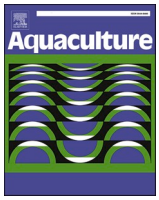
Aquaculture 587 (2024) 740853

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Improved traits of proximate composition, liver antioxidant capacity and feeding habits in diploid hybrids from female *Micropterus salmoides* × male *Lepomis cyanellus*

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ARTICLE INFO

Keywords:
Micropterus salmoides
Distant hybridization
Antioxidant enzyme activities
Pharyngeal tooth
Proximate composition

ABSTRACT

Distant hybridization has been widely used as an effective strategy for improving fish germplasm. The largemouth bass (*Micropterus salmoides*, LB) is a popular fish worldwide due to its excellent performance in aquaculture, edibility, and sport fishing. However, the carnivorous nature of LB increases the cost and difficulty of culture and poses a threat to wild fishery resources. At present, there is a lack of research on improving the germplasm of LB. In this study, hybrid offspring (LG) were produced by crossing female LB and male green sunfish (*Lepomis cyanellus*, GS). The biological characteristics of the LG, such as muscle proximate composition, liver antioxidant enzyme activity, and pharyngeal tooth structure, were analyzed to evaluate their potential for aquaculture. The proximate composition analysis revealed that LG has a higher crude protein content of 20.07% compared to LB (18.1%) and GS (18.7%) ($P < 0.05$), and a lower crude fat content of 0.57% than LB (1.17%) ($P < 0.05$). Furthermore, LG had a significantly higher essential amino acid content (7.20%) than LB (6.43%) ($P < 0.05$), but not significantly different from GS (6.82%) ($P > 0.05$). In terms of fatty acid content, LG had significantly lower levels of saturated fatty acids (0.083%) compared to LB (0.153%) and GS (0.169%) ($P < 0.05$). Additionally, the amount of arachidonic acid (C20:4n6, ARA) in LG (0.058%) was significantly higher than LB (0.019%) and GS (0.043%) ($P < 0.05$). Regarding antioxidant enzyme activities in the liver, LG exhibited significantly higher superoxide dismutase (SOD) activity than LB and GS ($P < 0.05$). Electron microscopic observation of the pharyngeal tooth showed that the tooth number of LG (160.67 ± 3.93) was significantly lower than that of LB (181.67 ± 4.97) ($P < 0.05$) and significantly higher than that of GS (108.00 ± 3.58) ($P < 0.05$), while the tooth diameter of LG (0.076 ± 0.023) was significantly higher than that of LB (0.057 ± 0.016) ($P < 0.05$) and significantly lower than that of GS (0.108 ± 0.044) ($P < 0.05$). In conclusion, this study found that LG exhibited improved traits in proximate composition and hepatic antioxidant enzyme activity. The pharyngeal tooth characteristics indicated that LG may have different food selection and processing capabilities. This is the first report on biological characteristics in offspring produced from a distant hybridization between LB and GS, providing new insights into the genetic improvement and germplasm innovation of LB.

1. Introduction

Largemouth bass (*Micropterus salmoides*, LB) is a popular freshwater fish, noted for its lack of intermuscular bones, rapid growth and favorable meat quality. Since its introduction to China, the scale of aquaculture and production has increased year by year (Bai et al., 2008; Du et al., 2021; Hussein et al., 2020). The total production of LB in China in 2022 was about 802,486 tons (Yu et al., 2024). However, several

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https://doi.org/10.1016/j.aquaculture.2024.740853
Received 26 January 2024; Received in revised form 7 March 2024; Accepted 20 March 2024
Available online 21 March 2024

problems have attracted widespread attention but have not been effectively addressed in the aquaculture of LB. On the one hand, extended inbreeding of fish can lead to genetical characterization decline of LB, such as reduced growth and weaker resistance to disease and stress (Bondari and Dunham, 1987; Deng et al., 2011; Hu et al., 2021). On the other hand, the LB is carnivorous fish in feeding habits. Although formulated feed can be used to domesticate commercially cultivated LB, a significant quantity of fish meal must be included to fulfil the protein needs of the LB. Unfortunately, the prices of fish meal are escalating persistently (Hu et al., 2022; Ma et al., 2020). Furthermore, early domestication can effectively reduce the problem of cannibalism in juvenile fish, but there is limited research on the dietary changes of LB. Consequently, breeding new varieties is advantageous for the sound progress of LB aquaculture.

Distant hybridization is a prevalent technique in fish breeding, heterologous genomes from different species can be promptly integrated into hybrid offspring through artificial fertilization, yielding phenotypes that exhibit significant variability (Chen et al., 2018). For example, some hybrid fish demonstrate faster growth rates and increased disease resistance (Gu et al., 2023; He et al., 2013; Ou et al., 2019), while others exhibit more cost-effective herbivorous feeding habits, which are anticipated to decrease farming expenses and enhance farming efficacy (Hu et al., 2023). Furthermore, there are hybrids that exhibit heterosis in terms of crude protein, essential amino acids, and unsaturated fatty acid content (Wang et al., 2018; Zeng et al., 2023). Extensive studies have demonstrated that distant hybridization combinations need to be meticulously designed. Generally, the phylogenetic relationship, chromosome number and trait diversity should be taken into account (Wang et al., 2019; Zhang et al., 2014).

Despite the various methods used for LB breeding research worldwide, there are still many shortcomings. For instance, the long breeding cycles and small changes in target traits in selective breeding are obvious drawbacks. In the 40 years since LB was introduced to China, only two new varieties of LB have been certified through selective breeding, named “Youlu No. 1” and “Youlu No. 3” (He et al., 2022; Li et al., 2017). Regarding cross breeding, limited research into the hybridization of LB has primarily concentrated on close hybridization with Florida subspecies (Khosa et al., 2022; Kleinsasser et al., 1990; Li et al., 2015; Williamson and Carmichael, 1990). Distant hybridization with LB as parents is restricted due to the limited availability of suitable species. Up to now, LB have successfully bred with *Lepomis cyanellus*, *Lepomis mearchirus*, *Micropterus punctulatus, Micropterus dolomieu* and *Micropterus sp. cf. cataractae* to produce viable offspring, either through artificial insemination or natural reproduction (Bangs et al., 2018; Barthel et al., 2010; Godbout et al., 2009; Li et al., 2020; Whitt et al., 1977). However, whether close hybridization or distant hybridization of LB, there has been a lack of systematic research or divergent views on the biological characteristics and commercial aquaculture potential of the progeny.

