Harnessing the Strategy of Metagenomics for Exploring the Intestinal Microecology of Sable (Martes Zibellina), the National First-Level Protected Animal

Jiakuo Yan
Qufu Normal University
Xiaoyang Wu
Qufu Normal University
Jun Chen
Ocean University of China
Yao Chen
Ocean University of China
Honghai Zhang (zhanghonghai67@126.com)
Qufu Normal University

Original article

Keywords: Sable (Martes zibellina)Metagenomics, Gut microbiota, Functional database, Gene function annotation

Posted Date: June 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-28506/v1

Version of Record: A version of this preprint was published on September 18th, 2020. See the published version at https://doi.org/10.1186/s13568-020-01103-6.

Abstract

Sable (*Martes zibellina*), belongs to Carnivora, Mustelidae and *Maretes*, was mainly distributed among the cold northern zone of Eurasia. The purpose of this study is to explore the intestinal flora of the sable by the method of the metagenomic library-based technique, libraries were sequenced on an Illumina HiSeq 4000 instrument. Effective Data volume of each sample is above 6000M, the ratio of the Effective Data (the Clean Data) to original Data (Raw Data) is over 98%. According to the analysis of statistical data, the Total length of ORF is about 603,031, which is 347.36 Mbp. We contrast the unique function of genes with KEGG database, we acquire 7140 genes (KO), a total of all the samples KO is 129788. We selected higher abundance genes to draw cluster heat maps, and according to the results of the KEGG metabolic pathway annotations, we acquire the gene function including metabolism, environmental information processing, genetic information processing, cellular process and organismal systems. We contrast the unique function of genes with CAZy database, the functional carbohydrate hydrolases have corresponding genes in the intestinal microorganisms of the sable. This is closely related to the fact that the sable is adapted to cold environments and requires a large amount of energy to maintain its metabolic activity. We contrast the unique function of genes with eggNOG database the main functions of genes included gene duplication, recombination and repair, transport and metabolism of amino acids, transport and metabolism of carbohydrates, etc.

Introduction

The sable (*Martes zibellina*), one of the species of carnivorous mammals, ranging across the cold northern zone of Eurasia (Li et al. 2013), is famous for its precious and thermal fur (Guan et al. 2016; Monakhov et al. 2018; Svishcheva and Kashtanov 2011). As a highly commercial valuable species, about 400000–450000 pelts were produced by sable furs in Russian fur hunting and fur farming each year (Monakhov 2015; Rozhnov et al. 2013). In China, the sable is mainly distributed in the needle-leaved forests in the north and northeast (Zhang et al. 2017). Because of its precious pelt, the wild sable has historically been hunted on a large scale, after a hunting bloodbath in some Western Siberian regions brought the sable to the brink of extinction (Kashtanov et al. 2011). In addition, the sable depends closely on their habitat, however, large areas of forests have been destroyed by human activity. Several factors had combined to result in a sharp fall of population quantities since the 1950s (Yan et al. 2017). The Chinese Government has decreed fierce bans on hunting and fur trade of the sable in recent years. The sable has been rediscovered in some places, but the population size has not recovered to its previous level (Li et al. 2018). This species has been listed on the Red List of Threatened Species of International Union for Conservation Nature.

Sable is not strictly carnivorous and feed on a wide variety of small mammals, birds, amphibians, reptiles, insects and fruits (Brzeziński 1994), and sable is an opportunist in selecting various kinds of foods in natural state (W. Buskirk et al. 1996). To the sexual dimorphism of sable, there are differences in body size and weight, however, there was no statistical significance in different diet between males and females (Dubinin 2010). Sable has a slender body and a high surface-to-volume ratio which maintain a

low body fat rate of about 8 percent even during particularly cold winters (Mustonen and Nieminen 2006), thus a high energy metabolic rate should be required for survival (Mustonen et al. 2006).

In natural condition, communities of microorganisms have the complex structure and abundant species, their evolution and ecology is determined by the interaction between external environmental factors and internal microbial communities (Ellegaard and Engel 2019). More researchers are beginning to study the overall microbial community structure, it is essential for understanding the genetics and function of microbial communities. Research indicated that coevolution processes between hosts and their microbial communities led to adaptability benefits to both partners (Federici 2019; Yu et al. 2019). The present findings confirm that various effect factors on the microbial diversity resulting from alimentary habits, photoperiod, season, age, elevation and region (Feng et al. 2019). In contrast, microbial community and their metabolites were all effective to host, and they participated in nutrient digestion, absorption and metabolic function (Tang et al. 2019; Zhang et al. 2018), other researchers have found that the diversity and stability of intestinal microbial affected host immune function (Liu et al. 2019; Willson et al. 2018). So far, it has been possible to improve the health of the host by regulating gut microbes (Gao et al. 2019). In a latest study, germ- free mice was treated with a transfusion of faecal microbials from healthy infants to reduce reactions of cow's milk allergy, and symptoms improved dramatically (Bunyavanich 2019). In order to understand the potential effects of intestinal microbiota on the host, it is important to identify the inner nature of its constituting constructs (Lyu et al. 2018).

Traditionally, the sort of microflora was determined through the methods of bacteria culture (Wu et al. 2017). However, the growth environment of many bacteria cannot be replicated, which hinders the study of microbial diversity and function (Tully et al. 2018). In recent years, the progress of high-throughput sequencing technologies has been a fundamental advancement in DNA sequencing (Shui et al. 2020). With the declined expense of DNA sequencing, metagenomics has been in the stage of rapid development (Johnson 2019). The technique is usually used to sequence and analyze the whole genome of microbes in an entire sample, without the necessity for cell cultures, it is widely applied to probe environmental microbial diversity from the all levels, in consequence further study of the microbial community structure and ecosystem function is possible (Martin et al. 2018).

Though there was a great number of international and domestic research on sable, they remain with the fields of morphology and biologic behavior as well as habitat environment, little was performed from molecular biology. The normal and balanced gut microbiota plays an important role in metabolism and immune regulation (Kc et al. 2019; Wang et al. 2019). Metagenomics, as the new orientation of genetic engineering is known, has vastly extended detection of diverse microorganisms and antimicrobial resistance genes (Ma et al. 2019; Vijayvargiya et al. 2019). To find out the relationship of gut microbes with hosts in metagenomic data, in this study, the high-throughput profiling of microbial taxa in sable feces were comprehensively characterized by utilizing Next-generation sequencing and metagenomic assembly analysis. The wild and farmed sable face many dangerous threats in China, the objectives of our study were to provide the necessary theoretical basis for the preservation of sables.

Materials And Methods

All fecal samples were collected from Dalian Mingwei Marten Industry Company Limited, these wild sables imported from Mohe County of Heilongjiang Province and Greater Khingan Range. When collecting stool samples, we recorded the details of host gender, sample date and cage number. We carried sterile collection device and a portable refrigerator and put the collected samples into the sterile bags, then all fecal samples were immediately frozen in the refrigerator. These samples were categorized into three groups: the five female sable fecal samples were named MZF.1–MZF.5, the four male sable fecal samples were named MZM.1–MZM.4, and intestinal contents were named MZS.1, MZB.1 and MZB.2 (Table 1). Before DNA extraction from fecal samples, we guarantee the freshness of samples and reduce the pollution of the environment on the samples.

