Veillonellaceae;g__Veillonella	269	232		OTU_42
k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Dorea;s__		87	425	OTU_44
k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides_vulgatus		147	332	OTU_46
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Paracoccus	332	289		OTU_47
k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Lachnospiraceae_NK3A20_group	423	195		OTU_48
k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Ruminococcus_gnavus_group;s__	1	5	579	OTU_49
k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Anaerostipes;s__Anaerostipes_hadrus		15	766	OTU_51
k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Fusicatenibacter;s__		52	384	OTU_53
k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae		26	520	OTU_57
k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__Bacteroides_fragilis		10	526	OTU_60
k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Blautia		36	417	OTU_71
k__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__Ruminococcus_torques_group;s__		79	442	OTU_171
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus	111	245	109	OTU_207
k__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides		24	435	OTU_230
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas		3	2323	OTU_1164
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1;s__	416	2164	50	OTU_1336

Figure 4: Heatmap of OTU-based taxonomic annotations of the gut microbiome in local pigs: A = Minahasa, B = Island, C = Bolaang Mongondow.

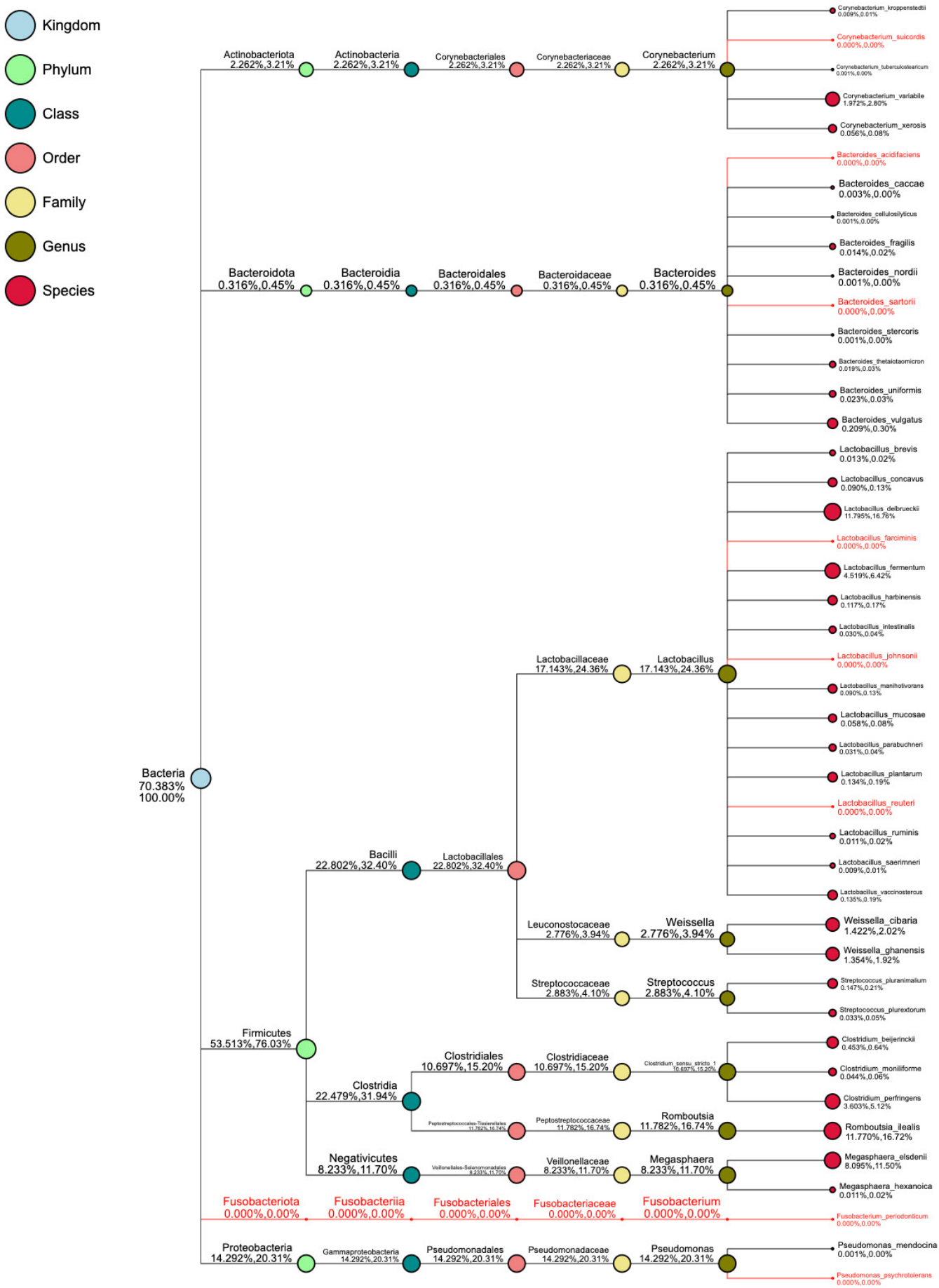

Figure 5: Phylogenetic tree of metagenomic samples: A= Minahasa local pig, B= Island local pig, C= Bolaang Mongondow local pig. Note: Different colors represent distinct taxonomic ranks.

Bacterial community composition of the local pig gut microbiome

Relative abundance

Relative abundance analysis of the top ten taxa at different taxonomic levels revealed distinct microbial profiles among indigenous pigs from Minahasa, Island, and Bolaang Mongondow. Variations were more pronounced at the family and genus levels, indicating population-specific differences in gut microbial composition (Figure 7).

The relative abundance profiles revealed distinct taxonomic distributions across the three local pig populations. At the class level, several dominant bacterial groups were consistently shared, while notable differences in proportional representation were observed among locations. At the family and genus levels, clear variation emerged, with certain taxa enriched in specific populations, reflecting potential geographic and ecological influences on microbial community composition.

Heatmap of taxonomic abundance clusters

Genus-level heatmap analysis revealed distinct clustering patterns among samples, indicating similarities within populations and differences among indigenous pig populations. Both shared microbial signatures and population-specific variations contributed to genus-level community structure (Figure 8).

Figure 6: KRONA visualization of the gut microbiome in North Sulawesi local pigs.

