

Article

SNP Genotyping Chip Reveals Genetic Structure and Selection Signatures of the “Huangxuan No 2” Population of *Portunus trituberculatus*

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Abstract

The swimming crab (*Portunus trituberculatus*) is a commercially important marine aquaculture species. After multiple generations of selective breeding, the third improved variety in China, “Huangxuan No 2” (HX2), was successfully developed in 2018. Compared to the original wild populations, HX2 exhibits enhanced resistance to low salinity stress and growth RATE. However, the genomic characteristics underlying these selected traits remain largely unexplored. To investigate the genetic variation associated with artificial selection, we genotyped 90 individuals from the HX2 strain and two wild populations (C and D) using a high-density SNP array. A total of 43,314 single nucleotide polymorphisms (SNPs) were identified, which were evenly distributed across the genome in 1 Mb windows. Genetic diversity analysis showed that HX2 and wild populations were similar but overall low in diversity levels. Population structure analysis and fixation index (*Fst*) values revealed low-to-moderate genetic differentiation between HX2 and the wild populations, whereas no differentiation was observed between the two wild populations. Using the wild populations as a reference, we identified 24 genomic regions under potential selection in HX2 based on the *Fst* between populations and the nucleotide diversity ratio (π -ratio), encompassing 425 candidate genes. Enrichment analysis indicated that these genes are primarily involved in pathways related to immune response, infection, signal transduction, and metabolism. Notably, genes associated with stress tolerance (e.g., *GPX3*, *HMGCS1*, *Duox*), immunity (e.g., *LAMB1*, *HSPG2*), and growth (e.g., *Cht5*) were identified. These findings provide valuable insights into the genomic signatures of artificial selection and offer fundamental resources for further genetic improvement of *P. trituberculatus*.



Academic Editor: Eric Hallerman

Received: 26 May 2026

Revised: 7 July 2026

Accepted: 7 July 2026

Published: 12 July 2026

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Keywords: SNP genotyping array; genetic structure; selective sweep

Key Contribution: This study provides new insights into the genomic characteristics of “Huangxuan No 2”, an improved variety of *Portunus trituberculatus*. Identifying functional genes associated with low salinity tolerance and immunity, it reveals genomic signatures shaped by artificial selection and provides references for new variety breeding and the formulation of germplasm conservation strategies.

1. Introduction

The swimming crab (*Portunus trituberculatus*) is primarily distributed in the coastal waters of China, Korea, and Japan. Due to its rapid growth and adaptability to coastal aquaculture environments, it has become an important aquaculture species in China, especially in the coastal regions of eastern China [1,2]. As farming areas have gradually expanded toward nearshore and even inland waters, decreasing salinity caused by precipitation and other environmental factors has become a major constraint affecting the survival and growth of *P. trituberculatus* [3]. Therefore, there is an urgent need to develop a new *P. trituberculatus* variety with rapid growth and low-salinity tolerance that can adapt to changing aquaculture environments and meet market demands. In 2018, the Yellow Sea Fisheries Research Institute of the Chinese Academy of Fishery Sciences successfully developed a new variety named “Huangxuan No 2” (HX2) (GS-01-006-2018), which is the third officially registered *P. trituberculatus* variety in China. Compared with unselected populations, HX2 exhibits significantly improved survival and growth performance, with a 31.2% increase in survival rate and an 18.8% increase in average body weight under the same rearing conditions, highlighting its superior stress-resistance traits. To date, an increasing number of studies have focused on the stress resistance of *P. trituberculatus*. Multi-omics analyses have elucidated the regulatory mechanisms of competing endogenous RNAs (ceRNAs) in response to *Vibrio parahaemolyticus* infection [2]. Whole-genome bisulfite sequencing (WGBS) has revealed epigenetic regulation through functional DNA methylation in the gill tissue under low-salinity stress [4]. These advances have been largely facilitated by the successful assembly of the *P. trituberculatus* reference genome [5]. However, the genetic structure, diversity, and selection signatures of this species under long-term artificial selection remain poorly understood.

Artificial selection targeting specific traits is an effective strategy for enhancing stress resistance and environmental adaptability in aquaculture species [6]. Understanding the genetic diversity and structure of the HX2 population is essential for the conservation and improvement of *P. trituberculatus* germplasm resources. Such knowledge is indispensable for enhancing aquaculture efficiency and stress resistance [7]. Moreover, long-term directional selection may also lead to a reduction in genetic diversity, genetic drift, and population bottlenecks [8,9]. Genetic diversity represents the total pool of genetic variation within a species. Higher diversity enhances adaptability to environmental challenges [10–12], whereas reduced diversity can adversely affect key traits such as growth, stress tolerance, and disease resistance [13]. Therefore, analyzing the genetic diversity and population structure among different *P. trituberculatus* populations are of great significance for the detection of selection signals and the elucidation of their underlying molecular mechanisms.

With the advancement of high-throughput sequencing technologies, single nucleotide polymorphisms (SNPs) have become the most abundant type of genetic marker in the genome and are considered ideal tools for analyzing population structure and detecting selection signals [14]. SNP genotyping arrays, which utilize miniaturized probes arranged on chips, enable cost-effective genotyping of large sample sets [15]. This technology has been widely applied in various aquatic species, such as blotched snakehead (*Channa maculata*) [16], Atlantic salmon (*Salmo salar*) [17], mud crab (*Scylla paramamosain*) [18], and whiteleg shrimp (*Litopenaeus vannamei*) [19]. In this study, to investigate the genetic structure and genome-wide selection signals of the improved *P. trituberculatus* variety HX2, we performed SNP chip genotyping on 90 individuals, comprising one selectively bred strain and two wild populations. The resulting genomic data were used to evaluate population structure across the three groups. Furthermore, selection signals in the HX2 population were identified using pairwise fixation index (*Fst*) and nucleotide diversity ratio (π -ratio) methods. This study aims to provide valuable insights for the conservation of *P.*

trituberculatus germplasm resources and for enabling genomic-assisted breeding, offering a solid foundation for future genetic improvement.

2. Materials and Methods

2.1. Samples Collection

A total of 90 *P. trituberculatus* individuals were collected from three populations, with 30 individuals sampled from each group (Figure 1). Two of the populations were wild groups, designated as C and D, originating from Tangshan, China (39.6° N, 118.2° E) and Dandong, China (40.1° N, 124.4° E), respectively. The third population was a selectively bred strain, designated as HX2, obtained from Changyi, Weifang, China (36.8° N, 119.3° E). All individuals from the three populations were collected at the adult stage, and only healthy crabs with normal external morphology were selected for subsequent SNP genotyping analysis. The wild populations were collected with the assistance of local aquaculture farms outside the seasonal fishing moratorium in China. All sampling procedures complied with local regulations, and no specific collection permit was required for these samples.

