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The impact of host genetics on microbe assemblages in three types of mrigal carp (*Cirrhinus mrigala*)

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ABSTRACT

Host–microbiota interactions are molecular and physical interactions between the microbiota and the host that play significant roles in the lifespan of animals. In the present study, two types of gynogenetic mrigal carp (GMCC and GMCW) and the original maternal mrigal carp (MC) were used as a models to investigate host–microbiota interactions. The two types of gynogenetic mrigal carps showed faster growth and better cold tolerance than the MC fish. Compared to those in MC, a large number of goblet cells and higher activity of cellulase and pepsin were detected in the intestine of gynogenetic fish. The composition and abundance of the microbial communities significantly differed among the three types of mrigal carp and water, with Proteobacteria, Firmicutes and Chloroflexi being the most dominant microbes in the GMCC, GMCW and MC fish, respectively. *Pseudomonas*, *Lactococcus* and *Defluviicoccus* were the biomarker genera in the GMCC, GMCW and MC fish. Network analysis revealed no relationships between fish and water, but the dominant genera was positively correlated with the abundance of certain enzymes. Functional analysis revealed the dominant genera associated with amino acid metabolism, fatty acid metabolism and bile acid biosynthesis. Our results suggested that host genetics may affected gut microbe assembly and that the specific metabolic functions of gut microbes may contribute to growth performance through the microbe-gut-liver axis.

1. Introduction

The gut microbiota consists of bacteria, archaea, ciliated protozoa, fungi, and virus that are positioned in the host gut. The gut microbes associated with animals, profoundly influence host physiology and reproduction by regulating metabolism and immune function, as well as complex host behaviors (Lynch and Hsiao, 2019; Legrand et al., 2020; Bereded et al., 2021). Recent studies in zebrafish, mice, rats, and even human have demonstrated that alterations in the gut microbiome influence the development and function of endogenous and neurobiological pathways through the microbe-gut-liver axis, the microbe-gut-brain axis, or the microbe-gut-immunity axis (Stilling et al., 2014; Vuong et al., 2017; Hoban et al., 2018; Chakrabarti et al., 2022; Zhou et al., 2022).

Generally, hosts and their microbiome, coevolved through host–microbiota interactions to maintain the homeostasis of the holobiont throughout the lifespan (Shapira, 2016; Foster et al., 2017). In addition to the influence of microbiome on the host's life process, hosts are able to select and enrich special microbial communities that are different from those in their surrounding environment. Diet has been shown to play the main role in shaping the gut microbiota, but a growing body of evidence suggests that host genetics is another important factor in determining the composition of the gut microbiome assembly (Goodrich et al., 2014; Goodrich et al., 2016; Roehe et al., 2016; Li et al., 2018; Martínez-Álvaro et al., 2022; Naya-Català et al., 2022; Ryu and Davenport, 2022; Small et al., 2023). In wild and experimental fish,

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extensive research has demonstrated that several factors in the host, including genetic variation, genomic regions, subgenomic interactions, and the immune system, regulate the recruitment of specific bacterial genera that possess antibacterial activity in fishes (Boutin et al., 2014; Smith et al., 2015; Tarnecki et al., 2017; Li et al., 2018; Small et al., 2023; Thormar et al., 2024). However, the current knowledge on the effect of the host on microbiome composition has focused mainly on intraspecific information, and the interaction between the host and gut microbiota enrichment in gynogenetic fish has not been studied.

In our previous study, two types of gynogenetic mrigal carp species, GMCC ($2n = 50$) and GMCW ($2n = 50$), were obtained by using ultraviolet-irradiated sperm from common carp (*Cyprinus carpio*, CC, $2n = 100$) and Japanese white crucian carp (*Carassius cuvieri*, WCC, $2n = 100$), respectively, to activate the maturation eggs from mrigal carp (*Cirrhinus mrigala*, MC, $2n = 50$). The appearance of the two types of gynogenetic mrigal carp are highly similar to that of the maternal mrigal carp. The gonad of GMCC and GMCW fish were also observed successfully developed to yolk accumulation stage (stage III) in 1-year-old, respectively. Interestingly, the two types of gynogenetic mrigal carp exhibited rapid growth and better cold tolerance, as the fish successfully survived winter when the water temperature was less than 10 °C for more than 50 days (the maternal fish was death) (Li et al., 2023a; Li et al., 2024; Su et al., 2024). In the present study, the three groups of fish were used as models to investigate the interaction between host genetics and the gut microbiota. The growth, intestinal structure, intestinal enzyme activity and microbial communities were comparatively analyzed. The results of this study are highly important for fish breeding practice and gynogenetic applications.

2. Materials and methods

2.1. Ethics approval and consent to participate

All experiments were approved by the Animal Care Committee of Hunan Normal University and followed the stated guidelines of the Administration of Affairs Concerning Animal Experimentation of China. The experimental fish used in this research were managed according to the guidelines of the Animal Ethics Committee of the Life Science Institute.

2.2. Experimental fish

The specimens, including 2-year-old MC ($n = 40$, each weighing approximately 2000 g) and male CC and WCC fish (each group $n = 10$) were provided by Chengyi Aquaculture Co., Ltd. (Guangzhou, China). All the fish were bred under natural environmental conditions (suitable water temperature ranging from 23 to 26 °C and dissolved oxygen level > 6.0 mg/L^{-1}) and maintained in a recirculating aquaculture tank for one month before the experiments.

