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Assessment of Genetic Diversity in Germplasm Resources of Cultured Marine Groupers

Hejun Peng [1]
Yuanhe Street Office, Yunhe County, Lishui City, Yuanhe 323600, Zhejiang, China
Corresponding author: 276529718@qq.com

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Abstract This study explores the classification characteristics, geographic distribution, and utilization status of grouper germplasm resources, and elaborates on the theoretical basis and major evaluation indices of genetic diversity. It focuses on summarizing the application progress of molecular techniques—such as microsatellite markers (SSR), single nucleotide polymorphisms (SNP), mitochondrial DNA, and high-throughput sequencing—in population genetic analyses, and analyzes the genetic structural differences between cultured and wild populations through representative case studies. The results indicate that wild grouper populations generally maintain relatively high genetic diversity, whereas cultured populations commonly exhibit reduced allelic richness, decreased heterozygosity, and increased genetic differentiation, mainly due to founder effects, genetic drift, and artificial selection. Meanwhile, genetic bottlenecks and inbreeding effects have gradually emerged in some cultured populations. Current research still faces limitations, including insufficient sample coverage, lack of a unified evaluation system, and inadequate integration of multi-omics approaches. Based on these findings, this study proposes strengthening genetic monitoring, optimizing broodstock management, establishing germplasm conservation systems, and promoting the application of high-throughput genomic technologies in genetic evaluation and molecular breeding. The results provide a theoretical basis for the conservation of grouper germplasm resources, genetic improvement, and the high-quality development of the aquaculture industry.

Keywords Grouper; Germplasm resources; Genetic diversity; Molecular markers; Population genetic structure

1 Introduction

Groupers (*Epinephelus* spp.), as one of the most important marine economic fish species, occupy a significant position in global mariculture due to their rapid growth, high flesh quality, and high market value. This group is mainly distributed and farmed in tropical and subtropical regions, with Asia—particularly China, Taiwan, and Indonesia—contributing over 90% of global production. Driven by market demand, the grouper industry has expanded rapidly, with global production increasing from approximately 60,000 tons in 1990 to nearly 200,000 tons in 2007. In China, coastal regions in the south have developed grouper aquaculture into a dominant specialty industry, supported by continuous advancements in farming technologies. Artificial breeding techniques have gradually matured, and superior varieties such as orange-spotted grouper, giant grouper, and their hybrids have been widely applied. However, the rapid expansion of the industry has, to some extent, outpaced the development of technology and management. Current farming systems still face challenges such as insufficient seed supply, high dependence on wild-caught juveniles, unbalanced feed structures, and frequent disease outbreaks (Ybanez and Gonzales, 2023). Meanwhile, long-term artificial selection and closed breeding practices have led to issues such as germplasm degradation, reduced growth performance, and weakened disease resistance, posing significant constraints on the sustainable development of the industry.

Germplasm resources constitute the fundamental basis for the sustainable development of aquaculture, and their level of genetic diversity directly determines a population's adaptability to environmental changes and resistance to pathogenic stress (Yang et al., 2024). High genetic diversity not only helps maintain population vitality but also provides potential resources for the genetic improvement of desirable traits. However, under the combined pressures of overfishing, habitat destruction, and aquaculture practices reliant on wild resources, the genetic diversity of wild grouper populations is declining. At the same time, non-standardized seed production processes can induce genetic bottlenecks, inbreeding accumulation, and allele loss, further weakening the genetic foundation of cultured populations (Ai et al., 2025). Therefore, protecting wild populations, optimizing broodstock management, and conducting continuous genetic monitoring are of great importance for maintaining germplasm stability and promoting selective breeding programs (Nousias et al., 2021; Li, 2022).

With the development of molecular biology technologies, research on genetic diversity in groupers has continued to deepen. Commonly used approaches include microsatellite markers (SSR), ISSR, mitochondrial DNA (mtDNA), DNA barcoding, as well as more recent high-throughput sequencing and environmental DNA (eDNA) technologies (Hassanien and Al-Rashada, 2020; Ai et al., 2025). Studies have shown that cultured populations generally exhibit lower genetic diversity compared to wild populations and often display significant genetic differentiation, mainly due to genetic mechanisms such as founder effects, genetic drift, and inbreeding. In addition, parent-offspring comparative studies based on microsatellite markers have revealed evident allele loss and genetic bottleneck signals during artificial breeding, highlighting the importance of genetic quality control in breeding programs. Molecular surveys at regional scales have also demonstrated significant genetic structure differences among geographically distinct populations, providing important evidence for resource conservation and stock enhancement programs (Tavakoli-Kolour et al., 2022; Nurdin et al., 2025).