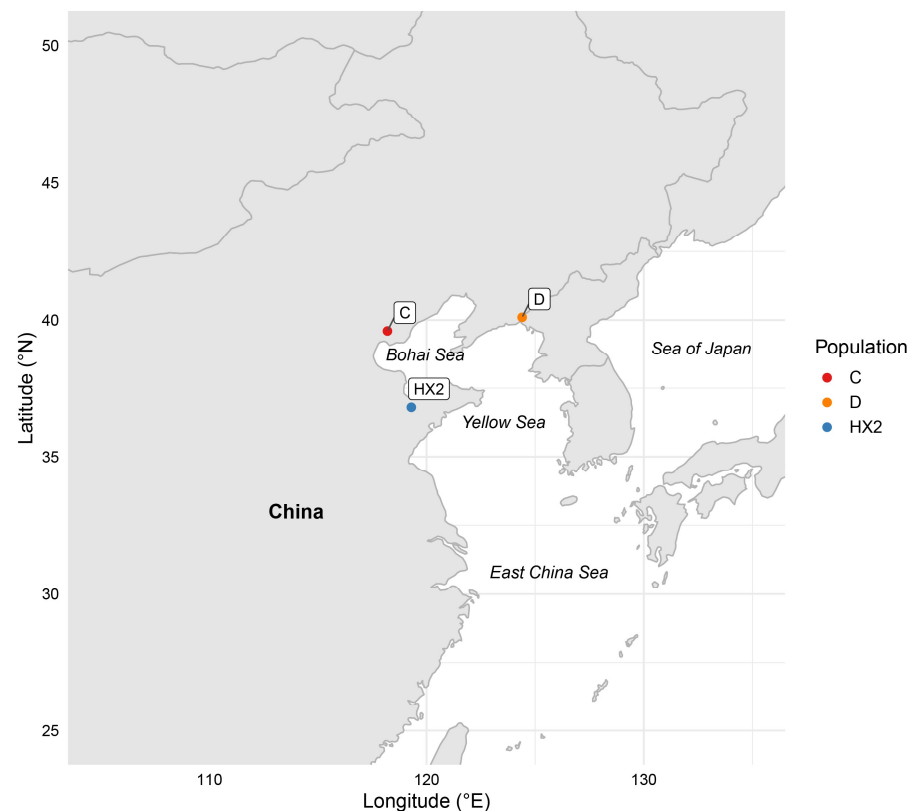


Figure 1. Geographic location of sampled *P. trituberculatus*. Each dot of a given color on the East Asian map represented the geographic location of sampled *P. trituberculatus*.

2.2. DNA Extraction, Library Construction, and Quality Control

For SNP genotyping, cheliped muscle tissue was collected from each *P. trituberculatus* individual. Briefly, the cheliped was removed from each crab, and muscle tissue was dissected using sterile scissors and forceps on ice. The collected muscle tissue was placed into a 1 mL cryotube and immediately snap-frozen in liquid nitrogen. Genomic DNA was then extracted from the cheliped muscle tissue using a magnetic bead-based method (TIANGEN, Beijing, China). DNA integrity was assessed via 1% agarose gel electrophoresis (voltage: 120 V; duration: 25 min; loading volume: 1 µL). DNA concentration and purity were determined using a spectrophotometer. All samples had DNA concentrations exceeding

10 ng/ μ L, with A260/A280 ratios between 1.8 and 2.2. Only DNA samples meeting quality control standards were used for library construction.

The prepared DNA libraries were sequenced on the MGI high-throughput platform (Higentec, Changsha, China) using a paired-end read length of 150 bp (PE150). All raw sequencing data generated in this study are available in the NCBI BioProject database under accession number PRJNA1300839. Raw reads were subjected to quality control and filtering using Fastp 0.21.0 [20] to remove adapter sequences and low-quality reads. Reads were discarded if more than 50% of bases had a quality score $\leq$ Q20, if they contained more than five ambiguous bases (Ns), or if the read length was <100 bp. After filtering, high-quality clean reads were retained. Clean reads were aligned to the reference genome (NCBI Accession No: GCA_043638975.1) using BWA 0.7.17 [21], and the resulting alignments were sorted to produce BAM files for subsequent variant calling.

2.3. Variant Calling

Genomic variation refers to DNA sequence polymorphisms arising from nucleotide changes at the genomic level, including single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels). Based on the alignment of clean reads to the reference genome, variant calling was conducted using the HaplotypeCaller module in GATK v4.0 [22] to detect genotypes at target loci. Variants were filtered using the following thresholds: QD < 2.0 , QUAL < 30.0 , MQ < 40.0 , FS > 60.0 , MQRankSum < -12.5 , and ReadPosRankSum < -8.0 . SNP loci corresponding to the chip were specified using the `--intervals` option. Functional annotation of the identified variants was conducted using ANNOVAR version 2020Jun08 [23].

2.4. Population Genetic Diversity and Structure Analysis

SNPs were further filtered using PLINK v1.9 (<https://www.cog-genomics.org/plink/1.9/>, accessed on 6 July 2026) with the parameters `--geno 0.1`, `--maf 0.01`, and `--biallelic-only strict`. Variants with a genotype missing rate $> 10\%$ or a minor allele frequency (MAF) $< 1\%$ were removed, and only strictly biallelic SNPs were retained for subsequent analyses.

Genetic diversity was assessed based on expected heterozygosity (H_e), observed heterozygosity (H_o), nucleotide diversity (π), inbreeding coefficient (F), and polymorphism information content (PIC). Nucleotide diversity (π) was calculated using Vcftools v0.1.16 with a 500 kb sliding window and a step size of 50 kb.

The phylogenetic tree was constructed using the neighbor-joining (NJ) method implemented in Fastme v2.0 (<https://www.atgc-montpellier.fr/fastme/>, accessed on 6 July 2026) based on genetic distance matrices generated by the VCF2Dis (<https://github.com/hewm2008/VCF2Dis>, accessed on 12 May 2025) toolkit, and visualized with the iTOL platform v7.0 (<https://itol.embl.de>, accessed on 6 July 2026). Principal component analysis (PCA) was performed using the `--pca` function in PLINK v1.9 (<https://www.cog-genomics.org/plink/1.9/>, accessed on 6 July 2026), and the top 10 principal components were visualized using the ggplot2 package in R v4.4.2. To estimate population genetic structure and admixture, individual ancestry proportions were inferred in ADMIXTURE v1.3.0 (<http://dalexander.github.io/admixture/>, accessed on 6 July 2026) under different assumed numbers of ancestral populations (K).

2.5. Selective Sweep Analysis and Functional Annotation

To investigate the genetic differentiation between the selectively bred HX2 population and wild populations, we applied two statistical approaches: the fixation index (F_{st}) and the nucleotide diversity ratio (π -ratio), both widely used to detect signals of positive selection. Based on the population structure results, the HX2 population was designated as the target group, with the C and D populations serving as reference groups. Sliding window analysis

was performed with a 500 kb window size and a 50 kb step size. Genomic regions within the top 5% of both *Fst* and π -ratio values were identified as candidate selective sweep regions. The results were visualized using the ggplot2 package in R. Candidate regions were merged using BEDtools v2.31.1 (<https://bedtools.readthedocs.io/>, accessed on 6 July 2026), and genes located within these regions were extracted. All coding genes of *P. trituberculatus* were annotated using eggNOG-mapper (<https://github.com/eggnogdb/eggno-mapper/>, accessed on 6 July 2026) and used as the background for subsequent enrichment analyses. Functional enrichment for Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was conducted using the *ClusterProfiler* package in R.

3. Results

A total of 1.21 billion reads were generated from liquid-phase SNP chip sequencing of 90 *P. trituberculatus* individuals. After alignment and quality control, 43,314 high-quality SNPs were identified. Of these, 28,043 SNPs (64.74%) were located in genic regions, including 15,328 (35.4%) in exons, 10,706 (24.7%) in introns, 655 (1.5%) in 5' untranslated regions (5' UTRs), and 1354 (3.1%) in 3' untranslated regions (3' UTRs). Library construction and sequencing were successful, with no evidence of contamination in any of the 90 samples. The genomic distribution of SNPs (Figure 2) indicated an even dispersion across all 53 chromosomes.



Figure 2. Number of SNPs per 1 Mb window based on the 43K SNP chip data of *P. trituberculatus*.

3.1. Genetic Diversity and Population Structure Analysis

Analysis of genetic diversity among the HX2, C, and D populations (Table 1) revealed that the expected heterozygosity (*He*) and observed heterozygosity (*Ho*) were generally similar across the three populations. The C population exhibited the highest *He* and *Ho* values (0.3069 and 0.3054, respectively), whereas the D population had the lowest (0.3021 and 0.2912, respectively). Notably, the *Ho* of the D population was substantially lower than its *He*, resulting in an inbreeding coefficient (*F*) of 0.0362, indicative of a small degree of inbreeding. In contrast, the HX2 population exhibited only a small difference between *Ho* and *He*, with the lowest *F* value (0.0045), indicating that genotype frequencies in HX2 were in close agreement with Hardy–Weinberg equilibrium expectations. Nucleotide diversity (π) showed minimal variation among the three populations, all approximately 1.15×10^{-5} ,

reflecting a generally low level of genetic variation. The polymorphism information content (PIC) was marginally higher in the C population (0.2472) and marginally lower in the D population (0.2436), indicating that the C population harbors somewhat higher polymorphism. As shown by the pairwise *Fst* values (Table 2), genetic differentiation between populations C and D was negligible, whereas HX2 showed low-to-moderate genetic differentiation from both wild populations.