Gynogenesis was induced as described in previous studies, with some modifications (Li et al., 2023a; Su et al., 2024). The semen of male CC and WCC fishes was collected, diluted with Hank's solution (1:10) and inactivated using ultraviolet (UV) lamp irradiation for 6–12 min. Then, the mature MC eggs were activated by UV-irradiated sperm from CC and WCC, respectively. At 2 min postfertilization, the two types of embryos were treated at 8–10 °C for 12–16 min and subsequently incubated in room temperature water. Moreover, self-crossing between female and male MCs was performed as a control. Finally, the three types of fish, GMCC, GMCW and MC, were successfully obtained.

2.3. Fish rearing and sampling

The larval fish of the MC, GMCW and GMCC groups were housed in three ponds (20 m × 10 m × 1.2 m) at the State Key Laboratory of Developmental Biology of Freshwater Fish, Hunan Normal University, Changsha, China. During the breeding process, the experimental fish were exposed to ambient light at a suitable pH (6.0–8.0) and dissolved oxygen content (5.5–7.0 mg/L). The three groups of fish were fed with artificial feed (per 1000 g contained fish meal 50.00 g, soybean meal 300.00 g, rapeseed meal 200.00 g, rice bran 350.00 g, and fish oil 35.00 g, among others) routinely two times per day at 9:00 and 15:00. The amount of food provided was gradually increased according to the fish's body weight. Body weight ($n = 40$ in each group) was recorded for 3, 6 and 12-month-old of the fish. In November 2022, the three groups of fish (6 months old) and the water (named MW, WW and CW belonging to MC, GMCW and GMCC, respectively) were collected and sampled ($n = 5$). The anterior intestine (25–30 cm of thick intestines near one end of the mouth) was excised for histological analysis as previously study described (Li et al., 2019). The mid-intestine (about 80–160 cm region of the intestine, total = 200 cm) was excised aseptically, washed with sterile saline to remove surface-associated contaminants, and the contents were gently removed in the phosphate-buffered saline (PBS, Thermo Fisher Scientific, USA), centrifugation at 10000 ×*g* for 5 min and then stored at −70 °C for bacteriome analysis and enzyme detection. In addition, a total of 10 L water was randomly collected in each tank and the microflora in the water samples was obtained by vacuum filtration with a 0.22 μm millipore filter membrane (Sangon Biotech, China).

2.4. Enzyme activity detection

The activity of four enzymes was measured by the double-antibody sandwich ELISA method with ELISA kits (SinoBestBio) according to the manufacturer's protocol as previous described (Li et al., 2018).

2.5. High-throughput sequencing

The bacteriome DNA from 15 intestine samples and 9 water samples was extracted using a Stool DNA Kit (Beijing Solarbio Science & Technology Corporation, China) according to the manufacturers' instructions. The hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified using the primer pair 338F and 806R as previously described (Li et al., 2023a). Sequencing libraries of the 24 samples were constructed and sequenced by Majorbio Biopharm Technology Co., Ltd. (Shanghai, China) using the MiSeq platform (2 × 300 bp, Illumina, San Diego, USA). The raw reads were uploaded to the National Center for Biotechnology Information Sequence Read Archive database (accession Number: PRJNA788359).

2.6. Bioinfomatics and statistical analysis

The raw reads were filtered, assembled and filtered again to obtain clean tags. The clean tags were clustered into operational taxonomic units (OTUs) of more than 97 % similarity using UPARSE (version 9.2.64) pipeline (Edgar, 2013). Classification was determined by comparing the abundant sequences (>5 OTUs in each sample) against the GreenGenes database (version 13.8). The data were subsequently analyzed on the online platform of Majorbio Cloud Platform (www.majorbio.com). Alpha diversity indices (including the Sobs, Shannon and ACE diversity indices) were calculated for each group of samples. The UniFrac distance matrix was used for the analysis of β-diversity, and the R package (version 2.15.3) was used to perform principal coordinate analyses (PCoA) and generate bar graphs and heatmaps. The Wilcoxon test and Kruskal–Wallis were applied to assess differences in the gut microbes among the groups. A Venn diagram of shared and unique genera was generated to visualize the similarities and differences among the three groups of fish. Redundancy analysis (RDA) and canonical correspondence analysis (CCA), and Spearman's correlation heatmap were used to analyze the relationships between enzyme content (including lipase, cellulase, amylase, and pepsin) and dominant microbial communities (at the genus level) (Guo et al., 2017). The strong correlation cutoff was $|r| > 0.6$ and $p < 0.05$. Significantly abundant phyla or genera were identified using linear discriminant analysis (LDA) effect size (LEfSe), which detected the significant ($p <$ value cutoff 0.05 and LDA cutoff 4.0) features of the respective groups. Using PICRUSt2 and FAPROTAX, 16S OTU information of 16S was used to predict the metabolic function of the microbiota based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Douglas et al., 2020). A combined total of 629,136 16S rRNA gene sequences (532.4 Mbp) were generated from 18 samples (3 from the water samples and 3 from the gut contents) was generated. These sequences represented a total of 4450 effective OTUs, 1095 genera, and 45 phyla (Supplemental file 1).