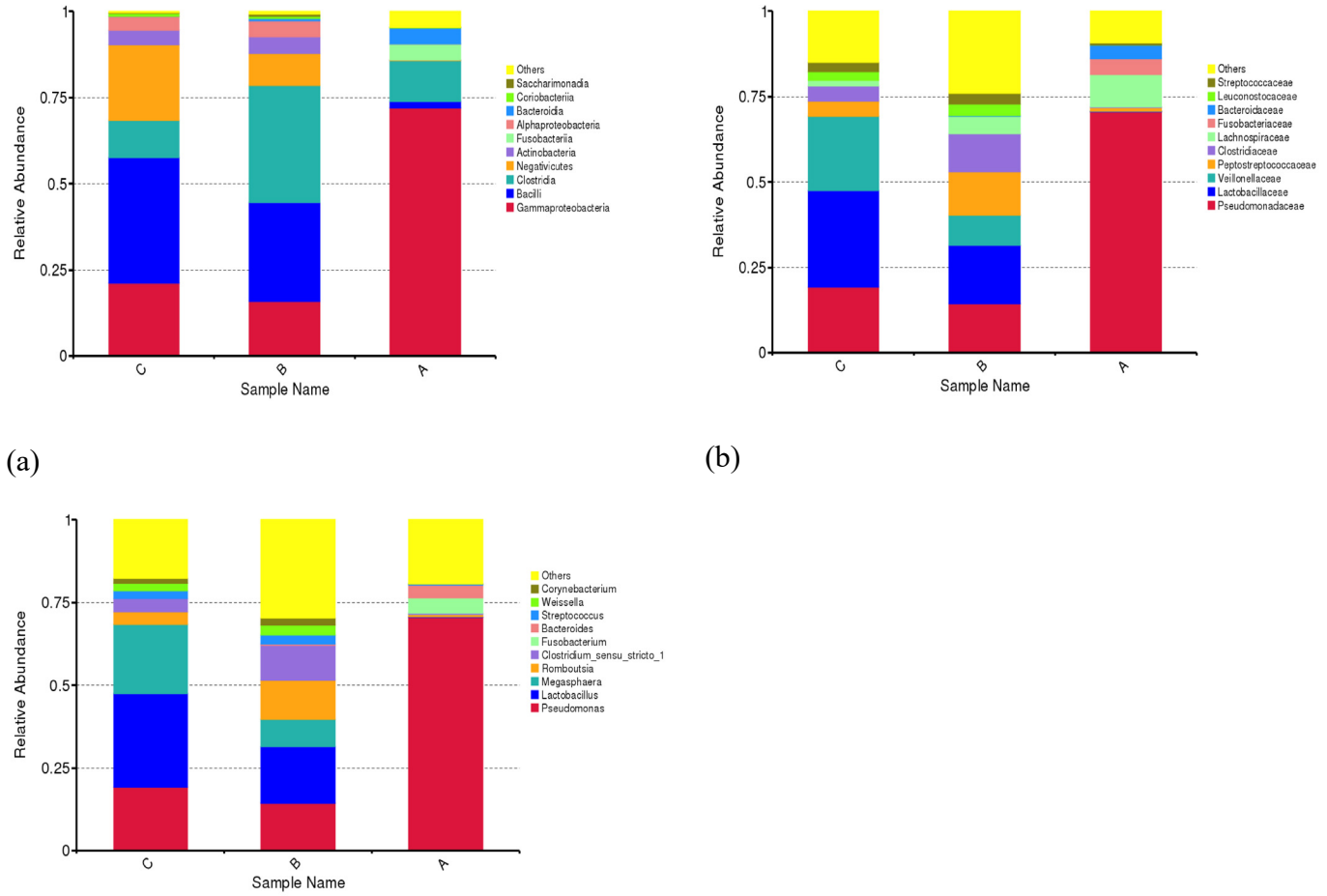

Figure 7: Relative abundance of bacterial taxa at different taxonomic ranks in the gut microbiome of North Sulawesi local pigs: (a) Class, (b) Family, (c) Genus. A = Minahasa local pig, B = Island local pig, C = Bolaang Mongondow local pig.

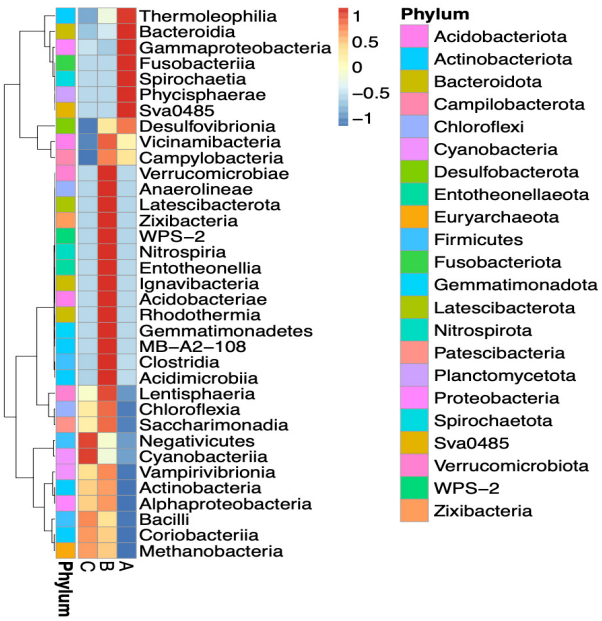

Figure 8: Heatmap of taxonomic abundance clusters in the gut microbiome of North Sulawesi local pigs: A = Minahasa, B = Island, C = Bolaang Mongondow.

Genus-level heatmap analysis showed closer clustering of samples within the same population, indicating similarities in gut microbial composition and potential influences of shared ecological or host-related factors. Ternary plot analysis further demonstrated differential enrichment of dominant taxa among Minahasa, Island, and Bolaang Mongondow pigs, highlighting population-specific patterns in microbial community structure (Figure 9).

Alpha Diversity Analysis

Alpha diversity analysis indicated variability in microbial richness and evenness among intestinal samples from North Sulawesi local pigs. While sequencing depth was sufficient across samples, differences in diversity indices suggested variation in community complexity among population (Supplementary Table 2).