Table 1. Genetic diversity statistics for three *P. trituberculatus* populations.

Population	He	Ho	π	F	PIC
HX2	0.3039	0.3024	1.160×10^{-5}	0.0045	0.2455
C	0.3069	0.3054	1.157×10^{-5}	0.0048	0.2472
D	0.3021	0.2912	1.150×10^{-5}	0.0362	0.2436

Table 2. Genetic differentiation coefficient for the three *P. trituberculatus* populations.

Population	HX2	C	D
HX2			
C	0.0409		
D	0.0387	−0.000039	

The kinship matrix revealed that most individuals exhibited low pairwise relatedness (Figure 3a). The neighbor-joining (NJ) tree further demonstrated substantial genetic divergence between the selectively bred HX2 population and the two wild populations (C and D) (Figure 3b). This result is consistent with the results of the principal component analysis (PCA), which separated all individuals into two distinct clusters: HX2 formed an independent clade, whereas populations C and D clustered together (Figure 3c). At $K = 2$, population structure analysis clearly distinguished HX2 from the wild groups, and the differentiation between HX2 and wild populations (C and D) became more pronounced with increasing K values (Figure 3d). The CV error (Figure S1) reached its lowest value at $K = 7$; however, we did not interpret $K = 7$ as indicating seven biological populations. In ADMIXTURE analysis, the K value represents the number of inferred ancestry components rather than the number of sampled populations, and higher K values may reflect fine-scale substructure within populations. Considering that only three populations were sampled in this study, and that PCA and NJ phylogenetic tree analyses showed a major separation between HX2 and the two wild populations, we focused on $K = 2$ and $K = 3$ for biological interpretation. Specifically, $K = 2$ represents the major differentiation between HX2 and the wild populations, whereas $K = 3$ provides additional resolution corresponding to the three sampled populations.

3.2. Detection of Selection Signature

To identify genomic regions potentially affected by selection pressure, we combined the population differentiation index (*Fst*) and the log-transformed nucleotide diversity ratio ($\log \theta/\pi$ ratio) between the selectively bred population HX2 and two wild populations (Figure 4). Genomic windows falling within the top 5% of *Fst* and π ratio values were defined as candidate regions under selection. A total of 24 putative selective sweep regions were identified in the HX2 population based on 20,408 sliding windows, encompassing 425 candidate genes. These regions were distributed across multiple chromosomes of *P. trituberculatus*. Notably, four selective regions were located on chromosome 12, exhibiting stronger selection signals (higher *Fst* and π ratio values) than other chromosomes.

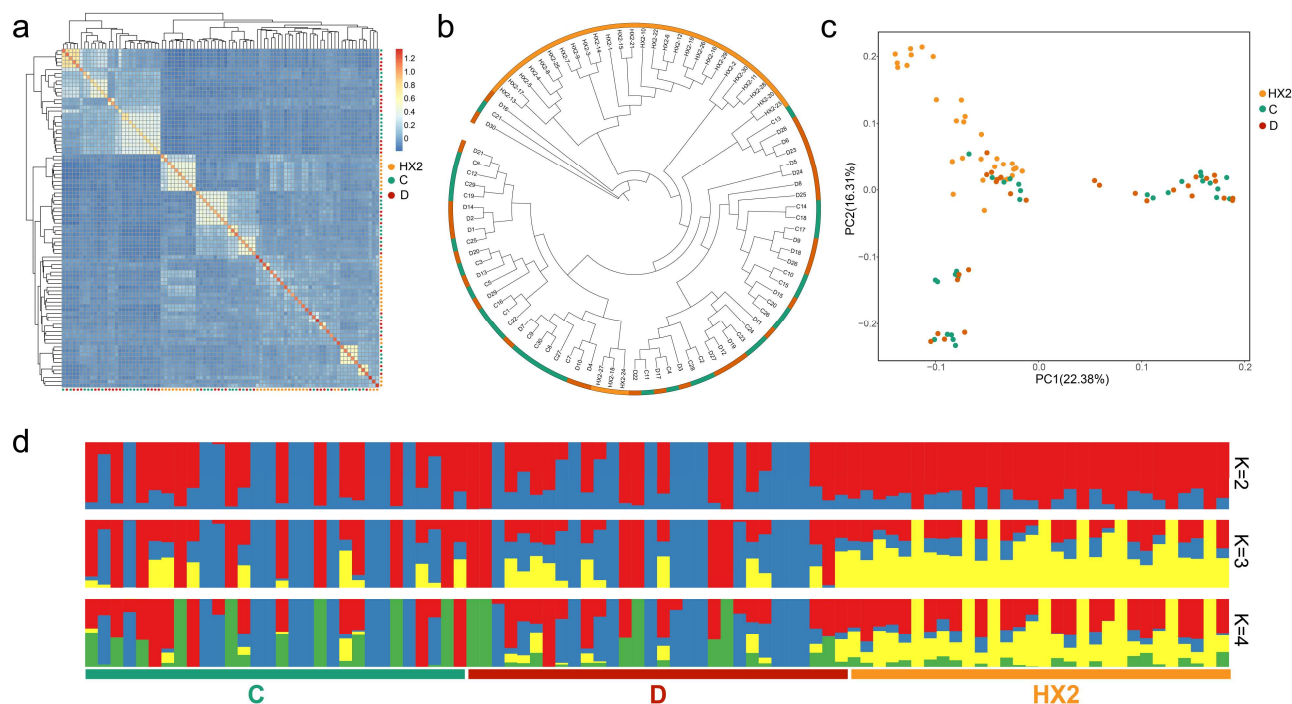


Figure 3. Genetic structure analysis of the three *P. trituberculatus* populations. (a) Kinship analysis among individuals from the three populations. (b) Neighbor-joining (NJ) phylogenetic tree constructed based on SNP genotyping data. (c) Principal component analysis (PCA) based on SNP chip genotyping data. (d) Population structure analysis of the three populations using the ADMIXTURE program. In panel (d), the ADMIXTURE bar plots correspond to K = 2, K = 3, and K = 4 from top to bottom. Each accession is represented by a bar, and the length of each coloured segment in the bar represents the proportion contributed by that ancestral population.