Table 1
Table of the information in samples

Species	Sample	Sex	Time
Sable	MZF.1	Female	2017.11
Sable	MZF.2	Female	2017.11
Sable	MZF.3	Female	2017.11
Sable	MZF.4	Female	2017.11
Sable	MZF.5	Female	2017.11
Sable	MZM.1	Male	2017.11
Sable	MZM.2	Male	2017.11
Sable	MZM.3	Male	2017.11
Sable	MZM.4	Male	2017.11
Sable	MZS.1	Male	2017.11
Sable	MZB.1	Male	2017.11
Sable	MZB.2	Male	2017.11

DNA extraction,library preparation and metagenomics sequencing

The QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) was exploited to extract microbial DNA from the stool of sable. The detection of DNA samples mainly includes two methods. Agarose gel electrophoresis (AGE) was used to analyze the purity and integrity of DNA and Qubit 2.0 (Invitrogen, USA) quantifies DNA concentrations precisely. During the library construction step, qualified DNA samples were randomly broken into a length of about 350 bp fragments with ultrasonic crusher (Covaris, UK), then the

fragments go through end repair and A-tailing, ligated with adapters including PCR primer hybridization sites, purified and PCR amplificated. After the library preparation,Qubit 2.0 (Invitrogen, USA) was used for initial quantification and the library was diluted to 2 ng/μl. Subsequently, using Agilent 2100 Bioanalyzer (Agilent, USA) to detect if the insert sizes of the library correspond to expectations. In order to ensure the quality of library, Real-time q-PCR was used to accurately quantify the effective concentration (> 3 nM) of library. After the library passed the inspection, sequencing was implemented on Illumina HiSeq xten platform (Illumina, USA). The raw reads are available at the NCBI Sequence Read Archive (BioProject ID PRJNA630144, SRA SRP265006).

Quality control and genome assembly

After sequencing, raw genome data that contain adapter information n and low-quality bases would interfere with the subsequent analysis. First of all, the original data should be controlled to remove interfering data so as to obtain clean data. There was the possibility of host genome contamination, which needs to be compared with the host gene database to filter out reads that may be host gene (SoapAligner parameter settings: identity ≥ 90%, -l 30, -v 7, -M 4, -m 200, -x 400). Reads with a quality value less than 38 (the default ≤ 40) and N (undetected bases) reached the set number (default set to 10) in the original data were removed. Remove reads if the overlap between adapter and the sequence exceeds a certain threshold (≥ 15 bp). Clean Data was obtained after pretreatment, and the SOAP denovo assembly software was used for assembly analysis (Luo et al. 2012). For a single sample, k-mer = 55 was selected for assembly to obtain the assembly result of the sample (Assembly parameters: -d 1, -M 3, -R, -u, -F) (Brum et al. 2015; Feng et al. 2015; Qin et al. 2014; Scher et al. 2013). The Scaffolds were interrupted from the N-junction to obtain N-free sequence fragments, called Scaftigs (i.e., continuous sequences within scaffolds) (Mende et al. 2012). The Clean Data of each sample was compared to Scaftigs of each sample by SoapAligner software to obtain PE reads (Alignment parameters: -u, -2, -m 200). Put reads of each sample that had not been utilized together, and k-mer = 55 was selected for mixed assembly (Karlsson et al. 2013), other assembly parameters are the same as single sample assembly parameters. The Scaffolds were broken from the N -junction to obtain the Scaftigs sequences without N. For Scaftigs generated by single sample and mixed assembly, fragments less than 500 bp were filtered out (Zeller et al. 2014), and statistical analysis and subsequent genetic prediction were performed.

Gene prediction and abundance analysis

Starting from the Scaftigs of each sample and mixed assembly (> = 500 bp), MetaGeneMark was used for ORF (Open Reading Frame) prediction (Li et al. 2014), and fragment length less than 100nt was filtered from the prediction results. For the ORF prediction results of each sample, the CD-HIT software was used to remove redundancy, so as to obtain the initial gene catalogue with non-redundancy. Clustering is conducted with identity 95% and coverage 90%, and the longest sequence is selected as the representative sequence (parameters: -c 0.95, -G 0, -aS 0.9, -g 1, -d 0). The Clean Data of each sample was compared with the initial gene catalogue by SoapAligner, and the number of reads of genes in each sample was calculated (Alignment parameters: -m 200, -x 400, identity ≥ 95%). The number of genes supporting reads in each sample < = 2 were filtered out to obtain the final gene catalogue for subsequent

analysis (Qin et al. 2012). The abundance information of each gene in each sample was calculated from the number of reads and gene length (Villar et al. 2015). Based on the abundance information of each gene in each sample, basic information statistics, core-pan gene analysis, correlation analysis between samples, and gene number Venn diagram analysis were conducted (blastp,evalue ≤ 1e-5).

Species annotation

DIAMOND software was used to compare unigenes with the sequences of Bacteria, Fungi, Archaea and Viruses extracted from NCBI NR database (Buchfink et al. 2015). Alignment filtering: for each sequence alignment, evalue ≤ minimum evalue * 10 is selected for subsequent analysis. After filtering, since there may be multiple alignment results for each sequence to obtain multiple different species classification information, in order to ensure its biological significance, LCA algorithm (systematic classification applied to MEGAN software) was adopted to take the classification level before the first branch as the species annotation information of the sequence (Huson et al. 2011). According to the LCA annotation results and gene abundance table, the abundance information of each sample at each classification level (genus and species) is obtained. The abundance of a species in a sample is equal to the sum of the gene abundance of the species annotated. Based on the LCA annotation results and gene abundance table, the gene number table of each sample at each classification level (genus and species) was obtained. For a species, the number of genes in a sample was equal to the number of genes whose abundance was not 0 in the annotated species. Krona analysis, relative abundance overview display, abundance cluster heat map display, PCA and NMDS dimensionality reduction analysis, Anosim inter-group (internal) difference analysis, Metastat and LEfSe multivariate statistical analysis of inter-group difference species were conducted from the abundance table of each classification level (genus and species).

Functional database and resistance gene annotation

Currently, the commonly used functional databases mainly include: Kyoto Encyclopedia of Genes and Genomes (KEGG), Evolutionary genealogy of genes: Non-supervised Orthologous Groups (eggNOG), Carbohydrate-Active enzymes Database (CAZy). KEGG database was introduced by Kanehisa Laboratories in 1995 with version 0.1. At present, it has developed into a comprehensive database, the core of which is KEGG pathway and KEGG Ortholog database. In the KEGG Ortholog database, genes performing the same function are clustered together and called Ortholog Groups (KO entries). In the KEGG pathway database, biological metabolic pathways are divided into 6 categories, which are Cellular Processes, Environmental Information Processing, Genetic Information Processing, Human Diseases, Metabolism, Organismal Systems, each of which is systematically classified as layer 2, 3, 4. The second layer currently includes 43 seed pathways, the third layer is the metabolic pathway diagram and the fourth layer is the specific annotation information of each metabolic pathway map. The database of eggNOG uses the smith-waterman comparison algorithm to annotate the Orthologous Groups of constructed genes. EggNOG V4.1 covers 2,031 species and about 190,000 Orthologous Groups are constructed. CAZy database is the study of carbohydrate enzyme professional database, mainly covers six functional categories: GHs (Glycoside Hydrolases), GTs (Glycosyl Transferases), PLs (Polysaccharide Lyases), CEs (Carbohydrate Esterases), AAs (Auxiliary Activities) and CBMs (Carbohydrate Binding

Modules). DIAMOND software was used to compare Unigenes with each functional database (blastp, evalue ≤ 1e-5). Alignment filtering: for alignment results of each sequence, the alignment results with the highest score (one HSP > 60 bits) were selected for subsequent analysis. Based on the result of functional annotation and gene abundance table, the number of genes in each sample at each classification level is obtained. The number of genes with a certain function in a sample is equal to the number of genes with a non-zero abundance in the genes with a certain function. Based on the abundance table at each classification level, carry out annotation gene number statistics, relative abundance overview display, abundance cluster heat map display, PCA and NMDS dimensionality reduction analysis, Anosim inter-group (internal) difference analysis based on functional abundance, metabolic pathway comparative analysis, Metastat and LEfSe analysis of inter-group functional difference. Use the Resistance Gene Identifier (RGI) software provided by CARD database to compare Unigenes with CARD database (RGI built-in blastp, evalue ≤ 1e-30) (Qin et al. 2010). According to the comparison results of RGI and the abundance information of unigenes, the relative abundance of ARO was calculated. According to ARO abundance, column diagram of abundance, cluster heat map of abundance, circle diagram of abundance distribution, ARO difference analysis between groups, and species attribution analysis of resistance genes (note to ARO unigenes) were conducted.