As an important feeding organ in fish, the pharyngeal tooth is involved in the capture, processing and transport of food, which correlates with the feeding habits of fish (Abbate et al., 2012; Lauder, 1983). For instance, straight or curved pharyngeal tooth is ideal for piercing meat and removing scales, while molar-shaped pharyngeal tooth is suitable for crunching shelled organisms and plant seeds. Spatulate tooth, on the other hand, is more advantageous for scraping algae and shredding plants (Tibbetts et al., 2008; Tibbetts and Carseldine, 2003). Our previously published study showed that hybrids (*Megalobrama amblycephala* × *Culter mongolicus*) were biased toward herbivory, as their pharyngeal tooth characteristics were similar to that of their herbivorous parents (Hu et al., 2023). Therefore, the structural adaptation of fish tooth to food, which can be used as an indicator of fish diet.

Fish liver is involved in important functions such as digestion and immunity (Long et al., 2021; Nakamura and Nishina, 2009). However, external factors such as temperature, ammonia, heavy metals, and imbalanced feed ratios can cause an overproduction of reactive oxygen species (ROS) in fish, leading to oxidative stress (Lu et al., 2017; Shahjahan et al., 2022; Yang et al., 2020). This can negatively impact growth and metabolism and has been linked to the development of various fish diseases. Like other organisms, fish also rely on antioxidant enzymes to scavenge ROS. For example, superoxide dismutase (SOD) catalyzes the formation of hydrogen peroxide (H_2O_2) and oxygen (O_2) from superoxide anion (O_2^-). Meanwhile, catalase (CAT) and glutathione peroxidase (GSH-PX) decompose hydrogen peroxide (H_2O_2), preventing the generation of the more cytotoxic hydroxyl radical (OH^-). Non-enzymatic antioxidants, such as glutathione (GSH), also perform similar functions. When the total antioxidant capacity (T-AOC) is insufficient to clear ROS, excessive ROS can cause lipid peroxidation (LPO) and produce malondialdehyde (MDA) (Bandeira Junior and Baldisserotto, 2021; Hoseinifar et al., 2021; Subaramaniyam et al., 2023). Therefore, the activity of antioxidant enzymes in the liver of fish may reflect their tolerance to harsh environments and diseases.

In this study, green sunfish (*Lepomis cyanellus*, GS) was selected as the male parent due to its omnivorous nature, gentle temperament compared to the LB, and tolerance of high temperatures and low oxygen levels (Babbington et al., 2023; Cortemeglia and Beitinger, 2008; Zheng et al., 2006). This species is valuable in commercial aquaculture and sport fisheries, as well as for its ornamental qualities. We successfully obtained a large number of hybrid offspring (LG) from a distant hybridization between female LB and male GS. During the farming period, we observed that LG was more easily domesticated by commercial feeds. Additionally, it was less prone to cannibalism and more disease-resistant. In order to evaluate the potential for aquaculture applications of the LG, we examined the appearance, chromosome number, muscle proximate composition, liver antioxidant enzyme activity and pharyngeal tooth structure. This is the first report on biological characteristics in offspring produced from a distant hybridization between LB and GS, providing novel insights into the genetic improvement and germplasm innovation of LB.

2. Materials and methods

2.1. Ethics statement

The animal welfare of the experimental fish in this study was provided in accordance with the recommendations in the Guidelines for the Care and Use of Laboratory Animals of the National Advisory Committee for Laboratory Animal Research in China and approval was granted by the Animal Care Committee of Hunan Normal University (Permit Number: 4236).

2.2. Experimental fish and crosses

Two-year-old LB and GS were used in hybridization experiments, provided by Hunan Normal University. During artificial induction of spawning, the water temperature was kept constant at 22–24 °C, 10 female LB received a single injection of mixed hormones including HCG (2000 IU/kg), LHRH-A2 (20 μg/kg) and DOM (5 mg/kg), while 10 female GS were given a single injection of HCG (500 IU/kg), LHRH-A2 (5 μg/kg) and DOM (2 mg/kg). In addition, 10 male LB and 10 male GS were injected with a halved dose of hormones without DOM. Due to the differences in body size, they were housed separately in a 300 L tank to avoid aggression and predation. After observing the spawning, the female LB were screened out and the mature eggs were quickly stripped into a stainless bowl by squeezing the abdomen. Then, the semen of the GS was stripped in the same way and thoroughly mixed. The fertilized eggs were placed in a glass culture dish and incubated in still water. The fertilization and hatching rates approximate 2000 fertilized eggs were randomly counted. All the above experiments have been the subject of more than three repetitions.

2.3. Morphological traits and ploidy levels

At one year of age, 20 LB, 20 GS and 20 LG were randomly selected for morphological detection. Detection of countable traits was based on previous research(Hu et al., 2018). For the measurable traits, we calculated the ratios of total length/body length (TL/BL), body length/body height (BL/BH), body length/head length (BL/HL), body length/tail length (BL/TL), tail length/tail height (TL/TH), head length/snout length(HL/SL), head length/eye diameter (HL/ED) and head length/head length behind the eyes (HL/HLBE).

Ploidy was determined by detecting the mean DNA content of erythrocytes using flow cytometry, with specific methods referring to previous studies. To further determine ploidy, we prepared blood cells chromosomes (Xiao et al., 2014), and randomly observed the morphology and number of chromosomes in 200 metaphase divisions under a microscope.

2.4. Proximate composition in back muscle

LB (10.57 ± 0.64 g), GS (10.22 ± 0.76 g), and LG (10.54 ± 0.75 g) with no significant difference in initial quality were cultured in an open pond at an appropriate density for 180 days, during which artificial fodder (crude protein >40%) was fed twice a day. Water quality parameters are maintained at pH higher than 7 and lower than 8.5, ammonia and nitrite concentrations were lower than 0.01 mg/L, and dissolved oxygen was higher than 5 mg/mL. When sampling, the muscles located at the back of LB, LG, and GS were precisely cut using sharp scissors, six replicates for each fish species. Care was taken to avoid any impurities, such as fish skin and scales, from being mixed. Back muscles were mixed with 50% hydrochloric acid in proportion and then hydrolyzed at 110 °C for 22 h. After filtration and drying, it was diluted with 0.02 mol/L hydrochloric acid. Subsequently, the content of 17 hydrolyzed amino acids was determined using an amino acid analyzer (Hitachi, Japan). 37 fatty acids were analyzed by gas chromatography–mass spectrometry. Meanwhile, the protein content was determined using a Kjeldahl nitrogen analyzer and the total fat was extracted using the Soxhlet extraction method, calculated by weight. The moisture and ash contents were determined according to GB 5009.3–2016 and GB 5009.4–2016, respectively.

Amino acid scoring standard model and egg protein amino acid standard model proposed by FAO/WHO were used to compare and evaluate the essential amino acids with the three types of fish species. Firstly, essential amino acid content was converted into milligrams per gram of crude protein. Then amino acid score (AAS), chemical score (CS) and amino acid index (EAAI) were calculated (FAO/WHO/UNU, 1985; He et al., 2023; Wang et al., 2022).