Venn and Flower Diagrams

Based on OTU clustering and normalized OTU tables, we analyzed the distribution of shared and unique taxa across the different pig populations. Venn and Flower

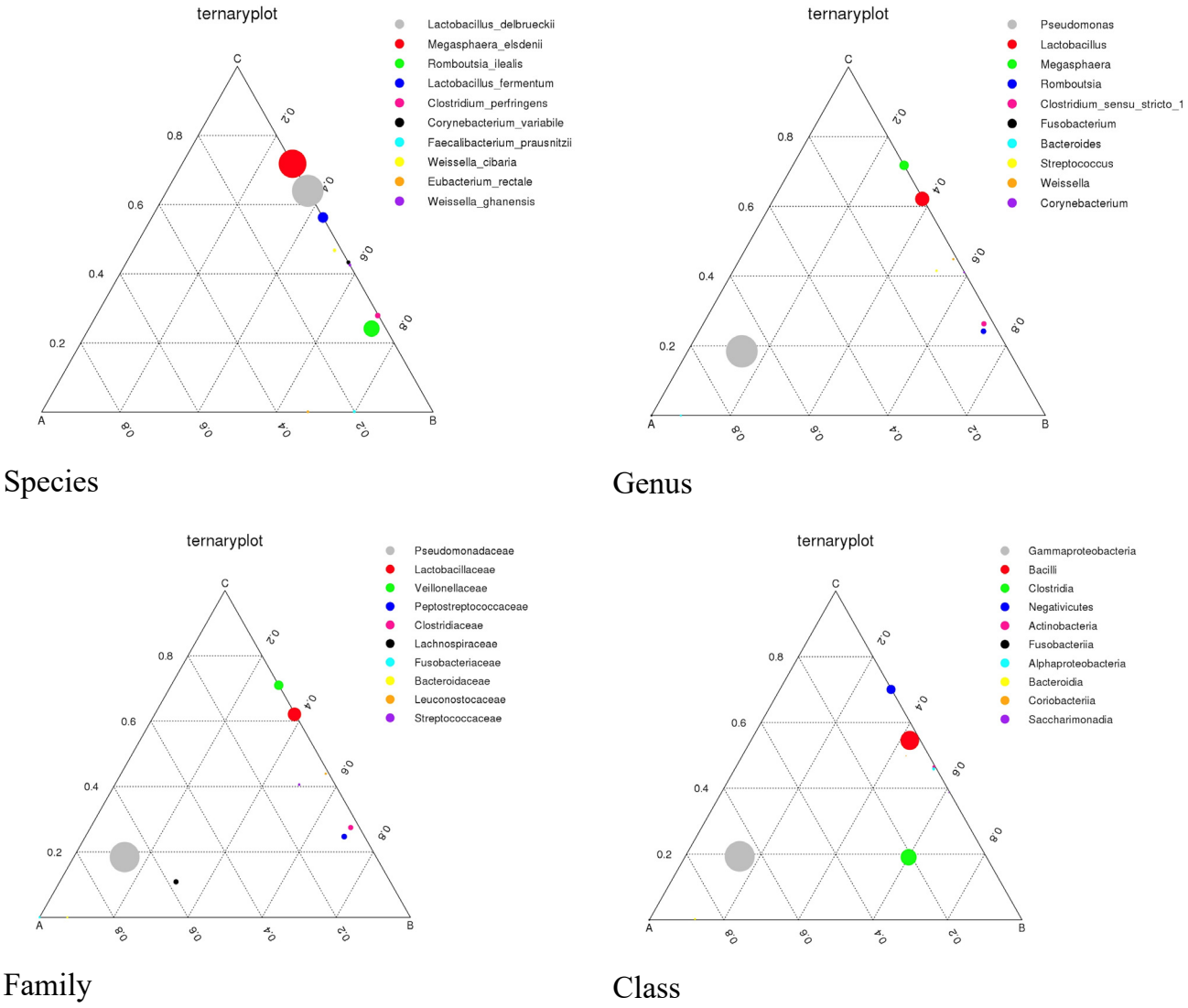

Figure 9: Ternary plots comparing microbial distribution at different taxonomic levels.

The biodiversity curve further illustrates sequencing depth adequacy and species richness across samples (Figure 10).