Results

Extraction of total microbial DNA from samples

The microbial genomes of the samples were extracted using QIAGEN specialized kit for DNA extraction of stool samples, and the total DNA was preliminarily detected by agarose-gel electrophoresis, and the total DNA concentration was detected by Qubit Fluorometer to check whether the samples met the requirements for database construction. The results were shown in Table 1.

Table 1
The detection report of DNA

Sample name	Concentration (ng/μl)	Volume (μl)	Total (ng)	Library type	Test Results
MZF.1	11.4	51	581	Meta	A
MZF.2	13	51	663	Meta	A
MZF.3	7.6	51	388	Meta	A
MZF.4	10	51	510	Meta	A
MZF.5	0.8	51	41	Meta	A
MZM.1	37	51	1887	Meta	A
MZM.2	5.7	51	291	Meta	A
MZM.3	16	51	816	Meta	A
MZM.4	2.54	51	130	Meta	A
MZS.1	7.2	51	367	Meta	A
MZB.1	4.7	51	240	Meta	A
MZB.2	21	51	1071	Meta	A

Sequencing data statistics

Using Illumina HiSeq 4000 sequencing platform to get the original data (Raw data), the sequencing results of the sample processing for data statistics (in millions of bases for the unit), first of all, the original data for data quality control and removal of sub base and low quality of information, filtering to get the follow-up analysis of valid data filtering after effective data volume of each sample is above 6000 m, and effective data (the clean data) and the original data (Raw data) as a percentage of (Effective rate) are over 98%, and we use the sequencing error rate is less than 0.01 (quality value is greater than 20) and 0.001 (quality values greater than 30) the percentage of the base number of the Clean Data monitoring, the sequencing quality percentage is over 90%, showing samples of valid Data conform to the requirements of the subsequent analysis of the quality and quantity of the specific results are shown in Table 2, the GC (%) said the percentage of the GC base content in the clean data.

Table 2
The statistical information of sample data

Sample	Raw Data	Clean Data	Clean_Q20	Clean_Q30	Clean_GC (%)	Effective (%)
MZF.1	6,423.43	6,348.62	97.36	95.16	52.27	98.835
MZF.2	6,400.51	6,371.69	97.28	95.07	49.81	99.55
MZF.3	6,337.84	6,290.67	97.46	95.33	51.35	99.256
MZF.4	6,792.60	6,769.70	97.12	94.95	41.59	99.663
MZF.5	6,390.60	6,341.12	96.89	95.33	45.32	99.226
MZM.1	6,272.72	6,260.54	97.11	94.76	47.25	99.806
MZM.2	7,024.25	7,010.36	96.72	94.53	38.97	99.802
MZM.3	6,252.62	6,230.76	97.25	95.01	50.56	99.65
MZM.4	6,612.68	6,556.58	95.95	94.00	41.27	99.152
MZS.1	6,438.21	6,360.45	96.71	94.22	42.41	98.792
MZB.1	6,266.57	6,244.48	96.58	94.82	47.00	99.648
MZB.2	6,809.10	6,693.22	96.75	91.59	47.48	98.298

Valid data assembly results

After quality control, assembly were performed. Firstly, we set the sequence similarity of the edge region of two sequences, then we through the overlap relationship building Contig sequences. Furthermore, Scaffold sequence were obtained by connecting Contig sequence sets according to reads with paired-end relationship, undetermined region Gap still exists in the Scaffold sequence, it's usually denoted by N or n. Finally, the scaffold assembled was interrupted from the N connection to obtain the scaftig sequence fragment without N

Step on each sample after operation, there will be some unused PE reads, at this time of each sample after quality control using SoapAligner software will compare the various samples after assembly Scaftigs, the k-mer value of 55 is selected for mixed assembly, other assembly parameters remain unchanged. The mixed Scaffolds were also interrupted from the N connection to obtain the Scaftigs sequence without N. We usually use NOVO_MIX to represent filtering out the Scaftigs fragments generated by assembly under 500 bp, and obtain the sequence for subsequent statistical analysis. The results of data assembly are shown in Table 3.

Table 3
The statistical information of sample assembled results

Sample	Total len. (bp)	Num.	Average len. (bp)	N50 Len. (bp)	Max len. (bp)
MZF.1	36,356,589	34,785	1,045.18	1,087	73,452
MZF.2	31,081,373	22,094	1,406.78	1,711	305,593
MZF.3	18,869,947	14,803	1,274.74	1,492	39,052
MZF.4	62,710,239	46,632	1,344.79	1,670	91,860
MZF.5	32,307,271	23,551	1,371.80	1,703	52,978
MZM.1	45,524,309	37,738	1,206.33	1,328	226,903
MZM.2	79,892,828	65,995	1,210.59	1,373	185,211
MZM.3	14,205,478	11,729	1,211.14	1,341	41,989
MZM.4	33,869,693	26,595	1,273.54	1,511	86,091
MZS.1	33,880,650	26,234	1,291.48	1,419	243,912
MZB.1	30,495,188	17,768	1,716.30	2,789	400,796
MZB.2	2,101,244	2,820	745.12	692	15,984
NOVO_MIX	205,647	273	753.29	700	5,562

Gene prediction

After data assembly, MetaGeneMark was used to make gene prediction, and the prediction results of length less than 100nt will be filtered, then we use the CD - HIT software to get rid of the redundant information (protein level), non-redundant initial gene catalogue was obtained, we usually choose identity by 95%, 90% coverage for the clustering, the longest sequence was selected as the representative sequence. Next, the Clean Data of each sample was compared with the original gene catalogue using the SoapAligner software, the number of reads of the gene compared in each sample was obtained, the genes with reads less than or equal to 2 in each sample were filtered out. we get genes in the samples than on the number of reads and then filtered reads in each sample. If the number of genes is less than or equal to 2, the gene catalogue(unigenes) for subsequent analysis will be finally obtained. Moreover, the number of reads and gene length were compared, the abundance information of each gene in each sample was obtained. Based on the statistical data, we get ORF (Open Reading Frame) for a total of 603,031, the number of genes with both start codon and stop codon accounted for 29.38%~49.66% in each sample, the number of genes with neither initiation codon nor termination codon accounted for 5.83%~12.09%. The total length of ORF predicted was 347.36 Mbp, we usually use Average length to indicate the ORF, The Average length of each sample is shown in Table 4.

Table 4
The statistical information of predicted gene

Sample	ORFs NO	Integrity: none	Integrity: all	Total length	Average length
MZB.1	39,513	3,004(7.6%)	19,623(49.66%)	25.86	654.52
MZB.2	1,766	103(5.83%)	718(40.66%)	0.5	284.09
MZF.1	57,458	6,949(12.09%)	16,882(29.38%)	31.42	546.78
MZF.2	43,961	3,734(8.49%)	18,541(42.18%)	26.64	606
MZF.3	27,858	2,972(10.67%)	10,265(36.85%)	16.63	597.07
MZF.4	91,228	8,049(8.82%)	37,356(40.95%)	54.37	595.93
MZF.5	47,236	3,832(8.11%)	20,673(43.77%)	27.95	591.7
MZM.1	67,006	7,037(10.5%)	24,051(35.89%)	39.32	586.82
MZM.2	105,623	8,052(7.62%)	46,010(43.56%)	54.35	514.59
MZM.3	21,266	2,107(9.91%)	8,287(38.97%)	12.07	567.54
MZM.4	50,935	4,540(8.91%)	20,947(41.12%)	29.4	577.3
MZS.1	49,063	4,797(9.78%)	20,141(41.05%)	28.82	587.47
NOVO_MIX	118	4(3.39%)	57(48.31%)	0.03	234.56

Species abundance

Based on the relative abundance table of different classification levels, the top 35 genera with abundance and their abundance information in each sample were selected to draw a heat map, and clustering was conducted at the level of species to facilitate the result display and information discovery, so as to identify the species with more aggregation in the sample (Fig. 1).