2.5. Detection of antioxidant enzyme activity in liver

LB (49.25 ± 1.24 g, $n = 30$), LG (49.43 ± 1.18 g, n = 30) and GS (49.31 ± 1.21 g, n = 30) were selected at random and placed in the aquarium (300*L*) for one week, with three replicates for each fish species. The fish were provided with a daily diet consisting of artificial fodder (crude protein >40%) that amounted to approximately 4% of body weight. Water quality parameters, including temperature (22–24 °C), dissolved oxygen (>5 mg/L), pH (7–7.5), ammonia and nitrite levels (<0.01 mg/L), were monitored and controlled. After one week, groups with a survival rate higher than 95% were sampled. Feeding was stopped 24 h before sampling. Six individuals from LB, LG and GS were randomly selected. Fresh liver tissue was extracted and placed onto an ice-cold culture dish. Exactly 0.1 g of liver tissue was weighed and transferred to a centrifuge tube. The sample was then diluted with pre-cooled physiological saline (g/9 ml) and homogenized carefully. Subsequently, centrifuge the sample at 4 °C for 10 min at a speed of 2500 r/min, and utilize the supernatant as the specimen for testing. Commercially available testing kits (Nanjing Jiancheng, China) were used to assess the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX), with a requisite sample concentration causing an inhibition rate of 50% in test results. Additionally, a total-protein detection kit (Nanjing Jiancheng, China) using bicinchoninic acid (BCA) was used to determine total protein concentration in the liver for enzyme activity calculation. Specific experimental steps are laid out in the corresponding instruction manual.

2.6. Scanning electron microscopic observation of pharyngeal tooth

The pharyngeal tooth of LB (29.93 ± 1.93 g, $n = 3$), GS (30.87 ± 1.43 g, n = 3) and LG (30.43 ± 1.72 g, n = 3) were excised using sharp scissors, trimmed to the appropriate size, and cleaned of blood stains with PBS (Sangon, Shanghai, China). They were then placed in electron microscope fixative and fixed for over 2 h at 4 °C, away from light. Following fixation, the samples were rinsed three times with PBS for 15 min each time. Subsequently, the samples were fixed with 1% osmium acid for 1 to 2 h at room temperature, protected from light. The samples were washed three times with PBS and then dehydrated gradually using 30% to 100% ethanol and isoamyl acetate for 15 min each time. After dehydration, the samples were dried in a K850 (Quorum) critical point desiccator and sputter gold plated (Hitachi, Japan) for 30 s. Finally, the samples were examined and photographed for documentation using an SU8100 scanning electron microscope (Hitachi, Japan). The ImageJ software (Version 1.50i, US National Institutes of Health, Bethesda, Maryland, USA) was used to count the tooth number, toothed area, tooth density and tooth diameter.

2.7. Statistical analysis

Statistical analysis was conducted using SPSS Statistics 18.0. Regarding the metrological data, a one-way ANOVA with homogeneity of variance test and multiple comparisons was used. A statistically significant difference was represented by $P < 0.05$. Regarding the attribute data, the raw data were subjected to normality tests using the Shapiro-Wilk test prior to conducting a one-way ANOVA (Soman et al., 2021).

3. Results

3.1. Embryonic development of LG

Fig. 1 shows the embryonic development of LG. There was no significant abnormality in the embryonic development of LG compared with LB (♀) × LB (♂). At a temperature of 20–22 °C, LG fertilized eggs took 50 h and 56 min to hatch. After approximately one week, the fry completed yolk absorption and began feeding. The fertilization rates (72.05 ± 8.00) and hatching rates (61.22 ± 8.68) were significantly lower ($P < 0.05$) than those of the parent LB (81.35 ± 10.80 and 70.47 ± 10.93, respectively) and significantly higher ($P < 0.05$) than those of the parent GS (61.37 ± 6.79 and 49.62 ± 6.14, respectively). The percentage of healthy fry for LG (83.90 ± 2.09) was significantly lower ($P < 0.05$) than that for LB (90.22 ± 1.90) and GS (89.18 ± 2.65). In the cross combination of GS (♀) × LB (♂), abnormal embryonic development was observed, with a very small number of fry hatching but not surviving (Table 1).

3.2. Morphological characteristics

The appearance characteristics of LB, GS and their hybrid offspring LG are shown in Fig. 2, which can be easily differentiated. Regarding countable traits (Table 2), there is a significant difference in the number of lateral scales between LB and LG ($P < 0.05$), while other ratios are not significantly different ($P > 0.05$). As for ratios of measurable traits (Table 3), between LG and LB, there was no significant difference ($P > 0.05$) in any other aspects except for TL/BL, HL/SL and HL/HLBE. All of the ratios except for the HL/SL and HL/ HLBE were significant difference

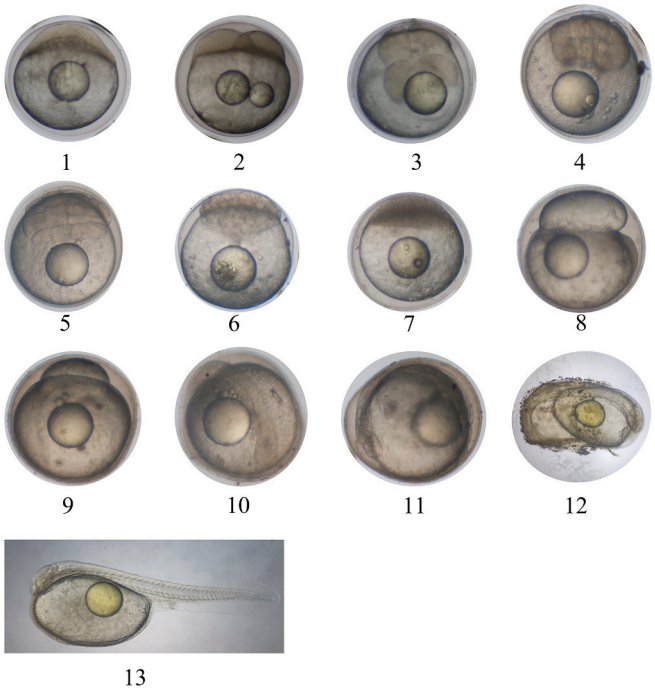

Fig. 1. Embryonic development of hybrid offspring.
1: blastoderm stage; 2: 2-cell stage; 3: 4-cell stage; 4: 8-cell stage; 5: 16-cell stage; 6: 32-cell stage; 7: morula stage; 8: mid-gastrula stage; 9: later gastrula stage; 10: somites stage; 11: muscular effect stage; 12: hatching stage; 13: 24 h after hatching stage.