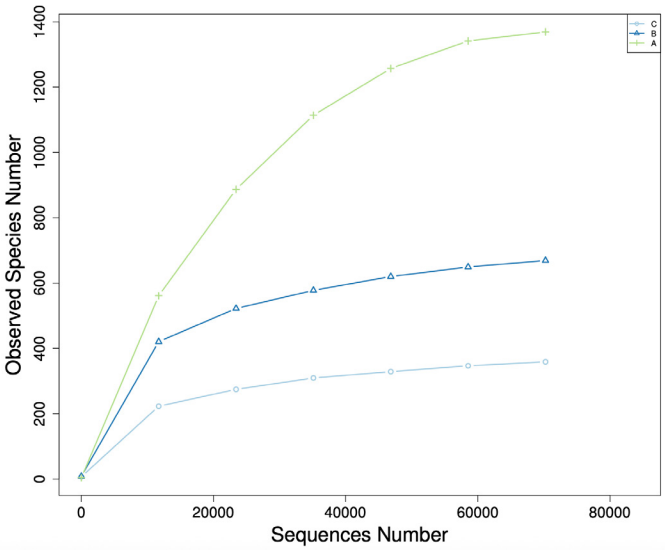

Figure 10: Biodiversity curve of metagenomic results of the digestive tract of local pigs in North Sulawesi.

diagrams were generated to illustrate the overlap and distinctiveness of microbial communities among groups, providing a clear visualization of core taxa common to all samples and population-specific taxa unique to each group (Figure 11).

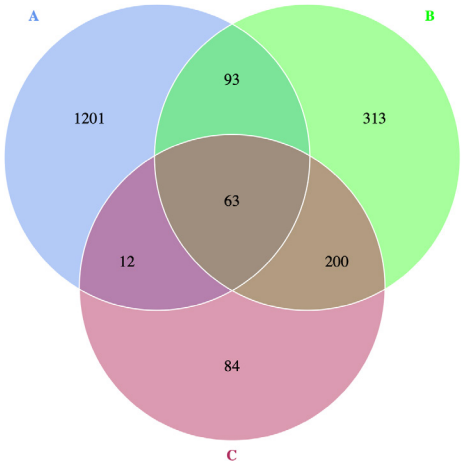

Figure 11: Venn diagram.

Beta diversity analysis

Beta diversity offers a direct comparison of microbial communities according to their taxonomic composition. This metric measures variations among microbial communities by computing pairwise distance or dissimilarity matrices, including unweighted UniFrac and weighted UniFrac distances. The distance matrices were further visualized by Principal Coordinates Analysis (PCoA), Principal Component Analysis (PCA), Non-metric Multidimensional Scaling (NMDS), and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering.

Beta diversity heatmap

Phylogenetic dissimilarity between paired samples was assessed using both weighted and unweighted UniFrac distances. These widely used metrics in microbial community sequencing projects enabled us to assess both presence/absence (unweighted) and abundance-weighted (weighted) differences among populations. The resulting heatmaps illustrate pairwise dissimilarities of gut microbial communities in North Sulawesi local pigs (Figure 12).

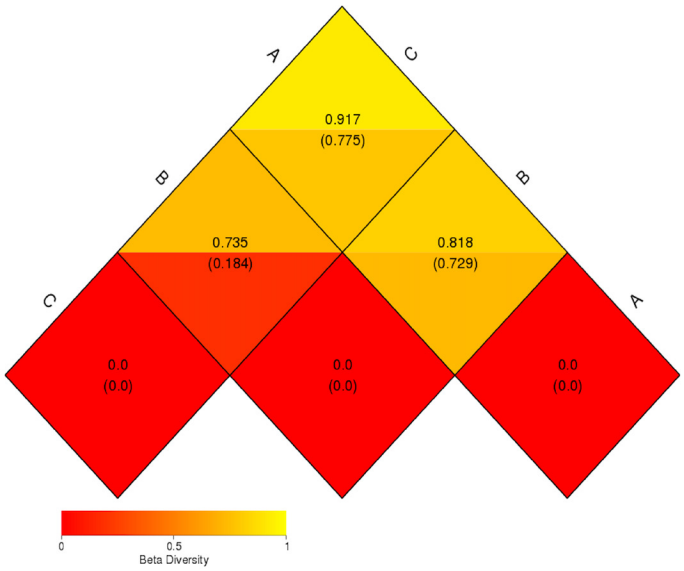

Figure 12: Beta diversity heatmap (each grid represents the pairwise difference coefficient between paired samples, where Weighted Unifrac distances are shown on top and Unweighted Unifrac distances on the opposite.