Transverse is the sample information, vertical is species information; The clustering tree on the left of the figure is a species clustering tree. The corresponding value of the middle heat map is the Z value obtained after the standardization of relative abundance of species in each row, the Z value of a sample in a certain classification is the difference between the relative abundance of samples in that classification and the average relative abundance of all samples in that classification divided by the standard deviation of all samples in that classification

KEGG annotation results

The predicted Unique Genes were compared with the KEGG functional database, and 7140 Genes (KO) were obtained. The total number of Genes (KO) in all samples reached 127,839. As shown in Fig. 2, the number of genes related to carbohydrate metabolism was the highest, accounting for 11.86%, which proves that carbohydrate, as the most important energy supplier, is the main energy source provided to

the host by intestinal flora in Environmental information. Among the processing functions, the number of genes related to membrane transportation is high proportion, accounting for 7.79% of the total number of genes in all samples, which proves that the replacement of nutrients and metabolites between the intestinal flora and the host through the continuous membrane transportation function, so as to provide the material transportation basis for the intestinal microorganisms to help the host digest food and provide vitamins and amino acids. According to the results of the KEGG metabolic pathway annotations, we acquire the gene function (Fig. 3),

CAZy annotation results

Comparing the Unique Genes with CAZy (carbohydrate enzyme professional database) database, the number of genes corresponding to the six carbohydrate enzymes was obtained, as shown in Fig. 3, the GH (Glycoside hydrolases) corresponding to the maximum proportion of genes, PL (Polysaccharide lyases) corresponding to the minimum number of genes. According to the results of the annotation to draw the samples six carbohydrate enzyme relative abundance bar chart (Fig. 4).

EggNOG annotates

EggNOG database combines COG, KOG and Orthologous Groups function database, mainly through the known protein to get corresponding functional annotation of the sequences, comparing the Unique Genes with eggNOG database, the main function of genes including gene replication and repair of amino acid transport and metabolism of carbohydrates, such as transport and metabolism, eggNOG database annotation results below (Fig. 5).

Resistant gene annotation

To reflect the distribution of ARO in each sample, a black-and-white heat map of ARO distribution was drawn. Meanwhile, according to the abundance information of ARO in each sample, the top 30 aros were selected to draw the abundance cluster heat map, and the results were shown as follows (Fig. 6). According to the annotation results of CARD database, the analysis circle of species belonging of resistance genes was drawn (Fig. 7).

Discussion

Analysis of intestinal flora structure of sable

Gut microbes are symbiotic with their hosts, it plays a unique role in host digestion, metabolism and immunity, such as maltodextrin, cellulose and hemicellulose are indigestible by the host itself, intestinal flora can break down these substances, which produces short chain fatty acid for the host, gut microbes and intestinal mucosa resist the invasion of foreign viruses and antigen, and effectively restrain the growth of the exotic harmful bacteria, promote the growth of host immune system. In terms of

macroecology, researchers have conducted research on the sable, however, from the perspective of metagenomics, there was blank in the research on the endangered species of sable.

The results from this study show that Proteobacteria and Firmicutes are the dominant bacteria of sable intestinal microorganisms at the phylum level. At the order level, Enterobacteriales, Lactobacillales and Clostridiales were the dominant bacterial community. It is found that the structure of host flora is closely related to its nutrient metabolism, in some carbohydrates catabolism and vitamin synthesis are usually performed by the intestinal flora. The dominant order of firmicutes in the intestinal microorganisms of sable, clostridium, is the main fiber-degrading order, Meanwhile, the abundance of clostridium was higher in the structure of human intestinal flora and the diversity of clostridium was the highest in the intestinal microorganisms of dogs (Liang et al. 2015). Proteobacteria are gram-negative bacteria, which participate in a variety of metabolism and have a good catabolic effect on some components of feed, it is often used as a marker of intestinal flora disorders (Harms et al. 2016). The study found that the dominant intestinal flora of the ferrets also varied when the animals were fed the same food.

Lactobacillaceae and Enterobacteriaceae as the dominant bacteria at the family level, they play important roles in assisting the host to break down carbohydrates and ferment sugars to maintain the host nutrition metabolism. Through further analysis at the genus level, lactobacillus and Escherichia have high abundance, Lactobacillus, as a beneficial bacterium, act as a barrier to foreign invaders, inhibit the growth of other pathogenic bacteria and synthetic vitamins and amino acids for host, maintaining the dynamic balance of gut microbes, also it plays an important role in tumor inhibition, in some hosts with disease, there may be a decrease in lactobacillus in the gut.

Analysis of intestinal flora function of sable

After data assembly and gene prediction, the Unique Genes were compared with the functional databases (KEGG, eggNOG, CAZy, CARD), and the corresponding annotation results were selected according to the matching score. According to KEGG annotation results, the number of genes corresponding to Metabolism function reached 77,891, accounting for 60.93% of the total number of genes. The highest number of genes related to carbohydrate catabolism was 15,397, accounting for 11.86%. The number of genes related to catabolism of synthetic secondary metabolites was 1,666, Among the functions of Environmental information processing, the number of genes related to membrane transport was the highest, accounting for 7.79% of the total genes of all samples, it indicates that the host and intestinal flora are constantly exchanging substances. The number of genes related to genetic information processing, such as gene replication, transcription, translation and repair, was 14,859, accounting for 11.62 percent of the total genes. According to annotation results, there are a large number of genes related to host diseases in intestinal flora, with the number of genes reaching 8167, accounting for 6.39%. There are also genes regulating cell processes in intestinal flora, among which the number of genes regulating cell growth and apoptosis is 1324, accounting for 1.04%. The number of genes regulating cell movement was 1336, accounting for 1.05%. The number of genes regulating the cell community was 3,542, accounting for 2.77%. The number of genes regulating the transport and catabolism of cells was 1004, accounting for 79%. The number of genes related to the biological system was 3849, accounting

for 3.01% of the total genes. In this function, the number of genes related to the endocrine system was up to 1372, accounting for 1.07%.

After CAZy database annotation, the number of genes corresponding to Glycoside hydrolases was 5267. Glycoside hydrolases are mainly composed of glycoconjugates and glycoconjugates. The corresponding number of Glycosyltransferase genes were 3,347, the main function is to attach the activated sugar groups to different receptor molecules. The corresponding number of Carbohydrate-binding modules genes were 1,421, the corresponding number of Carbohydrate Esterases genes were 542, the number of Polysaccharide lysates corresponding genes were 51, the corresponding number of genes in Auxiliary activities were 174. These enzymes complete the degradation and modification of carbohydrates and the formation of glycosidic bonds. CARD (the Comprehensive Antibiotic Research Database) as a relatively complete Database of Resistance genes, its core is the ARO (Antibiotic to hold Ontology), and ARO Database includes the target and mechanism of Antibiotic Resistance of Antibiotic Resistance genes and the relevant term. According to the results of the annotation found multiple drug-resistant Mexb protein has the highest number of genes encoding.

Declarations

Ethics approval and consent to participate

This work was carried out in compliance with the current laws in China.

Consent for publication

The written informed consent forms were taken from the volunteers.

Availability of data and materials

All the raw sequences were submitted to the NCBI Sequence Read Archive, under Accession Number SRP265006.

Competing interests

The authors declare that they have no competing interests.

Funding

the National Natural Science Fund of China

Authors' contributions

All authors have contributed to this research work. All authors read and approved the final manuscript.

Acknowledgements

We are grateful for support from the National Natural Science Foundation of China.