Table 1
The rate of fertilization, hatching and healthy fry in different groups.

Groups	Fertilization rate (%)	Hatching rate (%)	Rate of healthy fry (%)
LB (♀) × LB (♂)	81.35 ± 10.80[a]	70.47 ± 10.93[a]	90.22 ± 1.90[a]
GS (♀) × GS (♂)	61.37 ± 6.79[b]	49.62 ± 6.14[b]	89.18 ± 2.65[a]
LB (♀) × GS (♂)	72.05 ± 8.00[c]	61.22 ± 8.68[c]	83.90 ± 2.09[b]
GS (♀) × LB (♂)	4.43 ± 1.33[d]	0.53 ± 0.24[d]	0[c]

The identical superscript letters within the same column indicate no significant difference ($P > 0.05$), whereas different superscript letters within the same column indicate a significant difference ($P < 0.05$).

($P < 0.05$) between LG and GS.

3.3. Ploidy levels

The erythrocytes of LB and GS served as controls, and average DNA content of LG were determined through flow cytometry. The results indicated that the average DNA content in the erythrocytes of the LG did not differ from that of the parents, suggesting that the LG were diploid. Regarding the number of chromosomes (Fig. 3), metaphases owing 46 chromosomes accounts for 91.5% in LB, with a karyotype of 2 m + 2st + 42 t. A pair of metacentric chromosomes was readily identifiable within the metaphase. Meanwhile, 89.0% of metaphases indicated 48 chromosomes in GS, with a karyotype of 48 t. As for LG, 85% of metaphases consisted of 47 chromosomes, with a karyotype of 1 m + 1st + 45 t. A metacentric chromosome from LB can be clearly observed. Therefore, LG encompasses half of the genetic material from LB and the other half from GS.

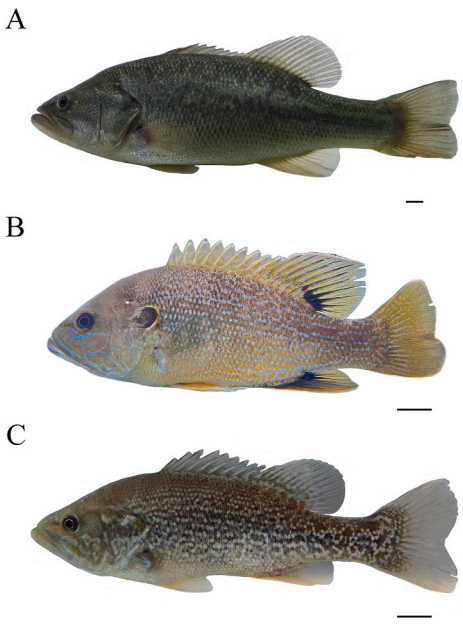

Fig. 2. The appearance of largemouth bass, green sunfish and their hybrid offspring.
(A) The appearance of LB; (B) the appearance of GS; (C) the appearance of LG. Bar = 1 cm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Proximate composition analysis

The proximate composition of the back muscles is shown in Table 4. LG has a crude protein content of 20.07%, which is significantly higher ($P < 0.05$) than both LB (18.1%) and GS (18.7%). Additionally, the crude fat content of LG (0.57%) was significantly lower ($P < 0.05$) than that of LB (1.17%), but not significantly different ($P > 0.05$) from GS (0.73%). In terms of amino acid composition (Table 5), LG exhibits significantly higher ($P < 0.05$) levels of 9 amino acids than the LB. Furthermore, the content of essential amino acids in LG (7.20%) was significantly greater ($P < 0.05$) than in LB (6.43%), but not significantly different ($P > 0.05$) from GS (6.82%). The assessment of essential amino acids is shown in Table 6 , the EAAI of LG (78.39) was higher than that of LB (76.81) and similar to that of GS (78.53). Regarding the fatty acid content (Table 7), LG showed significantly lower ($P < 0.05$) levels of saturated fatty acids (0.083%) when compared to LB (0.153%) and GS (0.169%). The analysis of unsaturated fatty acids revealed that LG (0.316%) had a significantly lower ($P < 0.05$) polyunsaturated fatty acid content than LB (0.506%), while there was no significant difference ($P > 0.05$) with GS (0.315%). Specifically, the docosahexaenoic acid (C22:6n3, DHA) content of LG (0.187%) was significantly lower ($P < 0.05$) than LB (0.213%) and significantly higher ($P < 0.05$) than GS (0.138%). In terms of eicosapentaenoic acid (C20:5n3, EPA) content, LG (0.007%) was not significantly different ($P > 0.05$) from LB (0.007%), but both were significantly lower ($P < 0.05$) than GS (0.015%). Regarding arachidonic acid (C20:4n6, ARA) content, LG (0.058%) had a significantly higher ($P < 0.05$) amount of ARA compared to LB (0.019%) and GS (0.043%).

3.5. Comparison of liver antioxidant enzyme activity

The activities of three antioxidant enzymes, SOD, CAT and GSH-PX, in the liver are shown in Fig. 4. The SOD activity of LG was significantly higher ($P < 0.05$) than that of the parental LB and GS. However, there was no significant difference ($P > 0.05$) in the CAT and GSH-PX activities among the livers of LB, GS, and LG. These results indicate that LG acquired a stronger antioxidant capacity through distant hybridization,

Table 2
Comparison of the countable traits in LB, LG and GS.

Groups	Lateral scales	Upper lateral scales	Lower lateral scales	Dorsal fin rays	Pectoral fin rays	Ventral fin rays	Anal fin rays
LB	65.60 ± 3.69[a] (64–69)	7.83 ± 0.41[a] (7–8)	17.50 ± 1.27[a] (16–20)	X + 12 ~ 14[a]	12 ~ 14[a]	I + 5[a]	III + 10 ~ 12[a]
LG	53.83 ± 1.94[b] 51–55	7.70 ± 0.52[a] (7–8)	17.10 ± 0.99[a] (16–19)	X + 12 ~ 13[a]	12 ~ 13[a]	I + 5[a]	III + 11 ~ 12[a]
GS	52.70 ± 2.41[b] (51–56)	7.60 ± 0.52[a] (7–8)	16.50 ± 0.84[a] (16–18)	X + 12 ~ 14[a]	11 ~ 12[a]	I + 5[a]	III + 10[a]

The identical superscript letters within the same column indicate no significant difference ($P > 0.05$), whereas different superscript letters within the same column indicate a significant difference ($P < 0.05$).

Table 3
Comparison of the measurable traits in LB, LG and GS.