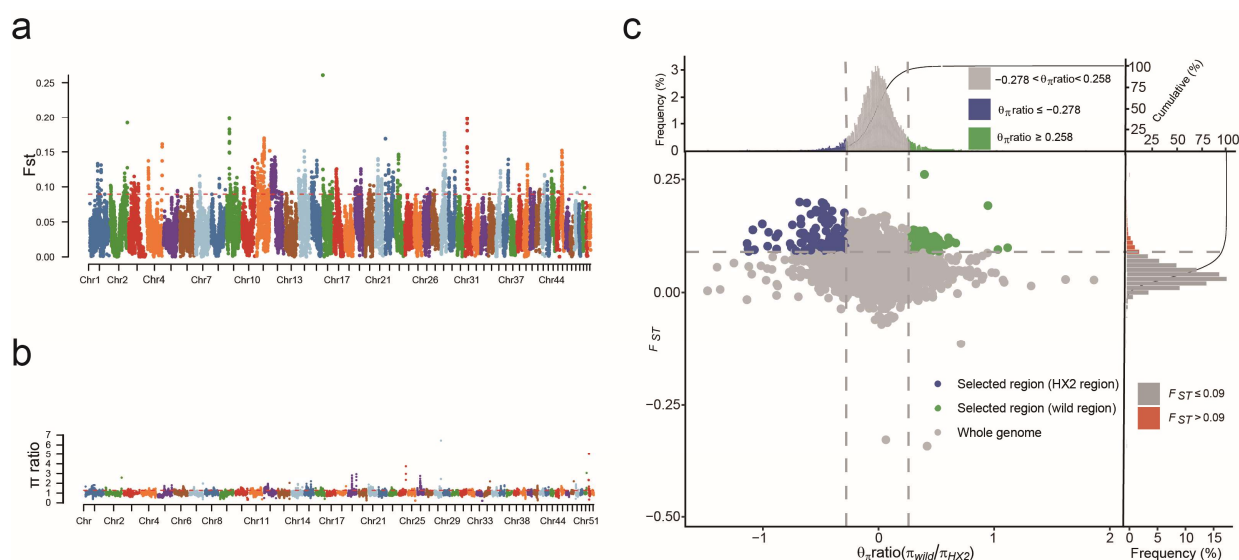


Figure 4. Genomic signature analysis of the HX2 population of *P. trituberculatus* compared with wild populations (C and D). (a) Plot of moving average F_{ST} (HX2 vs. wild) values of SNPs per chromosome. Different colors indicate different chromosomes; (b) Plot of moving average π -ratio (wild/HX2) values of SNPs per chromosome. (c) Distribution of π -ratio (wild/HX2) and F_{ST} values were calculated in 50-kb steps in a 500-kb window. Data points located on the left and right vertical dashed lines, respectively (corresponding to the 5% left and right tails of the empirical θ_{π} ratio distribution), and above the horizontal dashed line (the 5% right tail of the empirical F_{ST} distribution) was identified as selected region for the HX2 population (green point).

3.3. Functional Enrichment Analysis of Selected Genes

GO and KEGG enrichment analyses were performed on the candidate genes identified from selective sweep regions. GO pathways (Figure 5a) showed that, in the “Biological Process (BP)” category, significantly enriched GO terms included positive regulation of synaptic vesicle endocytosis, visual perception, molting cycle, chitin-based cuticle, and sensory perception of light stimulus, suggesting that these genes may be involved in critical physiological processes such as sensory responses, neural transmission, and exoskeleton formation. In the “Molecular Function (MF)” category, the enriched terms were primarily associated with glycine binding, integrin binding, and glycine-gated chloride ion channel activity, indicating that these genes may participate in transmembrane signaling and neural regulation, particularly involving amino acid-mediated neurotransmission. Within the “Cellular Component (CC)” category, candidate genes were significantly enriched in structures such as the mitotic spindle pole, lamellar body, chloride channel complex, and acrosomal vesicle, implying potential roles in mitotic regulation, membrane structure formation, and ion channel activity. KEGG pathway (Figure 5b) analysis further revealed that candidate genes were significantly enriched in several biologically important signaling pathways, including the PI3K-Akt signaling pathway, cGMP-PKG signaling pathway, ECM-receptor interaction, ABC transporters, and DNA replication. Additionally, genes were enriched in endocrine regulatory pathways such as thyroid hormone synthesis and cortisol synthesis and secretion. These pathways are likely to play key roles in cellular metabolism, signal transduction, neural activity, and environmental response.

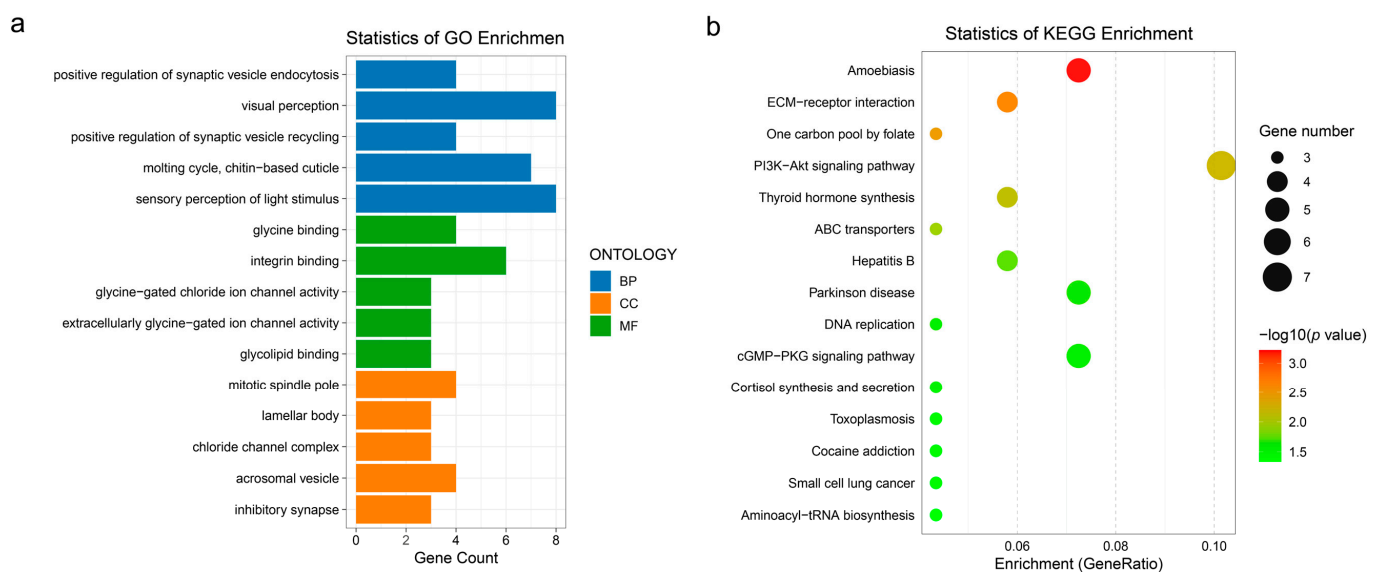


Figure 5. Enrichment analysis of the HX2 population compared with wild populations in *P. trituberculatus*. (a) GO enrichment analysis; (b) KEGG pathway enrichment analysis.

4. Discussion

P. trituberculatus is a commercially valuable crab species, widely appreciated for its high nutritional value and palatable taste [24]. To date, most studies on *P. trituberculatus* have focused on the species as a whole [25–27], with limited systematic investigations conducted on individual breeding strains. Although the selective breeding of *P. trituberculatus* was initiated later than that of many other crustaceans, three new strains had been developed by 2025. Among them, the HX2 population exhibits desirable traits such as rapid growth and strong tolerance to low salinity. However, the genomic changes associated with the selective breeding process of this strain remain largely unknown. In this study, a SNP genotyping chip was employed to identify genomic regions under selection in the HX2 population and

to assess the genetic structure and diversity between the selectively bred HX2 population and two wild populations (C and D). These findings aim to provide important insights informing future genetic improvement and genome-assisted breeding of *P. trituberculatus*.

Genetic diversity is essential for the conservation and utilization of germplasm resources [28], as it facilitates the evaluation of genetic variation, bottleneck effects, and inbreeding levels in breeding populations and underscores the broad prospects of genomic selection [29]. Heterozygosity is a key indicator of genetic variation, including expected heterozygosity (H_e) and observed heterozygosity (H_o), with higher values indicating greater genetic diversity [30]. In this study, all three populations exhibited higher H_e than H_o ($H_e > H_o$), which is consistent with the findings of Duan [13] and has also been reported in other species such as the Pacific oyster (*Crassostrea gigas*) [31] and grass carp (*Ctenopharyngodon idella*) [32], suggesting a certain degree of heterozygosity deficiency. This phenomenon may be attributed to deviations from Hardy–Weinberg equilibrium (HWE), including inbreeding effects [33].