The data were analyzed in Microsoft Excel 2010 and SPSS 25.0 (SPSS, Inc., USA). The data are expressed as the mean ± standard error. One-way analysis of variance was used to evaluate the significance of differences between groups. The threshold for statistical significance was $p < 0.05$.

3. Results

3.1. The formation and growth of the three types of mrigal carp

The production of the two types of gynogenetic mrigal carp, GCMW and GMCC, is outlined in Fig. 1. There was no difference in body weight or length between the MC and GMCW fish at either 3 or 6 months of age. However, the body weight and length of the GMCC fish were significantly greater than those of the MC and GMCW fish ($p < 0.05$). In addition, no MC data were recorded for the 12-month old fish, while the body weight and length of the GMCC were significantly greater than those of the GMCW fish ($p < 0.05$) (Table 1).

3.2. Intestinal enzyme content and histology analysis

Enzymatic activity indicates the potential digestive ability of the fish. The activities of four enzymes, including cellulase, amylase, lipase and pepsin were measured in the intestinal of the MC, GMCW and GMCC fish (Table 2). No significant difference was observed in amylase content among the three groups of fish. The activities of cellulase and pepsin were greater in the GMCW and GMCC groups than in the MC group ($p < 0.05$). However, the lipase activity in the MC group was significantly greater than that in the intestines of the GMCW and GMCC groups ($p < 0.05$).

Table 1
The growth level of MC, GMCW and GMCC.

		MC	GMCW	GMCC
3 month	Body weight	15.36 ± 1.77	15.74 ± 1.31	16.44 ± 1.76
	Body length	9.75 ± 0.63	10.25 ± 0.57	11.03 ± 0.58
6 month	Body weight	75.25 ± 9.32	79.55 ± 8.74	121.45 ± 11.89^{a}
	Body length	17.63 ± 0.88	17.64 ± 0.76	18.73 ± 0.53
12 month	Body weight	–	494.05 ± 45.13	621.45 ± 37.18^{a}
	Body length	–	30.35 ± 0.96	36.23 ± 1.36

Different letters represent a significant difference between the three groups fish ($p < 0.05$, one-way ANOVA test). - mean no data recorded, fish was death. The p value was detected <0.001.

Table 2
Intestinal enzyme activity among MC, GMCC and GMCW.

	MC	GMCC	GMCW
Cellulase (U/g)	0.32 ± 0.03^{a}	0.61 ± 0.16^{b}	0.54 ± 0.16^{b}
Amylase (mgprot/mL)	0.40 ± 0.03	0.47 ± 0.12	0.42 ± 0.07
Lipase (U/gprot)	24.07 ± 2.13^{b}	15.80 ± 3.54^{a}	13.10 ± 3.82^{a}
Pepsin (U/mgprot)	13.52 ± 1.50^{a}	28.57 ± 5.14^{c}	21.56 ± 2.59^{b}

Different letters represent significant difference between the same groups ($p < 0.05$, one-way ANOVA test).

Histological analysis revealed that the number of villi in MC fish was greater than that in GMCC and GMCW fish, which were also thin and straight compared with the two types of gynogenetic fish (curly and thick in GMCW and GMCC). Moreover, there were more goblet cells in the intestine of the GMCC fish (180 ± 25) was large than that in the MC (132 ± 21) and GMCW fish (139 ± 17) (Fig. 2).

3.3. Biodiversity and composition analysis

A lower biodiversity (Shannon index at the OTU level) was calculated based on the distances between microbial communities in the fish groups compared to the water samples. Alpha diversity was no