Groups	TL/BL	BL/BH	BL/HL	BL/TL	TL/TH	HL/SL	HL/ED	HL/HLBE
LB	1.13 ± 0.01[a]	3.21 ± 0.13[a]	3.50 ± 0.20[a]	4.45 ± 0.19[a]	1.86 ± 0.13[a]	5.37 ± 0.47[a]	6.12 ± 0.42[a]	1.54 ± 0.05[a]
LG	1.15 ± 0.01[b]	3.07 ± 0.23[a]	3.32 ± 0.15[a]	4.54 ± 0.37[a]	1.79 ± 0.22[a]	4.11 ± 0.49[b]	6.08 ± 0.73[a]	1.80 ± 0.28[b]
GS	1.19 ± 0.02[c]	2.62 ± 0.10[b]	2.99 ± 0.08[b]	5.14 ± 0.30[b]	1.46 ± 0.19[b]	4.02 ± 0.47[b]	5.11 ± 0.66[b]	1.87 ± 0.11[b]

The identical superscript letters within the same column indicate no significant difference ($P > 0.05$), whereas different superscript letters within the same column indicate a significant difference ($P < 0.05$).

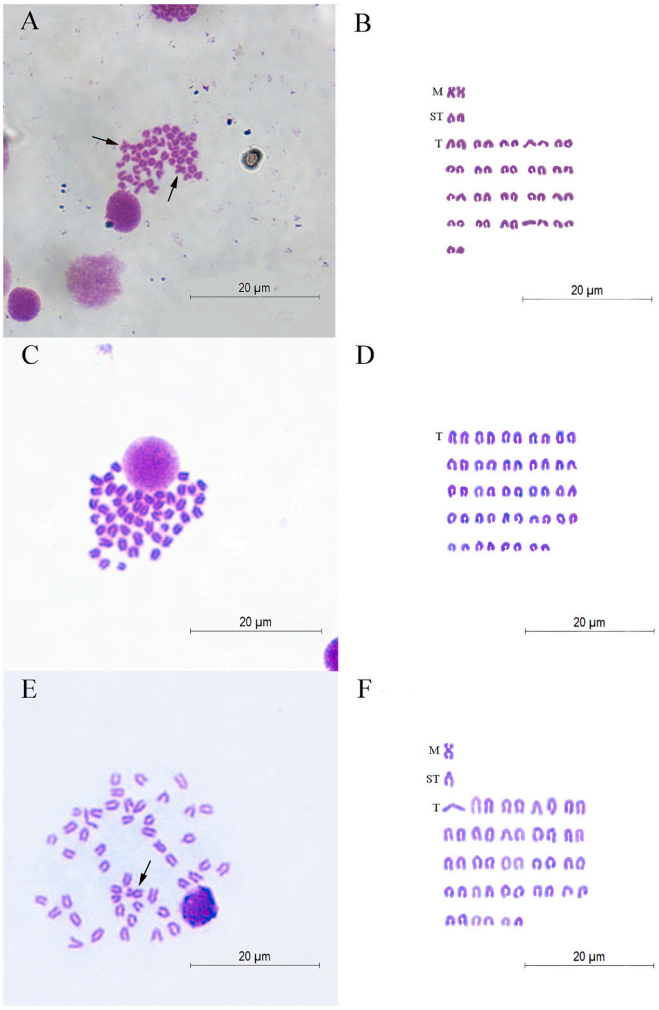

Fig. 3. Chromosomes at metaphase and karyotype in LB, GS and LG.
(A) the 46 chromosomes of LB; (B) the karyotype of LB: 2 m + 2st + 42 t; (C) the 48 chromosomes of GS; (D) the karyotype of GS: 48 t; (E) the 47 chromosomes of LG; (F) the karyotype of LG: 1 m + 1st + 45 t. The metacentric chromosomes are marked by the arrow.

Table 4
Proximate composition of back muscles in LB, LG and GS.

Nutritional composition	LB(g/100 g)	LG(g/100 g)	GS(g/100 g)
Crude protein	18.10 ± 1.02[a]	20.07 ± 0.31[b]	18.70 ± 0.26[a]
Crude fat	1.17 ± 0.15[a]	0.57 ± 0.06[b]	0.73 ± 0.06[b]
Moisture	78.8 ± 1.22[a]	77.47 ± 0.29[a]	78.33 ± 0.25[a]
Ash	1.13 ± 0.06[a]	1.37 ± 0.06[b]	1.33 ± 0.06[b]

The identical superscript letters within the same row indicate no significant difference ($P > 0.05$), whereas different superscript letters within the same row indicate a significant difference ($P < 0.05$).

Table 5
Amino acid content of LB, LG and GS.

Amino acid types	LB	LG	GS
Asp (g/100 g)	1.50 ± 0.15[a]	1.71 ± 0.05[b]	1.57 ± 0.05[ab]
Thr (g/100 g)	0.67 ± 0.06[a]	0.77 ± 0.04[b]	0.71 ± 0.02[ab]
Ser (g/100 g)	0.49 ± 0.04[a]	0.55 ± 0.03[a]	0.49 ± 0.03[a]
Glu (g/100 g)	2.05 ± 0.20[a]	2.44 ± 0.08[b]	2.19 ± 0.06[ab]
Gly (g/100 g)	0.72 ± 0.05[a]	0.81 ± 0.06[a]	0.74 ± 0.03[a]
Ala (g/100 g)	0.95 ± 0.08[a]	1.11 ± 0.02[b]	1.05 ± 0.02[b]
Cys (g/100 g)	0.21 ± 0.06[a]	0.26 ± 0.03[a]	0.21 ± 0.03[a]
Val (g/100 g)	0.76 ± 0.06[a]	0.84 ± 0.04[a]	0.84 ± 0.01[a]
Met (g/100 g)	0.43 ± 0.06[a]	0.48 ± 0.03[a]	0.42 ± 0.04[a]
Ile (g/100 g)	0.71 ± 0.05[a]	0.79 ± 0.05[b]	0.78 ± 0.01[ab]
Leu (g/100 g)	1.28 ± 0.12[a]	1.45 ± 0.06[b]	1.36 ± 0.03[ab]
Tyr (g/100 g)	0.53 ± 0.06[a]	0.58 ± 0.04[a]	0.50 ± 0.03[a]
Phe (g/100 g)	0.70 ± 0.05[a]	0.77 ± 0.03[a]	0.72 ± 0.01[a]
Lys (g/100 g)	1.51 ± 0.14[a]	1.72 ± 0.06[b]	1.61 ± 0.05[ab]
His (g/100 g)	0.37 ± 0.04[a]	0.38 ± 0.02[a]	0.37 ± 0.03[a]
Arg (g/100 g)	0.92 ± 0.07[a]	1.06 ± 0.03[b]	0.99 ± 0.03[ab]
Pro (g/100 g)	0.51 ± 0.04[a]	0.60 ± 0.02[b]	0.55 ± 0.03[ab]
EAA (g/100 g)	6.43 ± 0.58[a]	7.20 ± 0.23[b]	6.82 ± 0.18[ab]
NEAA (g/100 g)	7.88 ± 0.71[a]	9.13 ± 0.18[b]	8.30 ± 0.23[a]

The identical superscript letters within the same row indicate no significant difference ($P > 0.05$), whereas different superscript letters within the same row indicate a significant difference ($P < 0.05$).

which implies that LG might have an advantage in traits related to disease and stress resistance.