Notably, the overall levels of genetic diversity were relatively similar between the selectively bred and wild populations ($\pi = 1.15\text{--}1.16 \times 10^{-5}$; $\text{PIC} = 0.2436\text{--}0.2472$). In general, reduced genetic diversity in selectively bred populations is often attributed to factors such as small breeding population sizes, directional artificial selection, and limited gene flow from external populations [34]. The genetic diversity of wild *P. trituberculatus* populations may also have been influenced by the boom-bust population dynamics characteristic of many marine fishes and invertebrates [35]. In this study, we hypothesize that the implementation of effective and scientifically guided breeding programs may have minimized the loss of genetic diversity in the improved strain. Conversely, the genetic diversity of wild *P. trituberculatus* populations may have declined due to reduced effective population sizes resulting from intensive fishing pressure, as wild stocks are primarily sustained through capture fisheries [36,37].

In addition, we calculated the genetic differentiation index (F_{st}) to evaluate the genetic relationships among *P. trituberculatus* populations. Extremely low genetic differentiation was observed between the two wild populations ($F_{st} = -0.000039$), which may be attributed to the hydrological connectivity between the Yellow Sea and Bohai Sea, as well as the long-distance migratory behavior of *P. trituberculatus*, which facilitates gene flow and reduces genetic divergence between populations [38]. In contrast, HX2 showed low-to-moderate genetic differentiation from the wild populations ($F_{st} = 0.0387\text{--}0.0409$), which may be associated with its selective breeding history and geographical isolation. Both the phylogenetic tree and principal component analysis (PCA) supported a clear separation of the crabs into two major clusters. Admixture was evident between the two wild populations, consistent with the low F_{st} value. ADMIXTURE analysis further revealed a complex ancestral composition within the HX2 population. This pattern may be related to the known breeding and conservation practice of maintaining a relatively large broodstock population during the continued selection of HX2. As an economically important species, understanding the genetic structure of *P. trituberculatus* provides valuable insights for genetic improvement, germplasm conservation, and sustainable utilization.

Artificial selection can leave detectable signatures across the genome of *P. trituberculatus*, providing valuable insights into the genetic consequences of selective breeding [39]. To identify potential genomic regions and mechanisms underlying long-term artificial selection in the “Huangxuan No 2” strain, we performed selective sweep analysis based on SNP data using two complementary approaches: pairwise population differentiation (F_{st}) and the nucleotide diversity ratio (π ratio). This combined strategy was employed to enhance the robustness and comprehensiveness of selection signal detection. Previous studies have reported that applying multiple genome-wide scan methods can improve

the accuracy of detecting true signals under selection and reduce the number of false positives [40]. Using both approaches in this study, we jointly identified 24 selective sweep regions encompassing 425 candidate genes. Given that HX2 was primarily bred for low-salinity tolerance and rapid growth, the biological relevance of these selection signatures was interpreted in the context of these two major breeding objectives. These genes were significantly enriched in pathways related to infection and immunity (e.g., amoebiasis, hepatitis B, toxoplasmosis), signal transduction (e.g., PI3K-Akt signaling pathway, cGMP-PKG signaling pathway), and metabolism (e.g., one carbon pool by folate, DNA replication, Aminoacyl-tRNA biosynthesis), suggesting potential involvement in stress resistance, immune response, and growth regulation. Notably, the top three most significantly enriched pathways were all immunity-related. Several putative candidate genes identified within these pathways included *LAMB1*, *HSPG2*, *MTFMT*, and *AMT*. Among them, *LAMB1* encodes laminin subunit beta-1, a critical component of the extracellular matrix, which was enriched in multiple pathways such as Amoebiasis and ECM–receptor interaction. *LAMB1* plays essential roles in cell adhesion, migration, and tissue morphogenesis during development and has been strongly associated with various cancers [41]. The *LAMB1* gene has been identified as a key regulator of skin immunity in half-smooth tongue sole (*Cynoglossus semilaevis*) [42]. In giant freshwater prawn (*Macrobrachium rosenbergii*), the *HSPG2* gene has been shown to promote the regeneration of chelipeds and pleopods [43]. Moreover, several studies have demonstrated that the heparan sulfate proteoglycan (*HSPG*) protein family plays a critical role in limb regeneration [44,45]. In *P. trituberculatus* aquaculture, limb autotomy [46] caused by competition during the molting period [47] is a major factor contributing to increased mortality. Therefore, *LAMB1* and *HSPG2* may represent candidate genes potentially associated with immune-related processes and injury repair in HX2, although their specific roles in *P. trituberculatus* remain to be experimentally validated.

In addition, this study identified several other candidate genes that may play important roles in stress resistance and growth in *P. trituberculatus*. For example, *GPX3*, *HMGCS1*, and *Duox* are potentially involved in stress tolerance, while *Cht5* is associated with growth regulation. *GPX3* encodes a glutathione peroxidase, which plays a critical role in salt stress resistance in rice (*Oryza sativa* L.) [48], and is essential for the defense mechanisms against *V. parahaemolyticus* in whiteleg shrimp (*L. vannamei*) [49]. *HMGCS1* is a key enzyme involved in the de novo synthesis of cholesterol, serving as an important precursor for the production of secondary metabolites such as steroids and sterols [50,51]. Previous studies have shown that *HMGCS1* is significantly upregulated in fishes [52–54] under low salinity and low temperature stress conditions. *Duox* is an essential enzyme for the generation of reactive oxygen species (ROS) [55]. In the Chinese mitten crab (*Eriocheir sinensis*), nitrite stress has been shown to induce *Duox*-mediated ROS production, which activates the Dorsal transcription factor and subsequently promotes the expression of antimicrobial peptides (AMPs) [56–58], thereby contributing to resistance against white spot syndrome virus (WSSV). Studies on the kuruma shrimp (*Marsupenaeus japonicus*) have also indicated that *Duox*-mediated ROS play a crucial role in the immune system. Moreover, *Duox* has been identified as a key gene responsive to low salinity stress in Bohai red scallop [59]. We found that the functional mechanisms of this gene are closely associated with reactive oxygen species (ROS) [60]. Crustaceans primarily rely on innate immunity to defend against microbial invasion [61]. ROS, including superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), are highly reactive oxygen radicals. These findings suggest that ROS-related pathways may be involved in the innate immune response of *P. trituberculatus*, but their exact roles in HX2 require further investigation.

Notably, among the candidate genes identified, we detected *Cht5* (chitinase), a gene strongly associated with growth, which is known to be essential for insect development [62].

Previous studies have shown that chitinases in crustaceans participate in a variety of physiological processes, including growth, development, and immune defenses, through the hydrolysis of chitin [63,64]. We hypothesize that during the molting phase of *P. trituberculatus*, which demands high energy consumption, chitinase may contribute to growth by breaking down chitin to supply energy [65]. However, the specific biological effects of artificial selection on these selected genes remain to be further elucidated. A limitation of this study is that the functional interpretation of candidate genes was mainly based on annotation results and previous studies in other species. Therefore, genes such as *GPX3*, *HMGC1*, *Duox*, *LAMB1*, *HSPG2*, and *Cht5* should be considered putative candidates. Further functional validation is required to confirm their roles in low-salinity tolerance, tissue repair, and growth-related traits in *P. trituberculatus*.

5. Conclusions

In this study, we analyzed the genetic diversity and population structure of the selectively bred *P. trituberculatus* population HX2 and wild populations (C and D) using a self-developed 43K liquid-phase SNP genotyping array. The genetic diversity of HX2 was comparable to that of the wild populations, indicating that genetic variation was effectively retained during the selective breeding process. However, low-to-moderate genetic differentiation was observed between HX2 and the wild populations. Furthermore, using two wild populations as reference groups, we conducted a selection sweep analysis and identified 425 candidate genes in the HX2 population. These genes were significantly enriched in pathways related to immunity and growth, suggesting their potential roles in stress tolerance and growth traits in HX2. Overall, our findings provide scientific guidance for the further genetic improvement of *P. trituberculatus* and offer insights into the molecular mechanisms underlying stress resistance and growth.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fishes11070410/s1>, Figure S1. The cross-validation error (vertical axis) corresponding to K values ranging from 1 to 8 (horizontal axis).