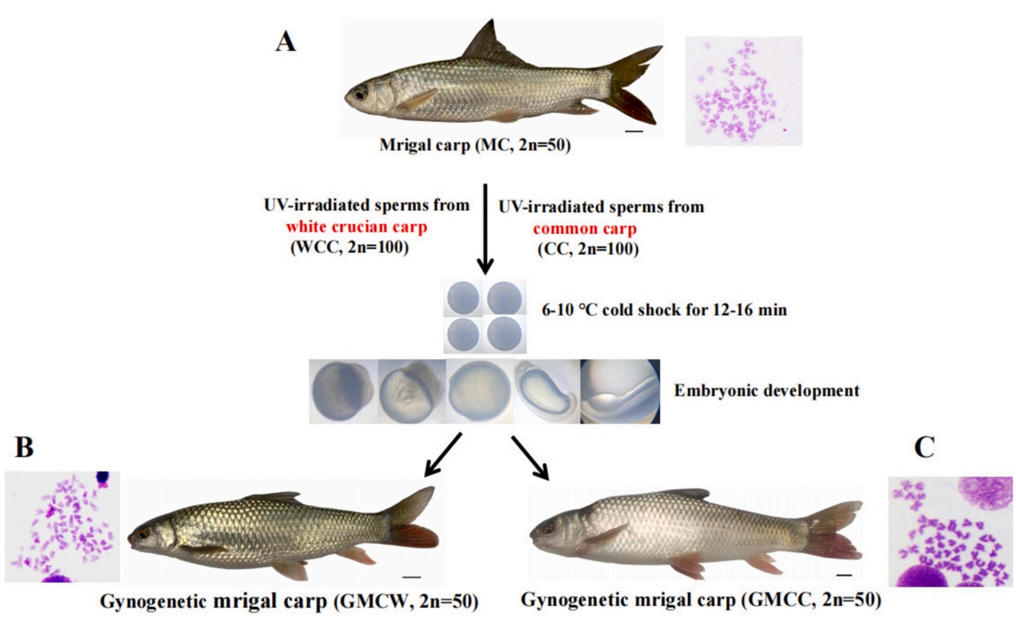

Fig. 1. The formation and phenotypes of the two gynogenetic mrigal carp types, GMCC and GMCW. UV-irradiated sperm from white crucian carp (WCC) and common carp (CC) were used to activate mature eggs of mrigal carp. After cold treatment at 6–8 °C for 16 min to double the chromosomes, two types of gynogenetic mrigal carp species, GMCW and GMCC, were successfully obtained. The phenotypes of 6-month old mrigal carp (A), GMCW (B) and GMCC (C). The chromosomes of the three groups of fish were $2n = 50$. Bar of the fish = 1 cm.

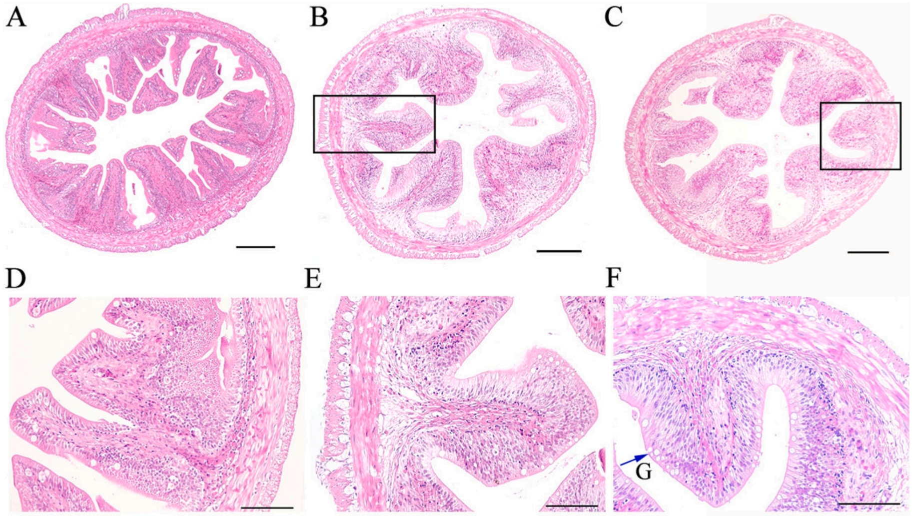

Fig. 2. Representative microstructure of the intestine in three types of mrigal carp. A–C, the intestinal microstructures of MC, GMCW and GMCC fish, D–F are magnified regions of A–C, respectively. G: goblet cells. A–C, bar = 200 μm; D–F, bar = 100 μm.

significantly different between the GMCC and GMCW fish and their water samples, while a greater diversity was detected in the MC fish than in the GMCW (Fig. 3A). Beta diversity (PCoA) analysis at the OTU level revealed that the microbial communities of all 18 samples could be broadly classified into three main clusters: water samples, MC fish and both gynogenetic mrigal carp species (Fig. 3C).

The composition and relative abundance of the microbial communities significantly differed at the phylum and genus levels between the three types of mrigal carp and the water samples. At the phylum level, Proteobacteria, Firmicutes and Chloroflexi were the most abundant phyla in the GMCC, GMCW and MC groups, respectively. Among the three groups of water samples, Proteobacteria, Cyanobacteria and Bacteroidota were stable and were the most abundant phyla (Fig. 3B). At the genus level, *Acinetobacter* and *Pseudomonas* were the most dominant microbiota communities in the GMCC group, *Latilactobacillus*, *Lactococcus*, and *bacilli* were the most dominant genera in the GMCW fish, and *Chloroflexaceae* and *Chloronema* were the most dominant genera in the MC fish (Fig. 3D). Moreover, more genera were detected in the water