Principal component analysis (PCA)

Principal Component Analysis (PCA) was utilized to decrease data dimensionality and identify the primary axes of variation in microbial community structure. By extracting the first two principal components, PCA provides a two-dimensional representation of high-dimensional OTU data, enabling visualization of overall community similarities and differences among samples. In the resulting ordination, samples with more similar gut microbial compositions cluster more closely, whereas those with distinct communities are positioned further apart. PCA of the gut microbiota from local pigs across Minahasa, Islands, and Bolaang Mongondow revealed clear patterns of community separation (Figure 13).

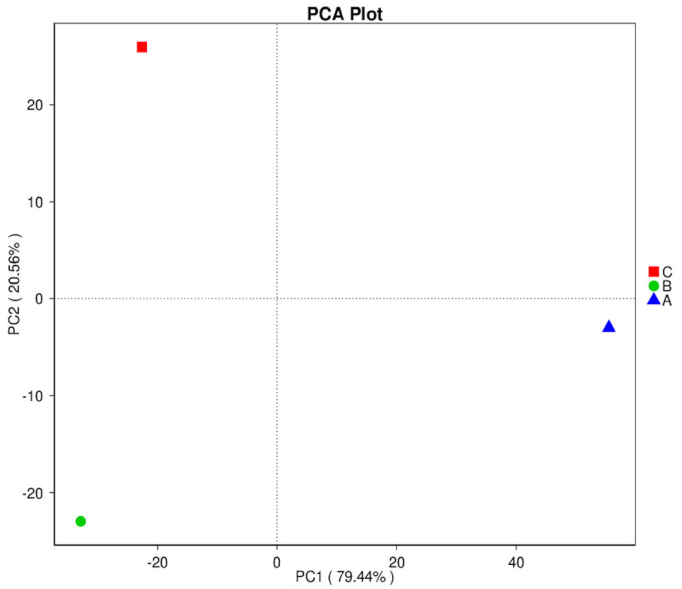

Figure 13: PCA Plot (Note: each point corresponds to a sample, plotted with the second principal component on the Y-axis and the first principal component on the X-axis, differentiated by group color).

UPGMA clustering

Hierarchical clustering was conducted to evaluate community similarity among samples, utilizing the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Distance matrices reflecting phylogenetic relationships were calculated using both weighted and unweighted UniFrac metrics, which were then utilized for constructing the tree. The UPGMA dendrograms produced, along with phylum-level relative abundance data, demonstrate the clustering patterns of gut microbial communities in local pigs from Minahasa, Islands, and Bolaang Mongondow (Figures 14 and 15).

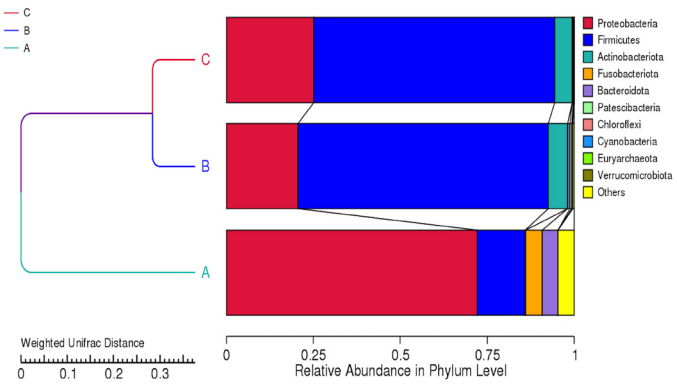

Figure 14: UPGMA cluster tree based on Weighted Unifrac distance.

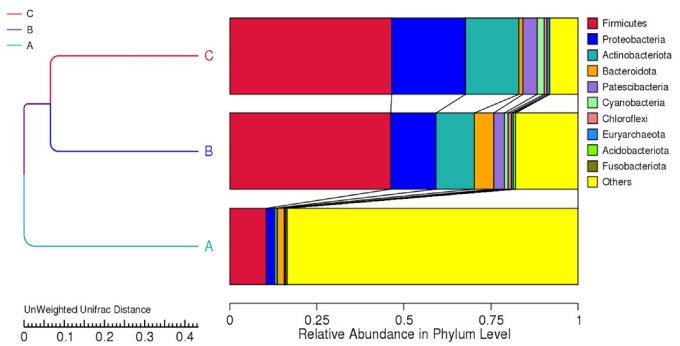

Figure 15: UPGMA cluster dendrogram derived using unweighted Unifrac distances, illustrated with the UPGMA tree on the left and relative abundances in the phylum map on the right.