Author Contributions: Conceptualization, H.P., Y.W., J.L. and S.S.; methodology, H.P., Y.W. and S.S.; formal analysis, H.P., J.P. and B.Z.; investigation, H.P., J.P. and B.Z.; resources, Y.W., J.L. and S.S.; data curation, H.P. and D.S.; writing—original draft preparation, H.P.; writing—review and editing, D.S. and B.G.; supervision, P.L. and B.G.; project administration, B.G.; funding acquisition, B.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Key R&D Program of Shandong Province, grant number 2024LZGC023; the earmarked fund for CARS-48; the Central Public-interest Scientific Institution Basal Research Fund, CAFS, grant numbers 2023TD50 and 20603022025009; and the Weifang Science and Technology Development Program 2024, grant number 2024ZJ1180.

Institutional Review Board Statement: Ethical review and approval were waived for this study because the specimens in this study are invertebrates.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw sequencing data generated in this study have been deposited in the NCBI BioProject database under accession number PRJNA1300839 (PRJNA1300839).

Conflicts of Interest: The authors declare no conflicts of interest.

References

- Duan, B.; Zhang, J.; Kang, T.; Zhang, C.; Mu, S.; Guan, Y.; Ren, Y.; Li, Z.; Kang, X. Association Analysis Reveals SNP Markers Associated with Growth Traits in Swimming Crabs (*Portunus trituberculatus*). *Comp. Biochem. Physiol. Part D Genom. Proteom.* **2025**, *55*, 101527. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sun, D.; Zhou, X.; Su, Y.; Gao, B.; Liu, P.; Lv, J. Multi-Omics Analysis Reveals an Endogenous Competitive Regulatory Mechanism for Resistance to *Vibrio Parahaemolyticus* in the Swimming Crab, *Portunus trituberculatus*. *Fish. Shellfish Immunol.* **2025**, *16*, 110403. [\[CrossRef\]](#) [\[PubMed\]](#)
- Lv, J.; Liu, P.; Wang, Y.; Gao, B.; Chen, P.; Li, J. Transcriptome Analysis of *Portunus trituberculatus* in Response to Salinity Stress Provides Insights into the Molecular Basis of Osmoregulation. *PLoS ONE* **2013**, *8*, e82155. [\[CrossRef\]](#) [\[PubMed\]](#)
- Guo, J.; Lv, J.; Sun, D.; Liu, P.; Gao, B. Elucidating the Role of DNA Methylation in Low-Salinity Adaptation of the Swimming Crab *Portunus trituberculatus*. *Aquaculture* **2025**, *595*, 741707. [\[CrossRef\]](#)
- Lv, J.; Li, R.; Su, Z.; Gao, B.; Ti, X.; Yan, D.; Liu, G.; Liu, P.; Wang, C.; Li, J. A Chromosome-Level Genome of *Portunus trituberculatus* Provides Insights into Its Evolution, Salinity Adaptation and Sex Determination. *Mol. Ecol. Resour.* **2022**, *22*, 1606–1625. [\[CrossRef\]](#) [\[PubMed\]](#)
- Yue, G.H. Recent Advances of Genome Mapping and Marker-Assisted Selection in Aquaculture. *Fish. Fish.* **2014**, *15*, 376–396. [\[CrossRef\]](#)
- Cárcamo, C.B.; Díaz, N.F.; Winkler, F.M. Genetic Diversity in Chilean Populations of Rainbow Trout, *Oncorhynchus mykiss*. *Lat. Am. J. Aquat. Res.* **2015**, *43*, 59–70. [\[CrossRef\]](#)
- Lind, M.I.; Ingvarsson, P.K.; Johansson, H.; Hall, D.; Johansson, F. Gene Flow and Selection on Phenotypic Plasticity in an Island System of *Rana temporaria*. *Evolution* **2011**, *65*, 684–697. [\[CrossRef\]](#) [\[PubMed\]](#)
- Nascimento-Schulze, J.C.; Bean, T.P.; Houston, R.D.; Santos, E.M.; Sanders, M.B.; Lewis, C.; Ellis, R.P. Optimizing Hatchery Practices for Genetic Improvement of Marine Bivalves. *Rev. Aquac.* **2021**, *13*, 2289–2304. [\[CrossRef\]](#)
- Markert, J.A.; Champlin, D.M.; Gutjahr-Gobell, R.; Grear, J.S.; Kuhn, A.; McGreevy, T.J.; Roth, A.; Bagley, M.J.; Nacci, D.E. Population Genetic Diversity and Fitness in Multiple Environments. *BMC Evol. Biol.* **2010**, *10*, 205. [\[CrossRef\]](#) [\[PubMed\]](#)
- Xiao, Q.; Zhang, Z.; Sun, H.; Yang, H.; Xue, M.; Liu, X.; Zhang, W.; Zhen, Y.; Zhu, M.; Wang, Q.; et al. Genetic Variation and Genetic Structure of Five Chinese Indigenous Pig Populations in Jiangsu Province Revealed by Sequencing Data. *Anim. Genet.* **2017**, *48*, 596–599. [\[CrossRef\]](#) [\[PubMed\]](#)
- Bravo, S.; Larama, G.; Quiñones, J.; Paz, E.; Rodero, E.; Sepúlveda, N. Genetic Diversity and Phylogenetic Relationship among Araucana Creole Sheep and Spanish Sheep Breeds. *Small Rumin. Res.* **2019**, *172*, 23–30. [\[CrossRef\]](#)
- Duan, B.; Mu, S.; Guan, Y.; Li, S.; Yu, Y.; Liu, W.; Li, Z.; Ji, X.; Kang, X. Genetic Diversity and Population Structure of the Swimming Crab (*Portunus trituberculatus*) in China Seas Determined by Genotyping-by-Sequencing (GBS). *Aquaculture* **2022**, *555*, 738233. [\[CrossRef\]](#)
- Guo, L.; Sun, L.; Li, C. Identification and Characterization of Disease Resistance-Related SNPs Using Transcriptome Analyses in Sea Cucumber *Apostichopus japonicus*. *Fish. Shellfish Immunol.* **2025**, *163*, 110387. [\[CrossRef\]](#) [\[PubMed\]](#)
- Shin, J.W.; Yoon, S.; Lee, S.H.; Park, J.-Y.; Park, J.-W.; Kim, H.-C.; Kim, W.-J.; Kim, Y.-O.; Seo, D.; Noh, E.S. Development and Validation of a Pacific Abalone (*Haliotis discus hannai*) Custom 60K SNP Array. *Aquac. Rep.* **2025**, *42*, 102770. [\[CrossRef\]](#)
- Cui, T.; Liu, H.; Zhang, J.; Luo, Q.; Fei, S.; Chen, K.; Zhao, J.; Ou, M. Development and Evaluation of a 50 K SNP Array for Blotched Snakehead (*Channa maculata*). *Aquaculture* **2025**, *598*, 742020. [\[CrossRef\]](#)
- Holborn, M.K.; Rochus, C.M.; Ang, K.P.; Elliott, J.A.K.; Leadbeater, S.; Powell, F.; Boulding, E.G. Family-Based Genome Wide Association Analysis for Salmon Lice (*Lepeophtheirus salmonis*) Resistance in North American Atlantic Salmon Using a 50 K SNP Array. *Aquaculture* **2019**, *511*, 734215. [\[CrossRef\]](#)
- Ye, S.; Zhou, X.; Ouyang, M.; Cui, W.; Xiang, Z.; Zhang, Y.; Yuan, Y.; Ikhwanuddin, M.; Li, S.; Zheng, H.; et al. Development and Validation of a 40 K Liquid SNP Array for the Mud Crab (*Scylla paramamosain*). *Aquaculture* **2025**, *594*, 741394. [\[CrossRef\]](#)
- Garcia, B.F.; Bonaguro, Á.; Araya, C.; Carvalheiro, R.; Yáñez, J.M. Application of a Novel 50K SNP Genotyping Array to Assess the Genetic Diversity and Linkage Disequilibrium in a Farmed Pacific White Shrimp (*Litopenaeus vannamei*) Population. *Aquac. Rep.* **2021**, *20*, 100691. [\[CrossRef\]](#)
- Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. Fastp: An Ultra-Fast All-in-One FASTQ Preprocessor. *Bioinformatics* **2018**, *34*, 884–890. [\[CrossRef\]](#) [\[PubMed\]](#)
- Li, H.; Durbin, R. Fast and Accurate Short Read Alignment with Burrows-Wheeler Transform. *Bioinformatics* **2009**, *25*, 1754–1760. [\[CrossRef\]](#) [\[PubMed\]](#)
- McKenna, A.; Hanna, M.; Banks, E.; Sivachenko, A.; Cibulskis, K.; Kernytsky, A.; Garimella, K.; Altshuler, D.; Gabriel, S.; Daly, M.; et al. The Genome Analysis Toolkit: A MapReduce Framework for Analyzing next-Generation DNA Sequencing Data. *Genome Res.* **2010**, *20*, 1297–1303. [\[CrossRef\]](#) [\[PubMed\]](#)
- Wang, K.; Li, M.; Hakonarson, H. ANNOVAR: Functional Annotation of Genetic Variants from High-Throughput Sequencing Data. *Nucleic Acids Res.* **2010**, *38*, e164. [\[CrossRef\]](#) [\[PubMed\]](#)