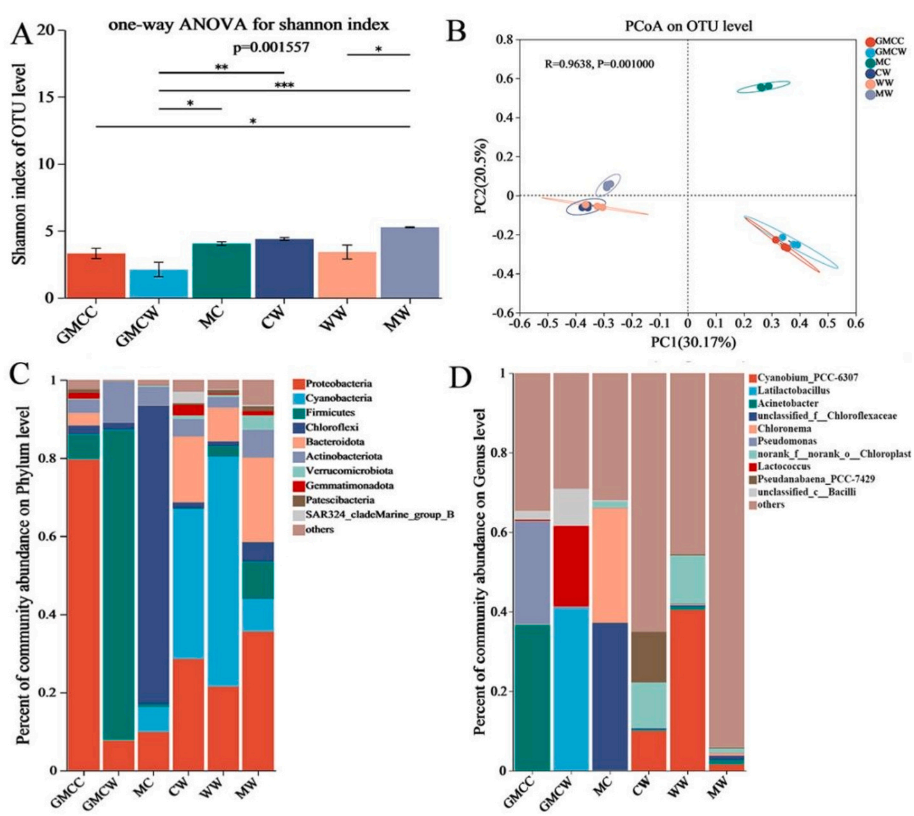

Fig. 3. Biodiversity and composition of the gut microbiota in the three types of mrigal carp. (A), Diversity (Shannon index) was estimated at the genus level between the three groups of fish and water samples. (B), PCA estimates for all microbial taxa in all individuals at the OTU level. (C), Relative abundance of microbial taxa (at the phylum level) between the three types of mrigal carp. (D), Relative abundance of microbial taxa (at the genus level) between the three types of mrigal carp. CW: water samples from the GMCC fish tank, WW: water samples from the GMCW fish tank, and MW: water samples from MC fish tank.

samples than in the three groups of fish (Fig. S1). The analysis of shared and special microbiota communities in the three types of mrigal carp revealed that 125 genera were common to the three groups of fish, with the most abundance being *Chloroflexaceae*, *Acinetobacter*, *Chloronema* and *Pseudomonas* (Fig. S2).

3.4. Biomarkers and correlation analysis between the three types of mrigal carp

A ternary plot (at the family level) showed that Moraxellaceae was a biomarker microbiota in both MC and GMCC fish, while Lactobacillaceae and Streptococcaceae were the biomarker microbiota in the GMCW fish (Fig. 4A). LEfSe analysis revealed that the Bacteroidia (class level) and *Pesudomonas* (genus level) were special biomarkers in the the GMCC fish. Firmicutes (phylum level), *Lactococcus* and *Rhodococcus* (genus level) were the biomarker microbes in the GMCW fish. There was no phylum level biomarker microbiota in the MC fish, however, *Caldilineaceae*, *Defluviicoccus* and *SJA*-15 (genus level) were biomarker microbes in MC fish (Fig. 4B).

RDA/CCA showed that the level of four enzymes strongly correlated with the microbial taxa in the three groups of fish. The levels of enzymes, including cellulase and amylase, were significantly positively correlated with microbiota at the genus level in the GMCW fish, whereas pepsin and lipase was positively correlated with microbiota at the genus level in the GMCC and MC fish (Fig. 4C). Spearman correlation coefficient analysis revealed positive ($R > 0.6$, $P < 0.05$) and negative correlations ($R < -0.6$, $P < 0.05$) between the thirty genera and the four enzymes. Lipase was strongly correlated with most of the dominant genera. Pepsin and cellulase were positively correlated with *Pseudomonas* and *Paracoccus*, and negatively correlated with *Chloroplast*, *Chloronema*, *TG-45*