This study aims to explore the research progress and existing issues in the genetic diversity of grouper germplasm resources. Although extensive studies have been conducted both domestically and internationally, limitations remain, including restricted sample coverage, the lack of a unified evaluation index system, and insufficient integration of multi-omics technologies, making it difficult to comprehensively characterize the overall genetic landscape of grouper germplasm resources. Taking marine-cultured grouper germplasm as the research subject, this study systematically reviews the theoretical foundations and technical systems for genetic diversity assessment and, combined with representative case studies, conducts a comprehensive analysis of current genetic diversity levels and existing challenges. The findings are expected to provide a theoretical basis for the scientific conservation and efficient utilization of grouper germplasm resources, offer references for optimizing molecular breeding strategies and developing improved varieties, and ultimately promote the high-quality and sustainable development of the grouper aquaculture industry.

2 Overview of Grouper Germplasm Resources

2.1 Classification of groupers and major cultured species

Groupers, in a broad sense, belong to the order Perciformes. Traditionally, they were classified under the family *Serranidae*, subfamily Epinephelinae. However, with advances in molecular phylogenetics, their classification has been progressively revised, and they are now recognized as an important group within the family *Epinephelidae*. This group is species-rich, with more than 160 species recorded worldwide, primarily distributed in tropical and subtropical waters, especially in the Indo-Pacific region (Ybanez and Gonzales, 2023). Nevertheless, due to both significant differences and certain convergent characteristics in body coloration, morphology, and ecological habits among species, traditional morphological methods have limitations in identifying closely related species, thereby affecting the accuracy of germplasm resource surveys and breeding practices.

With the development of molecular marker technologies, phylogenetic analyses based on mitochondrial genes such as Cyt b and COI have become important tools for grouper classification. Studies have shown that some morphologically distinct groups are not genetically independent; for example, Cromileptes is phylogenetically nested within the genus *Epinephelus*, revealing inconsistencies between traditional morphological classification and genetic relationships (Hassanien and Al-Rashada, 2020). Therefore, establishing a phylogeny-based classification system not only improves species identification accuracy but also provides a theoretical basis for pedigree management, hybrid design, and germplasm standardization.

In aquaculture applications, groupers have developed into high-value marine farming species. Globally, commercial aquaculture involves at least 47~48 grouper species and 15~16 hybrid combinations, with Asia playing a dominant role (Ybanez and Gonzales, 2023). In China, the main cultured species include orange-spotted grouper (*E. coioides*), Malabar grouper (*E. malabaricus*), giant grouper (*E. lanceolatus*), and yellow grouper (*E. awoara*). Additionally, species such as brown-marbled grouper, camouflage grouper, and coral trout also hold high economic value in regional aquaculture (Das et al., 2021). In recent years, hybrid breeding has developed rapidly; for instance, hybrids between giant grouper and orange-spotted grouper exhibit significant advantages in growth and stress resistance. However, differences in genetic background and pedigree stability among species and hybrids may lead to germplasm admixture and distortion of resource information if standardized classification and genetic monitoring are lacking. Therefore, systematic classification and phylogenetic organization of germplasm resources form the foundation for genetic evaluation and breeding utilization.

2.2 Distribution characteristics of different geographic populations

Groupers are widely distributed in tropical and subtropical seas, and in China they are mainly found in the South China Sea, East China Sea, and Taiwan Strait. Although their distribution range is broad, genetic studies indicate that natural populations are not randomly mixed but generally exhibit significant geographic structure (Chen et al., 2025). This differentiation is mainly influenced by ocean current systems, strait barriers, island distribution, and historical sea-level fluctuations during the Pleistocene, as well as species-specific dispersal capacity and reproductive behavior (Yang et al., 2022; Fadli et al., 2023).

Taking orange-spotted grouper (*E. coioides*) as an example, microsatellite analyses have revealed significant genetic differentiation among populations from China, Malaysia, and Indonesia, while populations within China’s coastal waters show relatively low differentiation. This suggests the formation of regional genetic structure at large spatial scales, with some degree of gene flow at local levels. Further studies indicate that this species may comprise two major evolutionary lineages, likely shaped by marginal sea isolation and Pleistocene sea-level changes (Chen et al., 2025). Similarly, yellow grouper (*E. awoara*) exhibits relatively high genetic diversity, but the Beibu Gulf population shows distinct characteristics, with the Qiongzhou Strait and Taiwan Strait acting as important barriers in its genetic differentiation (Yang et al., 2022).

At broader spatial scales, different grouper species exhibit diverse patterns of genetic structure. Studies in the Indo-Malay Archipelago show that some species display clear geographic differentiation, whereas others exhibit genetic homogeneity, closely related to larval dispersal capacity and habitat dependence (Fadli et al., 2023). In addition, regional genetic structure and low effective population sizes have been observed in species such as Nassau grouper and brown grouper in the Atlantic (Vaini et al., 2021). Notably, in aquaculture systems, frequent translocation of juveniles and artificial breeding have led to increasing mixing of geographic populations, gradually weakening original genetic structures. While this improves resource utilization efficiency, it also introduces risks of genetic contamination and loss of local adaptation. Therefore, both natural geographic structure and human intervention must be considered in germplasm utilization to ensure genetic compatibility and resource security.