References

1. Brum J, Ignacio Espinoza JC, Roux S, Doulcier G, Acinas S, Alberti A, Chaffron S, Cruaud C, de Vargas C, Gasol J, Gorsky G, Gregory A, Guidi L, Hingamp P, Iudicone D, Not F, Ogata H, Pesant S, Poulos B, Sullivan M (2015) Ocean plankton. Patterns and ecological drivers of ocean viral communities. Science 348 https://doi.org/10.1126/science.1261498
2. Brzeziński M (1994) Summer diet of the sable *Martes zibellina* in the Middle Yenisei taiga, Siberia. Acta Theriologica 39:103-107. https://doi.org/10.4098/AT.arch.94-14
3. Buchfink B, Xie C, Huson DH (2015) Fast and sensitive protein alignment using DIAMOND. Nature Methods 12(1):59-60. https://doi.org/10.1038/nmeth.3176
4. Bunyavanich S (2019) Food allergy: could the gut microbiota hold the key? Nature Reviews Gastroenterology & Hepatology https://doi.org/10.1038/s41575-019-0123-0
5. Dubinin EA (2010) On sexual dimorphism in the winter diet of the sable (*Martes zibellina* L.). Russian Journal of Ecology 41(3):244-248. https://doi.org/10.1134/S1067413610030082
6. Ellegaard KM, Engel P (2019) Genomic diversity landscape of the honey bee gut microbiota. Nature Communications 10(1):446. https://doi.org/10.1038/s41467-019-08303-0
7. Federici M (2019) Gut microbiome and microbial metabolites: a new system affecting metabolic disorders. Journal of Endocrinological Investigation https://doi.org/10.1007/s40618-019-01022-9
8. Feng Q, Liang S, Jia H, Stadlmayr A, Tang L, Lan Z, Zhang D, Xia H, Xu X, Jie Z, Su L, Li X, Li X, Li J, Xiao L, Huber-Schönauer U, Niederseer D, Xu X, Al-Aama JY, Yang H, Wang J, Kristiansen K, Arumugam M, Tilg H, Datz C, Wang J (2015) Gut microbiome development along the colorectal adenoma–carcinoma sequence. Nature Communications 6(1):6528. https://doi.org/10.1038/ncomms7528
9. Feng W, Zhang J, Jakovlić I, Xiong F, Wu S, Zou H, Li W, Li M, Wang G (2019) Gut segments outweigh the diet in shaping the intestinal microbiota composition in grass carp Ctenopharyngodon idellus. AMB Express 9(1):44. https://doi.org/10.1186/s13568-019-0770-0
10. Gao H, Chi X, Qin W, Wang L, Song P, Cai Z, Zhang J, Zhang T (2019) Comparison of the gut microbiota composition between the wild and captive Tibetan wild ass (*Equus kiang*). Journal of Applied Microbiology 126(6):1869-1878. https://doi.org/10.1111/jam.14240

11. Guan Y, Zhang H, Gao X, Shang S, Wu X, Chen J, Zhang W, Zhang W, Jiang M, Zhang B, Chen P (2016) Comparison of the bacterial communities in feces from wild versus housed sables (*Martes zibellina*) by high-throughput sequence analysis of the bacterial 16S rRNA gene. AMB Express 6(1):98-98. https://doi.org/10.1186/s13568-016-0254-4
12. Harms A, Maisonneuve E, Gerdes K (2016) Mechanisms of bacterial persistence during stress and antibiotic exposure. Science 354(6318):aaf4268. http://science.sciencemag.org/content/354/6318/aaf4268.abstract
13. Huson D, Mitra S, Ruscheweyh H-J, Weber N, Schuster S (2011) Integrative Analysis of Environmental Sequences Using MEGAN4. Genome research 21:1552-60. https://doi.org/10.1101/gr.120618.111
14. Johnson G (2019) High throughput DNA extraction of legume root nodules for rhizobial metagenomics. AMB Express 9(1):47. https://doi.org/10.1186/s13568-019-0771-z
15. Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B, Nielsen J, Bäckhed F (2013) Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature 498(7452):99-103. https://doi.org/10.1038/nature12198
16. Kashtanov SN, Afanasiev KI, Potapov SG, Lazebny OE (2011) Microsatellite analysis of two captive populations of sable (*Martes zibellina* L.). Russian Journal of Genetics 47(12):1438-1443. https://doi.org/10.1134/S1022795411120040
17. Kc D, Sumner R, Lippmann S (2019) Gut microbiota and health. Postgraduate Medicine:1-1. https://doi.org/10.1080/00325481.2019.1662711
18. Li B, Malyarchuk B, Ma Z, Derenko M, Zhao J, Zhou X (2013) Phylogeography of sable (*Martes zibellina* L. 1758) in the southeast portion of its range based on mitochondrial DNA variation: Highlighting the evolutionary history of the sable. Acta Theriologica 58 https://doi.org/10.1007/s13364-012-0100-2
19. Li B, Wu D, Cai Y, Vladimir G M, Zhang W, Xu Y (2018) Genetic individualization of sable *(Martes zibellina* L. 1758) using microsatellites. Animal Cells and Systems 22(4):253-258. https://doi.org/10.1080/19768354.2018.1494039
20. Li J, Jia H, Cai X, Zhong H, Feng Q, Sunagawa S, Arumugam M, Kultima JR, Prifti E, Nielsen T, Juncker AS, Manichanh C, Chen B, Zhang W, Levenez F, Wang J, Xu X, Xiao L, Liang S, Zhang D, Zhang Z, Chen W, Zhao H, Al-Aama JY, Edris S, Yang H, Wang J, Hansen T, Nielsen HB, Brunak S, Kristiansen K, Guarner F, Pedersen O, Doré J, Ehrlich SD, Pons N, Le Chatelier E, Batto J-M, Kennedy S, Haimet F, Winogradski Y, Pelletier E, LePaslier D, Artiguenave F, Bruls T, Weissenbach J, Turner K, Parkhill J, Antolin M, Casellas F, Borruel N, Varela E, Torrejon A, Denariaz G, Derrien M, van Hylckama Vlieg JET, Viega P, Oozeer R, Knoll J, Rescigno M, Brechot C, M'Rini C, Mérieux A, Yamada T, Tims S, Zoetendal EG, Kleerebezem M, de Vos WM, Cultrone A, Leclerc M, Juste C, Guedon E, Delorme C, Layec S, Khaci G, van de Guchte M, Vandemeulebrouck G, Jamet A, Dervyn R, Sanchez N, Blottière H, Maguin E, Renault P, Tap J, Mende DR, Bork P, Wang J, Meta HITC (2014) An integrated catalog of reference genes in the human gut microbiome. Nature Biotechnology 32(8):834-841. https://doi.org/10.1038/nbt.2942