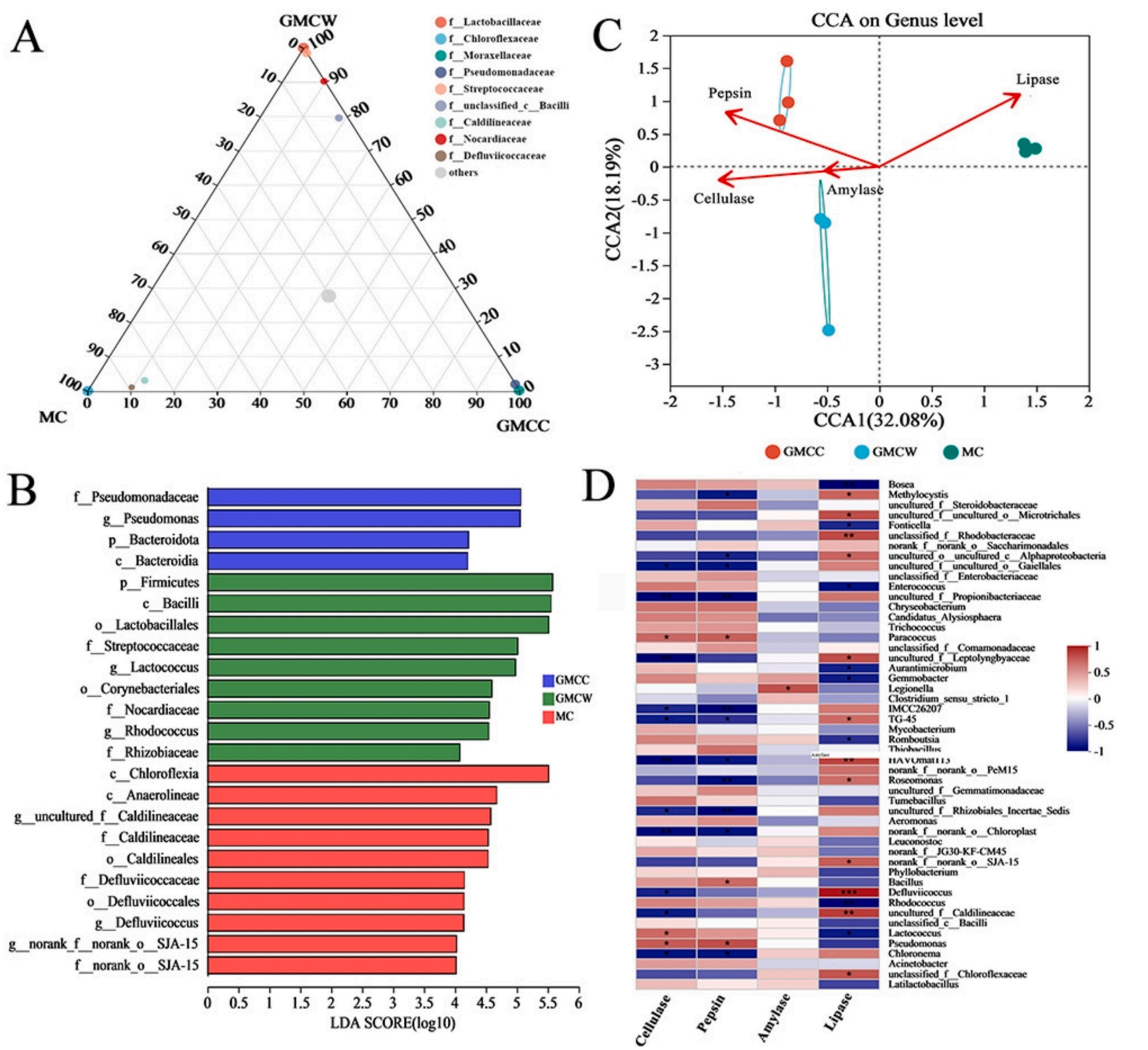

Fig. 4. Correlation analysis and the identification of biomarker microbes in the three types of mrigal carp. (A) Ternary plot showing the proportions and relationships of the gut microbial taxa (at the family level) among the three types of mrigal carp. (B) Bar chart showing the significantly abundant taxa in each group of fish, identified based on LEfSe analysis. (C), Redundancy analysis/Canonical correspondence analysis (RAD/CCA) showing the correlation of four intestinal enzymes and dominant microbial taxa among the three types of mrigal carp. (D), The Spearman correlation heatmap assesses the correlation between microbial taxa classification (30 dominant genera at the average level) and four intestinal enzymes; Blue: positive correlation, orange: negative correlation. *Significant difference between the four groups of fish samples, *$p < 0.05$, ** $p < 0.01$, ** $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and *Gaiellales*. However, amylase activity was less correlated with the thirty dominant genera (Fig. 4D). Network analysis of the 70 dominant microbial communities revealed no relationships between the three mrigal carp species and their waters, but some OTUs cooccurred among the three water samples (Fig. S3).

3.5. Functional prediction of the microbiota communities

To better understand the relationship between the fish and gut microbial communities, the functions of the microbial taxa in the three types of mrigal carp and water samples were predicted. The analysis revealed that a total of eleven metabolic pathways (level 2) were annotated, and the most enriched pathways were global and overview maps and xenobiotic biodegradation and metabolism in both GMCC and GMCW fish. Moreover, the pathways were more highly enriched in the gynogenetic mrigal carp than that in the MC fish (Fig. 5A). For the enriched pathways at level 3, nucleotide metabolism and primary bile acid biosynthesis were the most enriched pathways in both the GMCC and GMCW fish, and bile secretion and G protein-coupled receptors were the most enriched pathways in MC fish (Fig. 5B). Other pathways, such as lysine degradation, vitamin B6 metabolism and fatty acid metabolism and secondary bile acid biosynthesis were the significantly enriched in the GMCC and GMCW fish, respectively.

4. Discussion

Artificial gynogenesis is an important method for accelerating the selective breeding of varieties and populations. In many farmed fishes, artificial gynogenetic offspring exhibit superior traits such as rapid growth, good meat quality and improved stress resistance. Gynogenetic grass carp offspring showed 16.31 % faster growth rate than wild types by decrease input–output ratio (1.0:1.3) (Wang et al., 2022). The natural gynogenetic blunt snout bream is a high-quality gynogenetic fish that is more nutrition and has better muscle performance than maternal fish (Wu et al., 2022). Gynogenetic blunt snout bream can maintain normal liver parenchyma and activate some signaling pathways, such as the NF-kappa B signaling pathway, to resist hypoxia (Gong et al., 2020). After herpesvirus challenge, three gibel carp gynogenetic clones survived by altering the expression of innate and adaptive immune genes (Lu et al., 2019). Our previous study revealed that the GMCC population exhibited improved cold tolerance, with an increase in the number of mucus cells in the gill and the activity of pathways such as metabolism and immunity to cope with cold stress (Li et al., 2023a; Li et al., 2024). Recently, accumulating research has shown the incorporation of paternal genetic material in some gynogenetic fishes, which may result in hybrid effects (Long et al., 2020; Mao et al., 2020; Li et al., 2023b). In the present study, the two types of gynogenetic offspring showed a faster growth rate, a better cold tolerance, possibly because host genetics influence the development of gut and liver tissues which contributes to nutrient metabolism and host immune defense (Fig. 2) (Grootjans et al., 2013; Dawood et al., 2020).