3.6. Structural analysis of the pharyngeal tooth

The morphology of pharyngeal tooth of LB, GS and LG under scanning electron microscopic (SEM) is shown in Fig. 5. It is evident that the pharyngeal tooth of LB was slender and sharp, while the pharyngeal

Table 6
Essential amino acid composition and evaluation of muscles.

Amino acid	FAO/WHO standard	Egg protein standard	LB			LG			GS		
			content	AAS	CS	content	AAS	CS	content	AAS	CS
Ile	28	54	39.13	1.40	0.72	39.56	1.41	0.73	42.08	1.50	0.78
Leu	66	86	70.31	1.07	0.82	72.30	1.10	0.84	72.92	1.10	0.85
Lys	58	70	83.35	1.44	1.19	85.76	1.48	1.23	85.95	1.48	1.23
Met + Cys	25	57	35.14	1.41	0.62	36.88	1.48	0.65	33.72	1.35	0.59
Phe + Tyr	63	93	67.74	1.08	0.73	67.13	1.07	0.72	65.44	1.04	0.70
Thr	34	47	36.90	1.09	0.79	38.22	1.12	0.81	37.80	1.11	0.80
Val	35	66	41.87	1.20	0.63	41.89	1.20	0.63	44.75	1.28	0.68
EAAI				76.81			78.39			78.53	

Table 7
Fatty acid content of LB, LG and GS.

Fatty acid	Composition	LB(g/100 g)	LG(g/100 g)	GS(g/100 g)
Saturated fatty acid	C14:0	0.0047 ± 0.0007^{a}		0.0034 ± 0.0001^{b}
	C16:0	0.0973 ± 0.0240^{a}	0.0440 ± 0.0104^{b}	0.0990 ± 0.0040^{a}
	C18:0	0.0510 ± 0.0023^{a}	0.0400 ± 0.0073^{b}	0.0671 ± 0.0021^{c}
Monounsaturated fatty acid	C16:1	0.0134 ± 0.0023^{a}	0.0050 ± 0.0001^{b}	0.0090 ± 0.0006^{c}
	C18:1n9c	0.1330 ± 0.0344^{a}	0.0342 ± 0.0084^{b}	0.0614 ± 0.0050^{b}
	C20:1	0.0076 ± 0.0006^{a}		0.0045 ± 0.0002^{a}
	C24:1	0.0111 ± 0.0006^{a}	0.0142 ± 0.0013^{b}	0.0170 ± 0.0010^{c}
Polyunsaturated fatty acid	C18:2n6c	0.2020 ± 0.0060^{a}	0.0260 ± 0.0031^{b}	0.0700 ± 0.0070^{c}
	C18:3n3	0.0087 ± 0.0003^{a}	0.0041 ± 0.0005^{b}	0.0074 ± 0.0004^{c}
	C20:2	0.0101 ± 0.0007^{a}	0.0037 ± 0.0001^{b}	0.0059 ± 0.0003^{c}
	C20:3n6	0.0054 ± 0.0004^{a}	0.0040 ± 0.0004^{a}	
	C20:3n3	0.0043 ± 0.0002^{a}	0.0039 ± 0.0005^{a}	0.0056 ± 0.0001^{b}
	C22:1n9	0.0361 ± 0.0057^{a}	0.0224 ± 0.0034^{b}	0.0307 ± 0.0033^{a}
	C20:4n6	0.0193 ± 0.0021^{a}	0.0579 ± 0.0010^{b}	0.0430 ± 0.0014^{c}
	C20:5n3	0.0068 ± 0.0005^{a}	0.0070 ± 0.0007^{a}	0.0146 ± 0.0017^{b}
	C22:6n3	0.2134 ± 0.0170^{a}	0.1872 ± 0.0048^{b}	0.1384 ± 0.0101^{c}

The identical superscript letters within the same row indicate no significant difference ($P > 0.05$), whereas different superscript letters within the same row indicate a significant difference ($P < 0.05$).

tooth of GS was thick, short and blunt. Regarding the tooth number, LG (160.67 ± 3.93) was significantly higher ($P < 0.05$) than the male parent GS (108.00 ± 3.58) and significantly lower ($P < 0.05$) than the female parent LB (181.67 ± 4.97). However, there was no significant difference ($P > 0.05$) in tooth density between LB and LG. Both LB and LG had significantly higher ($P < 0.05$) tooth density than GS. Furthermore, LB (14.41 ± 0.34) had a significantly larger ($P < 0.05$) toothed area than LG (12.41 ± 0.52) and GS (12.24 ± 0.51), and the tooth diameter of LG (0.076 ± 0.023) was intermediate between the LB (0.057 ± 0.016) and GS (0.108 ± 0.044) (Table 8).

4. Discussion

Although the LB is not a native species, it is popular in the Chinese aquaculture market for its excellent economic traits. Nevertheless, the exorbitant and perpetually increasing breeding expenses, along with the persistent obstacles to farming technique, have impeded the sustainable progress of largemouth bass farming. At the core of these farming challenges lies the species' inherent carnivorous nature. This study investigated the appearance, chromosome number, muscle proximate composition, liver antioxidant enzyme activity and pharyngeal tooth structure of LG, which was produced by hybridizing carnivorous LB and omnivorous GS. The aim is to assess the potential for aquaculture applications of the LG.

Changes in appearance resulting from distant hybridization are the most intuitive and easily detectable traits. Visible differences in appearance help distinguish between parents and offspring. Some appearance traits, such as smaller heads and higher dorsal heights, are considered valuable morphological traits (Liu et al., 2019; Wang et al., 2022), while other appearance traits correlate with body weight and can serve as secondary indicators in fish production (Xia et al., 2022). In this study, the LG displayed several distinct physical differences from their parents. For example, LG inherits the iconic raised structure on the posterior edge of the gill cap from GS, but the color is lighter and the horizontal stripes on either side of the body are not as obvious as in LB, which can serve as a distinguishing feature. In terms of countable traits and measurable traits, the LG exhibited more pronounced differences from male ancestors GS and were closer to female ancestors LB.