DISCUSSION

The heatmap analysis identified a core gut microbial community that was consistently observed across the three local pig populations, comprising Lactobacillus, Megasphaera, and Bacteroides/Prevotella, which are essential taxa involved in carbohydrate fermentation and short-chain fatty acid (SCFA) production (Luo et al., 2022; Liao et al., 2024; Zhang et al., 2024; Jian et al., 2025). Even with this common foundation, notable variations in the relative abundance of various OTUs were detected across regions (A, B, and C), indicating the impact of dietary habits, environmental factors, and farming practices. Certain OTUs, including Pseudomonas, Romboutsia, Weissella, and Faecalibacterium, exhibited abundance variations based on location, suggesting potential interactions between the host and environment. The detection of potentially pathogenic taxa (*Clostridium perfringens, Escherichia coli*) and environmental genera (*Pseudomonas, Acinetobacter, Ralstonia*) warrants further validation through qPCR, quantitative read mapping, and shotgun metagenomics to determine whether their presence reflects contamination, opportunism, or genuine gut adaptation.

Phylogenetic reconstruction of metagenomic data demonstrated that the gut microbiota of North Sulawesi pigs is hierarchically structured, dominated by Bacteroidetes and Firmicutes. This is consistent with previous findings reporting these phyla as central to carbohydrate degradation, SCFA biosynthesis, and antibiotic resistance gene carriage in both local and commercial pig breeds (Endrakasih and Pazra, 2024; Yang et al.,2024; Xie et al., 2024). Genus-level clustering (e.g., Lactobacillus, Romboutsia, Faecalibacterium, Weissella) indicated the presence of fermentative and potentially probiotic lineages (Liao et al., 2024; Bernad-roche et al., 2021). Distinct phylogenetic clustering of Lactobacillus strains suggests strain diversification specific to local populations, underscoring the utility of metagenome-assembled genomes (MAGs) for capturing strain-level diversity and functional specialization. These results align with recent cataloging studies (e.g., PIGC) showing that a large proportion of pig gut microbial genomes remain uncharacterized, reinforcing the need for functional delineation at the strain level to better understand ecological and physiological implications in local breeds.

The KRONA visualization elucidated that the gut microbiota of local pigs is predominantly composed of Firmicutes (such as Ruminococcus, Clostridium sensu stricto 1, Lactobacillus, Faecalibacterium) and Bacteroidetes (including Bacteroides, Prevotella, Phocaeicola), exhibiting significant variability in abundance across the regions of Minahasa, Kepulauan, and Bolaang Mongondow. This composition aligns with global pig gut microbiota profiles, wherein Firmicutes and Bacteroidetes facilitate polysaccharide degradation, short-chain fatty acid production, and modulation of gut health (Hu et al., 2023; Pandey et al., 2023). The occurrence of opportunistic taxa, including Clostridium sensu stricto 1 and Escherichia-Shigella, underscores the necessity for comprehensive functional validation to evaluate virulence or antimicrobial resistance potential. The observed microbiota patterns illustrate the interaction among host genetics, feed composition, and environmental conditions, which together influence microbial diversity and function in indigenous pig populations.

This study provides novel insights into the gut microbiota of indigenous pigs from North Sulawesi, a population that has received limited attention compared to commercial breeds. While previous research has established *Firmicutes* and *Bacteroidetes* as dominant phyla in swine gut ecosystems (Liu et al., 2021; Yang et al., 2024), our results demonstrate unique microbial signatures in local populations, including the strain-level diversification of *Lactobacillus* and the location-specific abundance of genera such as *Weissella* and *Romboutsia*. These findings indicate that indigenous pigs harbor distinct microbial lineages not captured in existing reference databases or global pig gut catalogs, suggesting a previously undocumented reservoir of microbial genetic and functional diversity.

Furthermore, the detection of both beneficial taxa (e.g., *Faecalibacterium*, *Megasphaera*) and potential opportunistic/pathogenic lineages (*Clostridium perfringens*, *Escherichia-shigella*) within the same host populations highlights a complex ecological balance shaped by local diets, management practices, and environmental conditions. This dual presence underscores the need for strain-level metagenomic and functional characterization, as recommended in recent metagenome-assembled genome (MAG) studies, and positions North Sulawesi

pig microbiota as a critical model for understanding host microbe co-adaptation under traditional farming systems.