Human and animal model studies have shown that the host exerts control over the selection and regulation of its microbiota. Ongoing work in mice, cattle and UK twins has demonstrated that host genetics, including genotype, immune system genes and quantitative trait loci (QTLs), determine the abundance and composition of the gut microbiome (Benson et al., 2010; Goodrich et al., 2016; Li et al., 2019). This phenomenon is also found in fish, where even before hatching, the fish host indirectly exerts pressure on the selection of its microbiota through the apparent species-specific binding to the surface of the chorion. The amount of colonized microbes increases with fish development as a result of the increasing amount of gut space and the development of the immune system (Xiao et al., 2022). Studies in farmed fish also revealed that host genotype influences microbiota taxonomic composition and that specific host genomic regions regulate the recruitment of specific bacterial genera (Boutin et al., 2014; Small et al., 2023). In zebrafish, commensal microbe recognition was found to be mediated through TLR/MyD88 signaling pathway, and MyD88 modulates innate immune responses to microbes (Cheesman et al., 2011; Galindo-Villegas et al., 2012). Studies in hybrid fish have also shown that host hybridization has

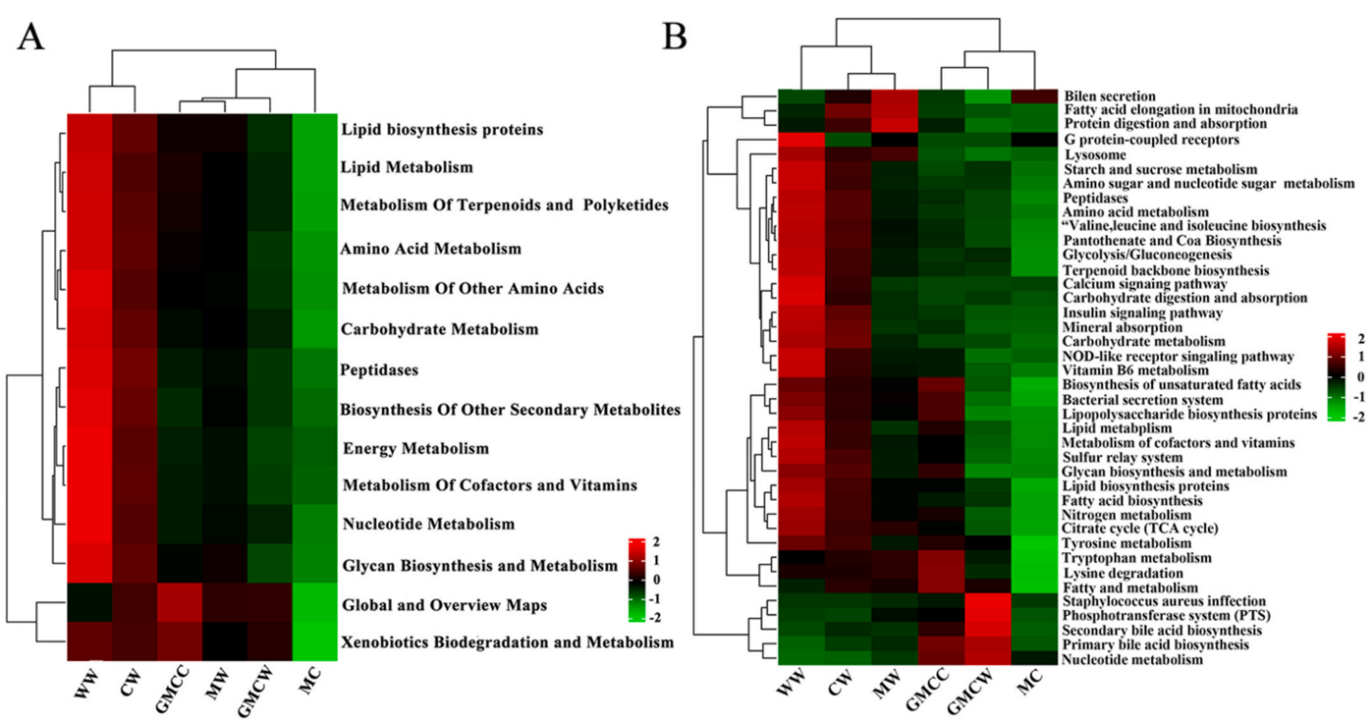

Fig. 5. Functional annotation clustering heatmap of microbial taxa in the three types of mrigal carp and in the water. KEGG functional pathway enrichment at level 2 (A) and level 3 (B). GMCC:Gynogenetic mrigal carp obtained from UV-irradiated sperm of common carp; GMCW: gynogenetic mrigal carp obtained from UV-irradiated sperm of white crucian carp, MC: mrigal carp, CW: water sample from the GMCC fish tank, WW: water sample from the GMCW fish tank, MW: water sample from the MC fish tank.

a sizeable effect on shaping the gut microbiota assemblages (Kokou et al., 2018; Li et al., 2019). Although host habitat is the major determinant of the gut microbiome of fish, namely the gut microbiome were generally colonized from the water and diet (Kim et al., 2021), we detected no relationship between the fish and water (Fig. S1 and Fig. S3), indicating water environment dynamic changes while gut microbiome relatively stable. This phenomenon was also observed in naturally spawned salmonid eggs, which have shown that there are egg-specific microbiome that do not correspond to the surrounding water environment (Wilkins et al., 2015). Moreover, the core microbes (at both in the phylum and genus levels) were significantly different among the three groups of mrigal carp and water samples (Fig. 3), indicating the colonists microbes may decline in abundance when they are excreted or reproduce poorly, and than the fish can harbor the core microbes that reside in the gut.