24. Yu, H.; Jiao, J.; Zhong, B.; Ye, Y.; Shi, C.; Wang, C.; Li, R.; Song, W.; Wang, H.; Mu, C. The Effect of the BMP Signaling Pathway on the Development of Secondary Sexual Characteristics of Swimming Crab *Portunus trituberculatus*. *Aquac. Rep.* **2025**, *42*, 102836. [\[CrossRef\]](#)
25. Zhang, Y.; Chen, Y.; Hou, C.; Wang, C.; Mu, C. Analysis of cDNA Microarrays Revealed the Effects of Mating on the Ovary and Hepatopancreas of Female Swimming Crab (*Portunus trituberculatus*) during the Late Stage of Ovarian Development. *Comp. Biochem. Physiol. Part D Genom. Proteom.* **2025**, *55*, 101520. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Imai, H.; Fujii, Y.; Karakawa, J.; Yamamoto, S.; Numachi, K. Analysis of the Population Structure of the Swimming Crab, *Portunus trituberculatus* in the Coastal Waters of Okayama Prefecture, by RFLPs in the Whole Region of Mitochondrial DNA. *Fish. Sci.* **1999**, *65*, 655–656. [\[CrossRef\]](#)
27. Jia, S.; Lan, W.; Huang, Y.; Su, Y.; Gao, B.; Liu, P.; Lv, J. Functions of the Transcription Factor *Myb-like* in Sex Determination and Differentiation in *Portunus trituberculatus*. *Aquaculture* **2025**, *599*, 742173. [\[CrossRef\]](#)
28. He, X.; Wang, S.; Teng, W.; Li, H.; Xie, X.; Liu, S.; Li, D.; Yu, Z.; Liu, X.; Zhou, Z.; et al. Genetic Diversity and Differentiation of Cultured and Wild Yesso Scallop (*Mizuhopecten yessoensis*) from China Revealed by Whole Genome Resequencing. *Aquac. Rep.* **2025**, *43*, 102901. [\[CrossRef\]](#)
29. Yáñez, J.M.; Newman, S.; Houston, R.D. Genomics in Aquaculture to Better Understand Species Biology and Accelerate Genetic Progress. *Front. Genet.* **2015**, *6*, 128. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Qin, Y.; Shi, G.; Sun, Y. Evaluation of Genetic Diversity in *Pampus argenteus* Using SSR Markers. *Genet. Mol. Res.* **2013**, *12*, 5833–5841. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Liang, Y.; Xu, C.; Li, Q. Heterosis and Genetic Diversity of Intraspecific Hybrids Crosses between Two Selected Lines of the Pacific Oyster *Crassostrea gigas*. *Aquaculture* **2023**, *569*, 739369. [\[CrossRef\]](#)
32. Zhang, D.-Y.; Liu, X.-M.; Huang, W.-J.; Wang, Y.; Anwarullah, K.; Luo, L.-F.; Gao, Z.-X. Whole-Genome Resequencing Reveals Genetic Diversity and Signatures of Selection in Mono-Female Grass Carp (*Ctenopharyngodon idella*). *Aquaculture* **2023**, *575*, 739816. [\[CrossRef\]](#)
33. Wang, X.; Yang, Z.; Jiang, L.; Liu, Z.; Dong, X.; Sui, M.; Yin, C.; Shen, X.; Zhao, A.; Hu, J.; et al. Assessment of Germplasm Resource and Detection of Genomic Signature under Artificial Selection of Zhikong Scallop (*Chlamys farreri*). *Aquaculture* **2023**, *574*, 739730. [\[CrossRef\]](#)
34. Lv, J.; Cai, Y.; Liu, P.; Huang, X.; Lv, Z.; Zhang, R.; Zhao, L.; Bao, L.; Wang, S.; Hu, J. Genomic Differentiation and Selection Signatures of Two Elite Varieties of Yesso Scallop *Mizuhopecten yessoensis*. *Aquaculture* **2022**, *550*, 737842. [\[CrossRef\]](#)
35. Hedgecock, D.; Pudovkin, A.I. Sweepstakes reproductive success in highly fecund marine fish and shellfish: A review and commentary. *Bull. Mar. Sci.* **2011**, *87*, 971–1002. [\[CrossRef\]](#)
36. Feng, M.; Fang, K.; Chen, S.; Liu, G.; Chen, B.; Guo, H.; Wu, K.; Wen, X. The COI Gene Analysis Reveals the Impact of Production Activities on Genetic Diversity: A Case Study of *Scylla paramamosain* Populations in Coastal China. *Aquac. Rep.* **2025**, *43*, 102915. [\[CrossRef\]](#)
37. de Croos, M.D.S.T.; Pálsson, S. Population Biology and Genetic Diversity of Two Adjacent Shrimp (*Parapenaeopsis coromandelica*) Populations Exploited under Different Fishing Pressures in the Coastal Waters of Sri Lanka. *J. Mar. Biol. Assoc. UK* **2012**, *92*, 819–829. [\[CrossRef\]](#)
38. Liu, S.; Sun, J.; Hurtado, L.A. Genetic Differentiation of *Portunus trituberculatus*, the World's Largest Crab Fishery, among Its Three Main Fishing Areas. *Fish. Res.* **2013**, *148*, 38–46. [\[CrossRef\]](#)
39. López, M.E.; Cádiz, M.I.; Rondeau, E.B.; Koop, B.F.; Yáñez, J.M. Detection of Selection Signatures in Farmed Coho Salmon (*Oncorhynchus kisutch*) Using Dense Genome-Wide Information. *Sci. Rep.* **2021**, *11*, 9685. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Dalongeville, A.; Benestan, L.; Mouillot, D.; Lobreaux, S.; Manel, S. Combining Six Genome Scan Methods to Detect Candidate Genes to Salinity in the Mediterranean Striped Red Mullet (*Mullus surmuletus*). *BMC Genom.* **2018**, *19*, 217. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Lee, H.; Kim, W.-J.; Kang, H.-G.; Jang, J.-H.; Choi, I.J.; Chun, K.-H.; Kim, S.-J. Upregulation of LAMB1 via ERK/c-Jun Axis Promotes Gastric Cancer Growth and Motility. *Int. J. Mol. Sci.* **2021**, *22*, 626. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Liu, H.; Jin, G.; Wang, R.; Lian, Z.; Hu, X.; Luo, Z.; Lv, A.; Jia, L.; Shang, X. Extracellular Matrix (ECM) Pathway Involved in Skin Immune Response of *Cynoglossus semilaevis* upon *Vibrio vulnificus* Infection. *Water Biol. Secur.* **2024**, *3*, 100277. [\[CrossRef\]](#)
43. Li, L.; Yu, J.; Xia, Z.; Gao, Q.; Tang, Q.; Yi, S. Unveiling the Epigenetic Signatures: DNA Methylation Patterns in the Social Hierarchies of Male Giant Freshwater Prawns (*Macrobrachium rosenbergii*). *Aquaculture* **2025**, *609*, 742838. [\[CrossRef\]](#)
44. Otsuka, T.; Phan, A.Q.; Laurencin, C.T.; Esko, J.D.; Bryant, S.V.; Gardiner, D.M. Identification of Heparan-Sulfate Rich Cells in the Loose Connective Tissues of the Axolotl (*Ambystoma mexicanum*) with the Potential to Mediate Growth Factor Signaling during Regeneration. *Regen. Eng. Transl. Med.* **2020**, *6*, 7–17. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Keil, S.; Gupta, M.; Brand, M.; Knopf, F. Heparan Sulfate Proteoglycan Expression in the Regenerating Zebrafish Fin. *Dev. Dyn.* **2021**, *250*, 1368–1380. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Wang, S.; Fu, Y.; Zheng, X.; Zhou, J.; Liu, L.; Wang, C. Spatiotemporal Expression and Functional Analysis of PtWnt7b During Limb Regeneration in *Portunus trituberculatus*. *Int. J. Biol. Macromol.* **2025**, *319*, 145219. [\[CrossRef\]](#) [\[PubMed\]](#)