21. Liang X, Bushman FD, FitzGerald GA (2015) Rhythmicity of the intestinal microbiota is regulated by gender and the host circadian clock. Proceedings of the National Academy of Sciences 112(33):10479. http://www.pnas.org/content/112/33/10479.abstract
22. Liu Y, Zheng Z, Yu L, Wu S, Sun L, Wu S, Xu Q, Cai S, Qin N, Bao W (2019) Examination of the temporal and spatial dynamics of the gut microbiome in newborn piglets reveals distinct microbial communities in six intestinal segments. Scientific Reports 9(1):3453. https://doi.org/10.1038/s41598-019-40235-z
23. Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y, Tang J, Wu G, Zhang H, Shi Y, Liu Y, Yu C, Wang B, Lu Y, Han C, Cheung DW, Yiu S-M, Peng S, Xiaoqian Z, Liu G, Liao X, Li Y, Yang H, Wang J, Lam T-W, Wang J (2012) SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. GigaScience 1(1):18. https://doi.org/10.1186/2047-217X-1-18
24. Lyu T, Liu G, Zhang H, Wang L, Zhou S, Dou H, Pang B, Sha W, Zhang H (2018) Changes in feeding habits promoted the differentiation of the composition and function of gut microbiotas between domestic dogs (*Canis lupus* familiaris) and gray wolves (*Canis lupus*). AMB Express 8(1):123. https://doi.org/10.1186/s13568-018-0652-x
25. Ma C, Chen C, Jia L, He X, Zhang B (2019) Comparison of the intestinal microbiota composition and function in healthy and diseased Yunlong Grouper. AMB Express 9(1):187. https://doi.org/10.1186/s13568-019-0913-3
26. Martin TC, Visconti A, Spector TD, Falchi M (2018) Conducting metagenomic studies in microbiology and clinical research. Applied Microbiology Biotechnology 102(20):8629-8646. https://doi.org/10.1007/s00253-018-9209-9
27. Mende D, Waller A, Sunagawa S, Järvelin A, Chan M, Arumugam M, Raes J, Bork P (2012) Assessment of Metagenomic Assembly Using Simulated Next Generation Sequencing Data. PloS one 7:e31386. https://doi.org/10.1371/journal.pone.0031386
28. Monakhov VG (2015) Geographic variation of the sable (*Martes zibellina* L., 1758) within the range, and phylogeography. Russian Journal of Ecology 46(3):279-288. https://doi.org/10.1134/S1067413615030078
29. Monakhov VG, Modorov MV, Ranyuk MN (2018) Genetic Effects of Sable (*Martes zibellina* L.) Reintroduction in Western Siberia. Russian Journal of Genetics 54(3):358-362. https://doi.org/10.1134/S1022795418030079
30. Mustonen A-M, Nieminen P (2006) Fatty acid composition in the central and peripheral adipose tissues of the sable (*Martes zibellina*). Journal of Thermal Biology 31:617-625. https://doi.org/10.1016/j.jtherbio.2006.08.004
31. Mustonen A-M, Puukka M, Seppo S, Paakkonen T, Aho J, Nieminen P (2006) Adaptations to fasting in a terrestrial mustelid, the sable (*Martes zibellina*). Comparative biochemistry and physiology Part A, Molecular & integrative physiology 144:444-50. https://doi.org/10.1016/j.cbpa.2006.03.008
32. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M,

Batto J-M, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Antolin M, Artiguenave F, Blottiere H, Borruel N, Bruls T, Casellas F, Chervaux C, Cultrone A, Delorme C, Denariaz G, Dervyn R, Forte M, Friss C, van de Guchte M, Guedon E, Haimet F, Jamet A, Juste C, Kaci G, Kleerebezem M, Knol J, Kristensen M, Layec S, Le Roux K, Leclerc M, Maguin E, Melo Minardi R, Oozeer R, Rescigno M, Sanchez N, Tims S, Torrejon T, Varela E, de Vos W, Winogradsky Y, Zoetendal E, Bork P, Ehrlich SD, Wang J, Meta HITC (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464(7285):59-65. https://doi.org/10.1038/nature08821

33. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, Peng Y, Zhang D, Jie Z, Wu W, Qin Y, Xue W, Li J, Han L, Lu D, Wu P, Dai Y, Sun X, Li Z, Tang A, Zhong S, Li X, Chen W, Xu R, Wang M, Feng Q, Gong M, Yu J, Zhang Y, Zhang M, Hansen T, Sanchez G, Raes J, Falony G, Okuda S, Almeida M, LeChatelier E, Renault P, Pons N, Batto J-M, Zhang Z, Chen H, Yang R, Zheng W, Li S, Yang H, Wang J, Ehrlich SD, Nielsen R, Pedersen O, Kristiansen K, Wang J (2012) A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 490(7418):55-60. https://doi.org/10.1038/nature11450
34. Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, Guo J, Le Chatelier E, Yao J, Wu L, Zhou J, Ni S, Liu L, Pons N, Batto JM, Kennedy SP, Leonard P, Yuan C, Ding W, Chen Y, Hu X, Zheng B, Qian G, Xu W, Ehrlich SD, Zheng S, Li L (2014) Alterations of the human gut microbiome in liver cirrhosis. Nature 513:59. https://doi.org/10.1038/nature13568
35. Rozhnov VV, Pishchulina SL, Meschersky IG, Simakin LV, Lazebny OE, Kashtanov SN (2013) Genetic structure of sable (*Martes zibellina* L.) in Eurasia—analysis of the mitochondrial lineages distribution. Russian Journal of Genetics 49(2):220-227. https://doi.org/10.1134/S1022795413020105
36. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, Rostron T, Cerundolo V, Pamer EG, Abramson SB, Huttenhower C, Littman DR (2013) Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. Elife 2:e01202-e01202. https://doi.org/10.7554/eLife.01202.001
37. Shui Y, Guan Z-B, Liu G-F, Fan L-M (2020) Gut microbiota of red swamp crayfish Procambarus clarkii in integrated crayfish-rice cultivation model. AMB Express 10(1):5. https://doi.org/10.1186/s13568-019-0944-9
38. Svishcheva GR, Kashtanov SN (2011) Reproductive strategy of the sable (*Martes zibellina* Linnaeus, 1758): An analysis of litter size inheritance in farm-raised populations. Russian Journal of Genetics: Applied Research 1(3):221-225. https://doi.org/10.1134/S2079059711030129
39. Tang W, Zhu G, Shi Q, Yang S, Ma T, Mishra SK, Wen A, Xu H, Wang Q, Jiang Y, Wu J, Xie M, Yao Y, Li D (2019) Characterizing the microbiota in gastrointestinal tract segments of Rhabdophis subminiatus: Dynamic changes and functional predictions. MicrobiologyOpen:e789. https://doi.org/10.1002/mbo3.789
40. Tully BJ, Graham ED, Heidelberg JF (2018) The reconstruction of 2,631 draft metagenome-assembled genomes from the global oceans. Scientific Data 5:170203.

https://doi.org/10.1038/sdata.2017.203

41. Vijayvargiya P, Jeraldo PR, Thoendel MJ, Greenwood-Quaintance KE, Esquer Garrigos Z, Sohail MR, Chia N, Pritt BS, Patel R (2019) Application of metagenomic shotgun sequencing to detect vector-borne pathogens in clinical blood samples. PLOS ONE 14(10):e0222915. https://doi.org/10.1371/journal.pone.0222915
42. Villar E, Farrant GK, Follows M, Garczarek L, Speich S, Audic S, Bittner L, Blanke B, Brum JR, Brunet C, Casotti R, Chase A, Dolan JR, d'Ortenzio F, Gattuso J-P, Grima N, Guidi L, Hill CN, Jahn O, Jamet J-L, Le Goff H, Lepoivre C, Malviya S, Pelletier E, Romagnan J-B, Roux S, Santini S, Scalco E, Schwenck SM, Tanaka A, Testor P, Vannier T, Vincent F, Zingone A, Dimier C, Picheral M, Searson S, Kandels-Lewis S, Acinas SG, Bork P, Boss E, de Vargas C, Gorsky G, Ogata H, Pesant S, Sullivan MB, Sunagawa S, Wincker P, Karsenti E, Bowler C, Not F, Hingamp P, Iudicone D (2015) Environmental characteristics of Agulhas rings affect interocean plankton transport. Science 348(6237):1261447. https://science.sciencemag.org/content/sci/348/6237/1261447.full.pdf
43. W.Buskirk S, ma Y, Xu L, Jiang Z (1996) Diets of, and Prey Selection by, Sables (*Martes zibellina*) in Northern China. Journal of Mammalogy 77:725. https://doi.org/10.2307/1382677
44. Wang Z, Wang Q, Zhao J, Gong L, Zhang Y, Wang X, Yuan Z (2019) Altered diversity and composition of the gut microbiome in patients with cervical cancer. AMB Express 9(1):40. https://doi.org/10.1186/s13568-019-0763-z
45. Willson N-L, Nattrass GS, Hughes RJ, Moore RJ, Stanley D, Hynd PI, Forder REA (2018) Correlations between intestinal innate immune genes and cecal microbiota highlight potential for probiotic development for immune modulation in poultry. Applied Microbiology and Biotechnology 102(21):9317-9329. https://doi.org/10.1007/s00253-018-9281-1
46. Wu X, Zhang H, Chen J, Shang S, Yan J, Chen Y, Tang X, Zhang H (2017) Analysis and comparison of the wolf microbiome under different environmental factors using three different data of Next Generation Sequencing. Scientific Reports 7(1):11332. https://doi.org/10.1038/s41598-017-11770-4
47. Yan H, Xu Y, Zhang W, Li B (2017) Complete mitochondrial genome reveals the phylogenetic relationship of sable *Martes zibellina* linkouensis Mitochondrial DNA Part A 28(2):263-264. https://doi.org/10.3109/19401736.2015.1118070
48. Yu Z, Liu C, Fu Q, Lu G, Han S, Wang L, Song L (2019) The differences of bacterial communities in the tissues between healthy and diseased Yesso scallop (Patinopecten yessoensis). AMB Express 9(1):148. https://doi.org/10.1186/s13568-019-0870-x
49. Zeller G, Tap J, Voigt A, Sunagawa S, Kultima J, Costea P, Amiot A, Böhm J, Brunetti F, Habermann N, Hercog R, Koch M, Luciani A, Mende D, Schneider M, Schrotz-King P, Tournigand C, Tran Van Nhieu J, Yamada T, Bork P (2014) Potential of fecal microbiota for early-stage detection of colorectal cancer. Molecular Systems Biology 10 https://doi.org/10.15252/msb.20145645
50. Zhang R, Yang L, Ai L, Yang Q, Chen M, Li J, Yang L, Luan X (2017) Geographic characteristics of sable (*Martes zibellina*) distribution over time in Northeast China. Ecology and evolution 7(11):4016-4023. https://doi.org/10.1002/ece3.2983