As mentioned above, the insertion of paternal DNA (fragments or base loci) results in genomic differences between gynogenetic offspring and original maternal fish (Li et al., 2023a). Therefore, we suspected that the differences in genomics alone are the main factor that affect phenotype variability (growth and development) and the gut microbiota composition. The latter seems to be much more complex. In this study, higher enzyme activity was detected in gynogenetic offspring (Table 2), and a greater number of goblet cells was observed in the intestine (Fig. 2). The host liver, can transports enzymes, bile salts and antimicrobial molecules to the intestinal lumen and then regulates the composition of microbes (Tripathi et al., 2018). Besides, goblet cells are thought to play a major role in regulating microbes at mucosal surfaces and restricting the growth of opportunistic pathogens (Molnár et al., 2018). However, determining the regulatory mechanism of genes in the gut-liver axis that influence microbiota assembly require further investigation.

The host-microbiota interactome, which describe the molecular and physical interactions between the microbiota and the host, has been demonstrated to function through the microbiota-gut-liver axis, the microbiota-gut-brain axis, etc. The gut microbiome can produce a variety of digestive enzymes and alter gut histology, thereby directly or indirectly impacting distal gut biomolecules and the expression of special genes and finial contribute to host health and growth (Zhu et al., 2011; Zhang et al., 2019; Henriques et al., 2020; Su et al., 2021). For example, probiotic bacteria including *Bacillus subtilis* and *Clostridium butyricum*, reported been an ideal probiotic bacterium to fish aquaculture that increase feed digestibility, prevent microbial diseases, and avoid water pollution (Olmos et al., 2020). Moreover, gut products such as host and/or microbial metabolites (short-chain fatty acids and secondary bile acids) and microbial-associated molecular patterns (MAMPs), are translocatd to the liver via the portal vein and influence liver metabolism and brain behaviors (Schoeler and Caesar, 2019). Several studies have reported that special microbes, such as *Clostridia*, *Pseudomonas*, *Lactococcus*, and *Bifidobacteria*, mediate crosstalk between the microbiota and host (Yukgehnaish et al., 2020). In mandarin fish (*Siniperca chuatsi*), certain taxa, including *Lactococcus*, *Klebsiella* and *Woeseia*, may be closely related to the digestion and absorption of compound diets and contribute to growth (Chen et al., 2022). In the Huanghe carp new strain, the gut bacterial community structure is associated with growth performance and gene expression through the microbiome-gut-brain axis (Su et al., 2021). In an allodiploid hybrid fish, the dynamic changes of the dominant gut microbial communities during host development contributed to dietary adaptation (Li et al., 2023a). In the present study, several biomarker microbes were also identified in the three types of mrigal carp, and the functions of the most dominant microbes contributing to bile acid, fatty acid and amino acid biosynthesis were predicted (Figs. 4 and 5). We suspected that the host genetics would impacted the microbes composition in the three types of fish, in turn, the microbes were influenced by diet and through the microbiome-gut-liver axis (Su et al., 2021; Lin et al., 2021). But, how the changes in gut microbiome drive the phenotypic variability, and how the changes of gut microbiome contribute to nutrition also need further investigation.

5. Conclusion

In the present study, we observed that the two types of gynogenetic fishes (GMCC and GMCW) exhibited faster growth and batter cold tolerance than maternal mrigal carp. We investigated interactions between the host and microbiota in the three mrigal carp. A greater number of goblet cells and more active enzymes were detected in the intestines of the GMCC and GMCW fish than in those of the MC fish. The composition and abundance of the microbial communities significantly differed among the three types of mrigal carp but not in waters, and the biomarker genera were identified. The dominant genera were predicted to be associated with amino acid metabolism, fatty acid metabolism and bile acid biosynthesis in the three groups of fish. Network analysis revealed no relationships between fish and water, but the dominant genera was positive correlated with intestinal enzymes. Our results suggest that host genetics greatly affected the gut microbe enrichment and that gut microbes possess specific metabolic functions that may contribute to growth performance through the microbiome-gut-liver axis.

CRediT authorship contribution statement

Wuhui Li: Writing – review & editing, Methodology, Funding acquisition. **Yongchun Li:** Writing – original draft. **Shujuan Chen:** Methodology, Data curation. **Yan Miao:** Visualization, Methodology. **Ye Yuan:** Visualization, Data curation. **Rongxi Xiang:** Methodology, Data curation. **Liang Kai:** Validation, Methodology. **Zexun Zhou:** Writing – original draft. **Hongqin Li:** Data curation. **Jisen Su:** Data curation. **Shi Wang:** Data curation. **Zhongyuan Shen:** Resources, Methodology. **Lei Zeng:** Software, Methodology. **Li Ren:** Software, Data curation. **Qinbo Qin:** Writing – review & editing. **Shaojun Liu:** Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.741696.

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