As lower vertebrates, the fish genome exhibits significant plasticity. It was suggested that the metacentric chromosome of this species arose from the fusion of two telocentric chromosomes (Sun et al., 2021). Heterologous genomes in fish also demonstrate good compatibility, yet it appears to be directional. Several studies have demonstrated that viable offspring can be produced through a hybrid combination with a higher number of maternal chromosomes than paternal chromosomes, whereas the reverse is difficult to achieve (Wang et al., 2019). In this study, three fish species maintain consistency in ploidy. The LB exhibits a chromosome number of 46 and a karyotype of 2 m + 2st + 42 t. By comparison, the GS has a chromosome number of 48 with a karyotype of 48 t. The number of chromosomes in the LG is 47, with a karyotype of 1 m + 1st + 45 t. Considering the results of Sun et al., this study is consistent with the perspective of Liu et al. on the correlation between the number of chromosomes in a species and the survival of hybrid offspring. The inability to produce viable fish fry in GL crossing combinations may be attributed to the small size of GS eggs, which is less than half of LB eggs. This size discrepancy results in an insufficient material basis for embryonic development, irrespective of chromosome numbers.

Proximate composition of muscle reflects the nutritional value of fish. Previous research has demonstrated that certain cross combinations, such as *Carassius auratus cuvieri* × *Carassius auratus* red var. (Liu et al., 2017), *Epinephelus fuscoguttatus* × *Epinephelus tukula* (Wang et al., 2023), can result in hybrid offspring with superior specific muscle nutrients compared to their parents. Therefore, the muscle quality of fish can appropriately be improved through hybridization. In the present study, LG exhibited a significantly elevated ($P < 0.05$) crude protein content compared to its parents. In addition, the crude fat content of LG was significantly lower ($P < 0.05$) than that of LB, but not significantly different ($P > 0.05$) from GS. The evaluation of the amino acids in the three fish showed that LG and GS had a higher NAAI than LB, indicating

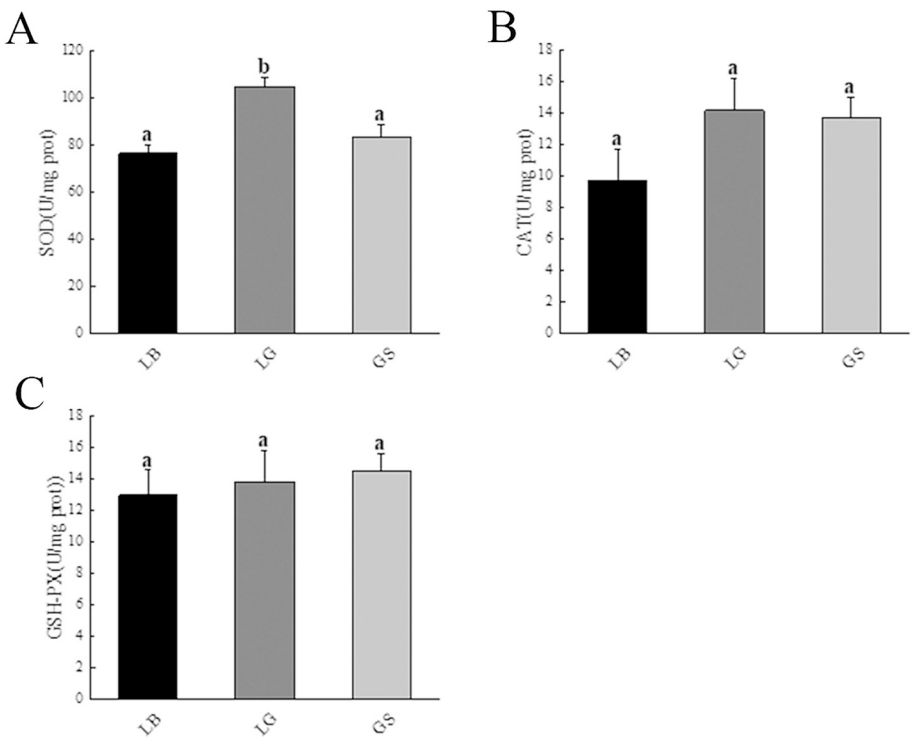

Fig. 4. Antioxidant enzyme activities of LB, LG and GS.
(A) SOD activity; (B) CAT activity; (C) GSH-PX activity. The identical letters indicate no significant difference ($P > 0.05$), whereas different letters indicate a significant difference ($P < 0.05$).

a more balanced amino acid composition in LG and GS. The analysis of fatty acid content revealed that LG had a significantly lower saturated fatty ($P < 0.05$) acid content than its parents, and a significantly lower ($P < 0.05$) polyunsaturated fatty acid content than LB, with no significant difference ($P > 0.05$) from GS. The DHA content of LG falls between LB and GS, while the EPA content of LG was not significantly different ($P > 0.05$) from LB, but both were significantly lower ($P < 0.05$) than GS. In terms of ARA content, LG was significantly higher ($P < 0.05$) than LB and GS. In general, saturated fatty acids can be synthesized endogenously, whereas unsaturated fatty acids are mainly obtained exogenously from food and cannot be synthesized endogenously or in small amounts. The difference in fatty acid content between LG and its parents indicated that they have distinct mechanisms for regulating fatty acid metabolism to support their individual growth and development, which may be related to genetic differences and gene interactions between heterologous genes in LB and GS.

Previous studies have shown that oxidative stress caused by diseases and environmental stresses poses a significant threat to aquatic animals. The antioxidant oxidase system plays a crucial role in scavenging free radicals and resisting oxidative stress. Therefore, increasing antioxidant enzyme activity can improve the survival rate of aquatic animals. In the present study, we determined the activities of SOD, GSH-PX, and CAT in the livers of LB, GS, and LG, and the results showed that the hepatic SOD activity of LG was significantly higher ($P < 0.05$) than that of its parents. During the culturing, it was observed that LG exhibited greater resistance to diseases, which may be attributed to their higher levels of hepatic antioxidant enzymes. This is supported by previous studies which have shown that higher antioxidant enzyme activities in hybrid fish (*Carassius cuvieri* ♀ × *Carassius auratus red var* ♂) and hybrid shrimp (*Macrobrachium nipponense*) are associated with increased resistance in the face of pathogenic infections and nitrite stress (Li et al., 2023; Xiong et al., 2022). Therefore, hybridization can potentially enhance offspring resistance to disease and adversity.