51. Zhang X-Y, Sukhchuluun G, Bo T-B, Chi Q-S, Yang J-J, Chen B, Zhang L, Wang D-H (2018) Huddling remodels gut microbiota to reduce energy requirements in a small mammal species during cold exposure. Microbiome 6(1):103. https://doi.org/10.1186/s40168-018-0473-9

Figures

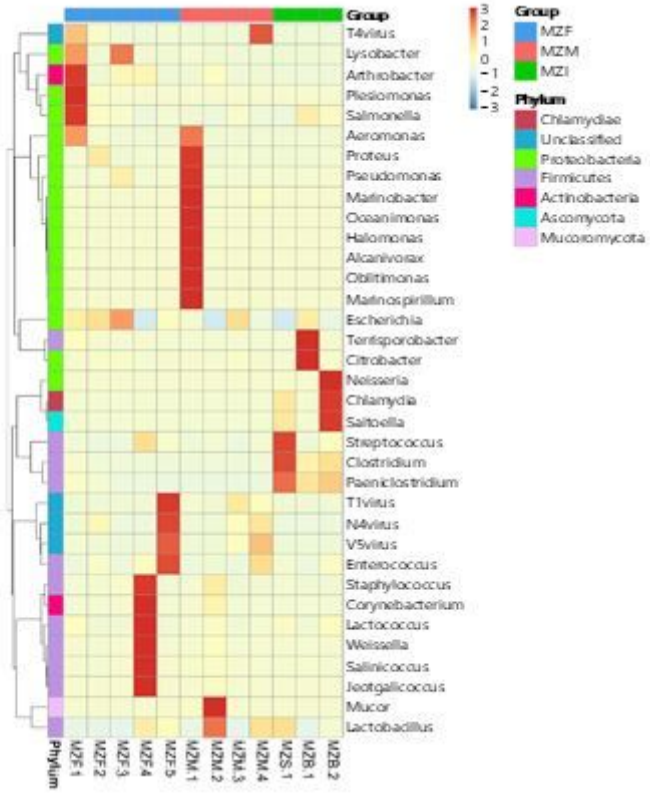

Figure 1

Cluster heat map of relative abundance at genus level

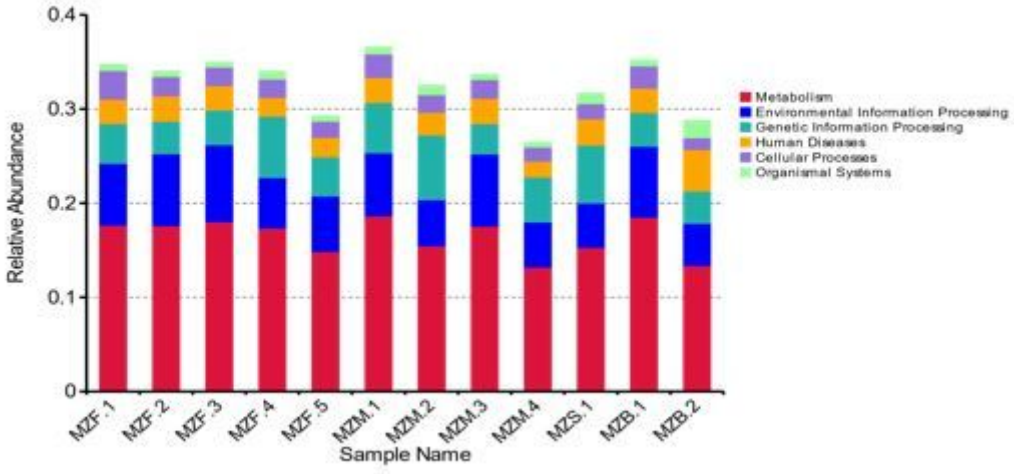

Figure 2

Relative abundance of pathways

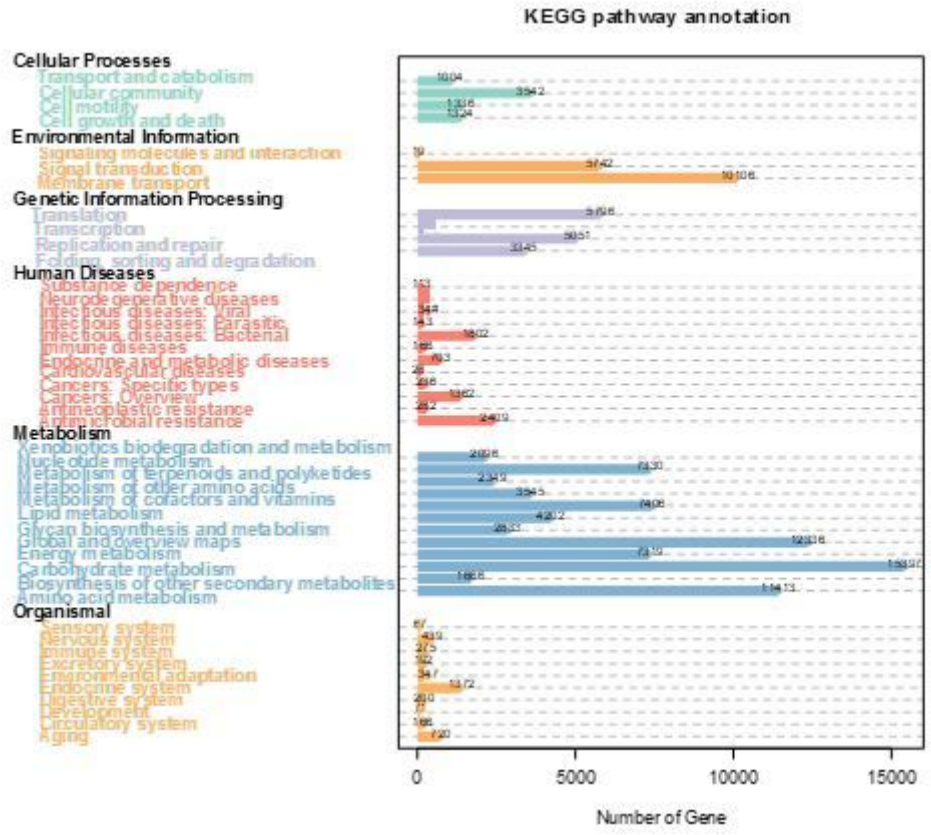

Figure 3

KEGG pathway annotation

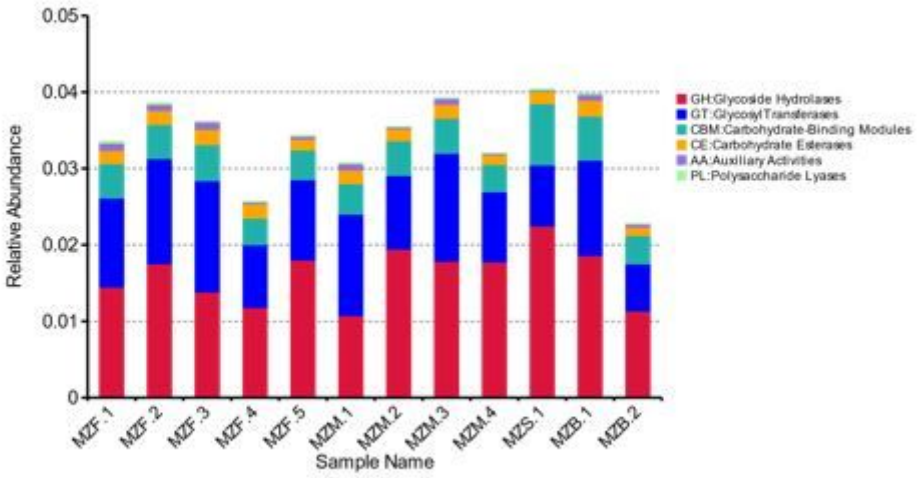

Figure 4