In summary, this study is unique in that it is (i) the first metagenomic characterization of gut microbiota in indigenous North Sulawesi pigs, (ii) there is evidence of strain diversification unique to local populations, and (iii) unexplored microbial lineages with potential probiotic or pathogenic functions were identified. Collectively, these findings contribute to a better knowledge of swine microbiota by discovering underrepresented microbial reserves in non-commercial pig populations, opening up new possibilities for animal health, conservation genetics, and microbiome-driven livestock management.

CONCLUSION

This research indicates that indigenous pigs from North Sulawesi possess gut microbial communities that vary significantly across regions at the class, family, and genus levels. The prevalence of Gammaproteobacteria, Bacilli, and Clostridia, along with the presence of families like Enterobacteriaceae, Ruminococcaceae, and Lachnospiraceae, indicates a variety of metabolic functions, encompassing both simple carbohydrate fermentation and complex polysaccharide degradation. This research presents the initial documentation of gut microbiota composition in three distinct subpopulations of North Sulawesi pigs, offering evidence that microbial diversity is significantly influenced by ecological adaptation and may enhance the resilience of local livestock. Future research should focus on functional metagenomic analyses to identify essential genes and metabolic pathways that underpin these microbial functions. Furthermore, trials focused on feed interventions utilizing locally available resources should be undertaken to assess the efficacy of microbiota-driven strategies in enhancing gut health, nutritional efficiency, and overall productivity in indigenous pig populations.

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NOVELTY STATEMENT

This study provides the first region-resolved metagenomic characterization of the gut microbiota of indigenous pigs from North Sulawesi, Indonesia, a genetically diverse and ecologically adapted porcine population that remains absent from global pig gut microbiome catalogs dominated by commercial breeds. By integrating 16S rRNA–based taxonomic profiling with multivariate and phylogeny-aware analyses (ternary plots and UniFrac-based UPGMA clustering), we demonstrate that gut microbial community structure is strongly shaped by geographical origin and local ecological conditions, revealing population-specific microbial signatures beyond core porcine taxa. Importantly, the identification of distinct enrichments of fermentative and potentially probiotic lineages, alongside opportunistic taxa, highlights a previously undocumented microbial reservoir associated with traditional feeding systems and environmental adaptation. These findings extend current porcine microbiome references and establish a foundational framework for microbiome-informed nutritional, health, and conservation strategies tailored to indigenous pig production systems.

AUTHORS' CONTRIBUTION

RAM and MYS devised the study, implemented the methodology, and conducted the investigation. IS and NM conducted a thorough data and formal analysis while also gathering the necessary resources. The original draft was composed by MYS. IS and NM engaged in the writing, reviewing, and editing of the manuscript, while RAM provided oversight. All authors have reviewed and granted their approval for the final manuscript prior to its publication.

Generative AI and AI assisted technology statement

The authors declare that no generative AI or AI-assisted technologies were used to generate, analyze, or interpret the research data. Generative AI tools were used only to assist in improving the grammar and readability of the manuscript during the revision stage, and all scientific in terpretations and conclusions are entirely the authors' own.

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Data availability

All datasets generated or analyzed during this study are included in the manuscript.

Ethics statement

Not applicable

Conflict of interest

The authors have declared no conflict of interest.

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Supplementary Table 1: Extraction and purification of total genomic DNA from Indigenous Sulawesi Pigs

No	Sample	Picogreen (ng/ul)	Nanodrop (ng/ul)	A260/A280	A260/A230	Description
1	A	22.1	22,41	1.54	0.25	Metagenomic sequencing continued
2	B	20,20	23,21	1.57	0.28	Metagenomic sequencing continued
3	C	23,22	22,15	1.52	0.24	Metagenomic sequencing continued

Supplementary Table 2: Alpha diversity index.

Nama sampel	spesies_yang diamati	Shannon	simpson	chao1	ACE	barang_cakupan	PD_seluruh_pohon
C	359	4,322	0,887	409.312	414.803	0,999	56,223
B	669	5,468	0,946	754.513	745.081	0,998	140.519
A	1369	3,068	0,546	1369.200	1376.397	1.000	430.479