In the present study, we found that pharyngeal tooth of LB was distributed with sharp and long chorionic tooth, while pharyngeal tooth of GS was blunter and shorter. These pharyngeal tooth characteristics are similar to those reported in previous studies (Lauder, 1983; Linser et al., 1998), suggesting that pharyngeal tooth characteristics of largemouth bass are more adapted to carnivory, whereas pharyngeal tooth characteristics of GS are more adapted to omnivory. During the farming process, it was observed that LG were less prone to fratricide and had a higher survival rate during the juvenile stage compared to LB. This suggests that the feeding habits of LG are different from those of LB, which is supported by our results. The number of pharyngeal tooth and tooth diameter of LG were significantly different ($P < 0.05$) from those of its parents, indicating that the ability of LG to capture, process and transport food is different from that of its parents. However, a systematic study of the differences in feeding habits between LG and its parents is still required.

In conclusion, this study we hybridized carnivorous LB and omnivorous GS, and diploid hybrid offspring with a high hatching rate were obtained. The results of muscle proximate composition and liver antioxidant enzyme activity indicated that LG exhibited improved traits with higher crude protein content and stronger stress resistance. Furthermore, the pharyngeal tooth characteristics indicated that LG may have different food selection and processing capabilities. Our study provides new insights into the genetic improvement and germplasm innovation of largemouth bass.

CRediT authorship contribution statement

Haitao Zhong: Writing – original draft, Visualization, Investigation, Data curation. **Hong Chen:** Writing – original draft, Validation, Investigation. **Mingli Liu:** Validation, Resources, Investigation. **Yu Sun:** Validation, Resources, Methodology. **Pengfei Yu:** Methodology. **Chiye Zhao:** Validation. **Chaoying Luo:** Visualization. **Chun Zhang:** Visualization, Methodology, Funding acquisition. **Chang Wu:** Data curation. **Xueyan Wang:** Formal analysis. **Yilin Wu:** Visualization. **Shi Wang:** Visualization. **Ming Wen:** Visualization. **Fangzhou Hu:** Writing – review & editing, Supervision, Methodology, Funding acquisition.

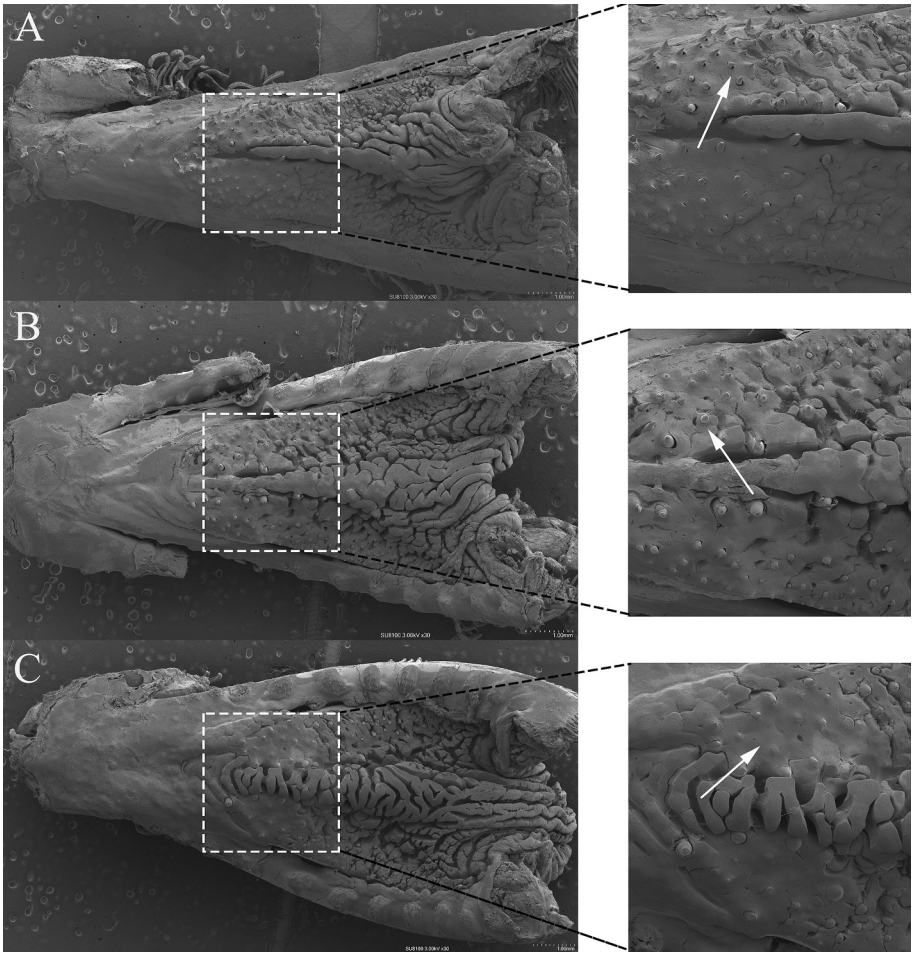

Fig. 5. Structure of the pharyngeal tooth under the electron microscope.
(A) pharyngeal tooth of LB, the tooth pointed out by the arrow is sharp and elongated, with mostly exposed; (B) pharyngeal tooth of LG; (C) pharyngeal tooth of GS, the tooth pointed by the arrow are thick, short, and blunt, with partially covered. Bar = 1 mm. The images within the dashed area were magnified.

Table 8
Amination of tooth number, toothed area, tooth density and tooth diameter in LB, LG and GS.

Fish type	Tooth number	Toothed area (mm^2)	Tooth density (n/mm^2)	Tooth diameter (mm)
LB	181.67 ± 4.97^a	14.41 ± 0.34^a	12.62 ± 0.48^a	0.057 ± 0.016^a
LG	160.67 ± 3.93^b	12.41 ± 0.52^b	12.97 ± 0.72^a	0.076 ± 0.023^b
GS	108.00 ± 3.58^c	12.24 ± 0.51^b	8.84 ± 0.52^b	0.108 ± 0.044^c

The identical superscript letters within the same column indicate no significant difference ($P > 0.05$), whereas different superscript letters within the same column indicate a significant difference ($P < 0.05$).

Shaojun Liu: Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2022YFD2400802), Youth Science and Technology Talents Lifting Project in Hunan Province (2022TJ-N18), the Youth Project of Hunan Provincial Education Department (22B0068), the National Natural Science Foundation of China (Grant No. 32293252, U19A2040), the Key Research and Development Program of Hunan Province of China (2023WK2001), Laboratory of Lingnan Modern Agriculture Project (Grant No. NT2021008), the earmarked fund for Agriculture Research System of China (Grant No. CARS-45), Cooperative Innovation Center of Engineering and New Products for Developmental Biology of Hunan Province (Grant No. 20134486), and Key Research and Development Program of Hunan Province (Grant No. 2021NK1010).

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