47. Ghanawi, J.; Saoud, I.P. Molting, Reproductive Biology, and Hatchery Management of Redclaw Crayfish *Cherax quadricarinatus* (von Martens 1868). *Aquaculture* **2012**, *358*, 183–195. [\[CrossRef\]](#)
48. Paiva, A.L.S.; Passaia, G.; Lobo, A.K.M.; Jardim-Messeder, D.; Silveira, J.A.G.; Margis-Pinheiro, M. Mitochondrial Glutathione Peroxidase (*OsGPX3*) Has a Crucial Role in Rice Protection against Salt Stress. *Environ. Exp. Bot.* **2019**, *158*, 12–21. [\[CrossRef\]](#)
49. Liu, C.-H.; Tseng, M.-C.; Cheng, W. Identification and Cloning of the Antioxidant Enzyme, Glutathione Peroxidase, of White Shrimp, *Litopenaeus vannamei*, and Its Expression Following *Vibrio alginolyticus* Infection. *Fish. Shellfish Immunol.* **2007**, *23*, 34–45. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Lan, M.; Wang, Y.; Mu, Y.; Li, Y.; Zhang, Z.; Guan, Y. Effects of a Chinese Herbal Medicine Mixture on Growth Performance, Immune Response, and Gene Expression in Chinese Soft-Shelled Turtles (*Pelodiscus sinensis*). *Anim. Feed. Sci. Technol.* **2024**, *316*, 116084. [\[CrossRef\]](#)
51. Chen, F.; Tholl, D.; Bohlmann, J.; Pichersky, E. The Family of Terpene Synthases in Plants: A Mid-Size Family of Genes for Specialized Metabolism That Is Highly Diversified throughout the Kingdom. *Plant. J.* **2011**, *66*, 212–229. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Si, Y.; Wen, H.; Li, Y.; He, F.; Li, J.; Li, S.; He, H. Liver Transcriptome Analysis Reveals Extensive Transcriptional Plasticity during Acclimation to Low Salinity in *Cynoglossus semilaevis*. *BMC Genom.* **2018**, *19*, 464. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Shi, M.; Zhang, Q.; Li, Y.; Zhang, W.; Liao, L.; Cheng, Y.; Jiang, Y.; Huang, X.; Duan, Y.; Xia, L.; et al. Global Gene Expression Profile under Low-Temperature Conditions in the Brain of the Grass Carp (*Ctenopharyngodon idellus*). *PLoS ONE* **2020**, *15*, e0239730. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Hua, J.; Tao, Y.; Lu, S.; Li, Y.; Dong, Y.; Jiang, B.; Xi, B.; Qiang, J. Integration of Transcriptome, Histopathology, and Physiological Indicators Reveals Regulatory Mechanisms of Largemouth Bass (*Micropterus salmoides*) in Response to Carbonate Alkalinity Stress. *Aquaculture* **2025**, *596*, 741883. [\[CrossRef\]](#)
55. Ha, E.-M.; Oh, C.-T.; Bae, Y.S.; Lee, W.-J. A Direct Role for Dual Oxidase in Drosophila Gut Immunity. *Science* **2005**, *310*, 847–850. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Jia, R.; Dai, X.; Li, Y.; Yang, X.; Min, X.; Quan, D.; Liu, P.; Huang, X.; Ge, J.; Ren, Q. Duox Mediated ROS Production Inhibited WSSV Replication in *Eriocheir sinensis* under Short-Term Nitrite Stress. *Aquat. Toxicol.* **2023**, *260*, 106575. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Nam, B.-H.; Park, E.-H.; Shin, E.-H.; Kim, Y.-O.; Kim, D.-G.; Kong, H.J.; Park, J.Y.; Seo, J.-K. Development of Novel Antimicrobial Peptides Derived from Anti-Lipopolysaccharide Factor of the Swimming Crab, *Portunus trituberculatus*. *Fish. Shellfish Immunol.* **2019**, *84*, 664–672. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Li, C.; Hong, P.-P.; Yang, M.-C.; Zhao, X.-F.; Wang, J.-X. FOXO Regulates the Expression of Antimicrobial Peptides and Promotes Phagocytosis of Hemocytes in Shrimp Antibacterial Immunity. *PLoS Pathog.* **2021**, *17*, e1009479. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Wen, L.; Bo, Q.; Zhang, J.; Zhu, P.; Tian, P.; Ning, J.; Wu, S.; Liu, G.; Liu, B.; Wang, C. Selection of Low-Salinity Resistant Strains and the Underlying Mechanisms in Bohai Red Scallops. *Aquaculture* **2025**, *608*, 742711. [\[CrossRef\]](#)
60. Yu, J.; Teng, S.; Yue, X.; Wang, H.; Liu, B. The Toll Pathway and Duox-ROS System Are Required for the Clam Antibacterial Immune Response in the Hepatopancreas. *Aquaculture* **2023**, *574*, 739637. [\[CrossRef\]](#)
61. Huang, Y.; Ren, Q. Research Progress in Innate Immunity of Freshwater Crustaceans. *Dev. Comp. Immunol.* **2020**, *104*, 103569. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Zhang, J.; Zhang, X.; Arakane, Y.; Muthukrishnan, S.; Kramer, K.J.; Ma, E.; Zhu, K.Y. Identification and Characterization of a Novel Chitinase-like Gene Cluster (*AgCht5*) Possibly Derived from Tandem Duplications in the African Malaria Mosquito, *Anopheles gambiae*. *Insect Biochem. Mol. Biol.* **2011**, *41*, 521–528. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Li, X.; Zhou, G.; Zhou, J.; Lin, H.; Li, Y.; Lu, Q.; Zhang, Y. Research Progresses in Function and Expression Regulation of Chitinase Gene Family of Aquatic Crustaceans. *J. Aquac.* **2017**, *38*, 26–30. [\[CrossRef\]](#)
64. Song, L.; Lv, J.; Wang, L.; Sun, D.; Gao, B.; Liu, P. Characterization of a Chitinase-1 Gene (*PtCht-1*) from a Marine Crab *Portunus trituberculatus* and Its Response to Immune Stress. *Gene* **2020**, *741*, 144523. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Zhou, K.; Zhou, F.; Huang, J.; Yang, Q.; Jiang, S.; Qiu, L.; Yang, L.; Zhu, C.; Jiang, S. Characterization and Expression Analysis of a Chitinase Gene (*PmChi-4*) from Black Tiger Shrimp (*Penaeus monodon*) under Pathogen Infection and Ambient Ammonia Nitrogen Stress. *Fish. Shellfish Immunol.* **2017**, *62*, 31–40. [\[CrossRef\]](#) [\[PubMed\]](#)

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