Relative abundance of carbohydrates

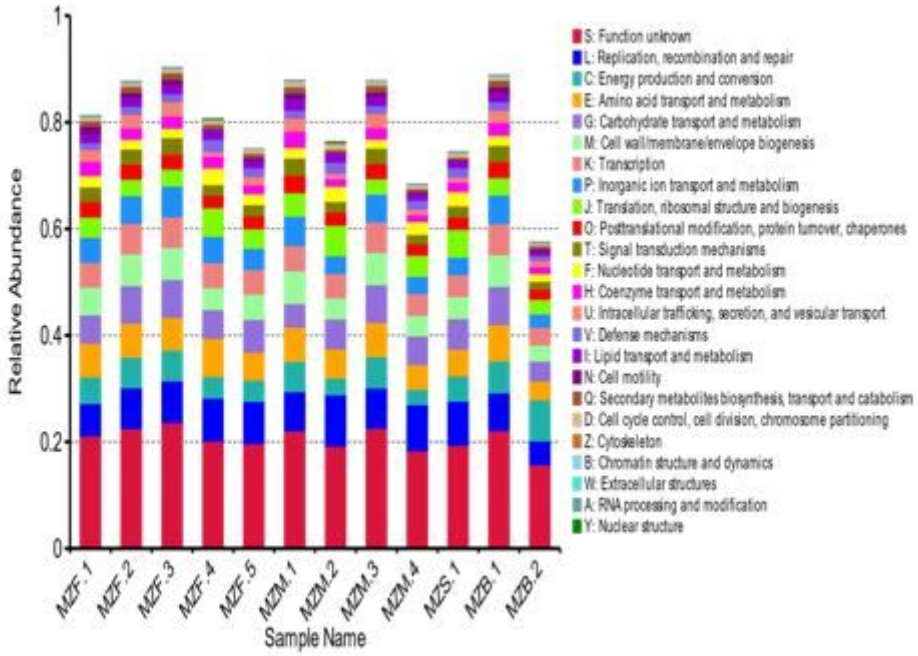

Figure 5

Relative abundance of function class

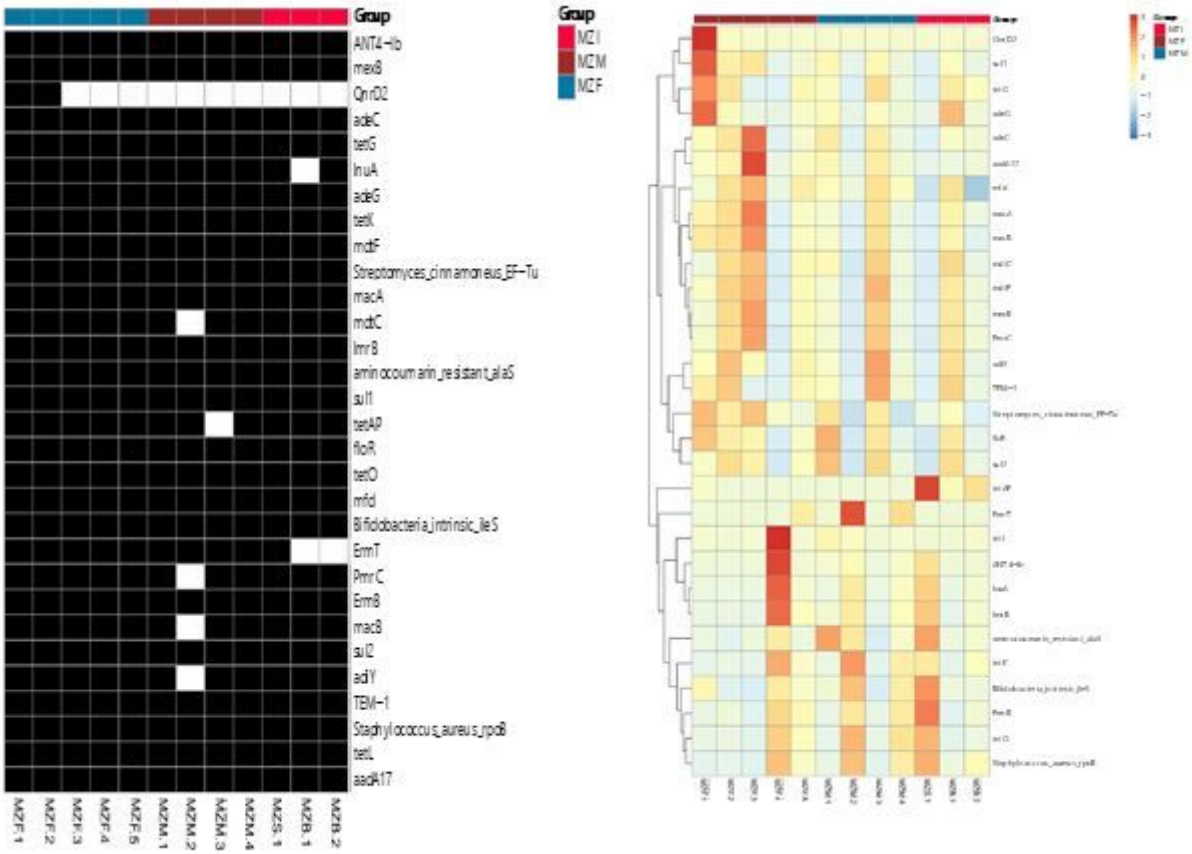

Figure 6

ARO distribution and abundance cluster heat map A) is the heat map of ARO distribution. The horizontal axis is the name of the sample, and the right vertical axis is the name of ARO of the resistance gene type.B) is the ARO abundance cluster heat map. The right vertical axis is the ARO name, and the left vertical axis is the ARO cluster tree.

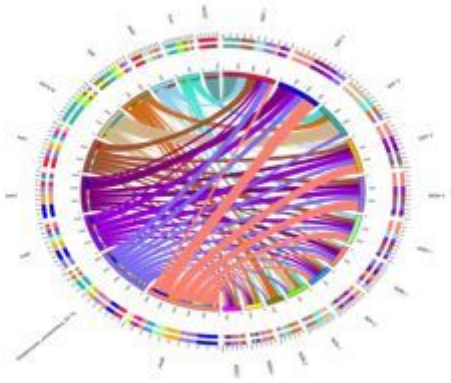

Figure 7

The overview circle graph of resistance gene