2.3 Current status and challenges of germplasm resources

At present, grouper germplasm resources are characterized by “rich diversity but uneven utilization.” Wild populations are highly diverse with complex genetic backgrounds, providing important genetic sources for breeding; however, aquaculture mainly relies on a limited number of dominant species and their hybrids (Ybanez and Gonzales, 2023). Although Asia contributes over 90% of global production, germplasm utilization is relatively concentrated. While this improves production efficiency, it also exacerbates issues such as a narrow genetic base and germplasm homogenization. Under long-term artificial selection and closed breeding systems, some cultured populations have exhibited genetic degeneration, including reduced growth performance, weakened stress resistance, and increased disease susceptibility (Li, 2022; Sonesson et al., 2023).

From a genetic perspective, these problems mainly arise from founder effects, genetic drift, and the accumulation of inbreeding. Studies have shown that in species such as orange-spotted grouper, kelp grouper, and sevenband grouper, cultured populations often experience the loss of rare alleles and reduced allelic richness. Even when heterozygosity does not change significantly, their genetic potential may still decline. In addition, insufficient broodstock numbers and inadequate pedigree management can lead to excessive contributions from certain families, accelerating the loss of genetic diversity (Sonesson et al., 2023). Meanwhile, although hybrid breeding can enhance growth performance, the absence of molecular marker-assisted management may result in genetic background confusion and biased evaluation.

On the other hand, wild germplasm resources are also under continuous pressure. Overfishing, habitat degradation, and environmental disturbances have led to population declines and reduced genetic diversity in some species (Fadli et al., 2023; Ybanez and Gonzales, 2023; Nurdin et al., 2025). Some populations have shown signals of historical contraction and even low levels of genetic variation (Vaini et al., 2021; Chen et al., 2025). Additionally, management challenges remain, including insufficient genetic assessment, incomplete broodstock renewal mechanisms, lack of germplasm databases, and inadequate coordination of conservation policies (Li, 2022; Sonesson et al., 2023). Therefore, it is necessary to establish a full-chain management system covering “wild resources—conservation populations—breeding populations—commercial seed,” integrating molecular monitoring, broodstock management, and ecological conservation to ensure the long-term stability and sustainable utilization of grouper germplasm resources (Li, 2022).

3 Theoretical Basis for Genetic Diversity Assessment in Groupers

3.1 Concept and evaluation indices of genetic diversity

Genetic diversity refers to the quantity and distribution patterns of genetic variation within a species and among different populations. It is an important component of biodiversity and forms the basis for aquatic organisms to adapt to environmental changes, maintain population stability, and support genetic improvement (Hassanien and Al-Rashada, 2020; Yang et al., 2022). For marine aquaculture species such as groupers, genetic diversity is primarily reflected at the DNA level, including the number and frequency distribution of alleles, haplotype composition, and variation at polymorphic loci across the genome. These genetic differences may further manifest as phenotypic variations in traits such as growth rate, body shape, disease resistance, and environmental adaptability. Therefore, a high level of genetic diversity generally indicates stronger environmental adaptability, greater evolutionary potential, and lower risk of population decline, making it an important criterion for evaluating the quality and breeding potential of grouper germplasm resources (Chen et al., 2025).

Compared with traditional morphological indicators, molecular marker technologies can more directly reflect genetic variation and are less affected by environmental factors; thus, they have become core tools in studies of genetic diversity in groupers. Currently, commonly used methods include microsatellite markers (SSR), mitochondrial DNA (mtDNA) haplotype analysis, single nucleotide polymorphisms (SNPs), ISSR, as well as rapidly developing high-throughput genotyping and whole-genome resequencing technologies (Hassanien and Al-Rashada, 2020; Hsu et al., 2023; Wu et al., 2024). These techniques have enabled genetic diversity assessment to evolve from early low-resolution morphological or limited-locus analyses to comprehensive multi-locus, genome-wide, and functionally associated analyses, improving both the accuracy of population comparisons and the interpretation of the genetic basis of germplasm resources (Houston et al., 2020).

In practice, genetic diversity assessment typically relies on a set of quantitative indices to form a comprehensive analytical framework. Common within-population diversity indices include the number of alleles (Na), effective number of alleles (Ne), allelic richness, observed heterozygosity (Ho), and expected heterozygosity (He), which reflect variation at the allele and genotype levels (Hassanien and Al-Rashada, 2020). Among these, Na represents the total number of alleles detected at a locus, whereas Ne emphasizes the contribution of allele frequency distribution to effective variation. Ho and He represent the actual proportion of heterozygous individuals and the theoretically expected heterozygosity, respectively. In addition, polymorphism information content (PIC), nucleotide diversity (π), and haplotype diversity (Hd) are commonly used to evaluate marker polymorphism and sequence-level variation (Chen et al., 2025). For comparisons among populations, indices and methods such as FST, ΦST, GST, DEST, genetic distance, principal component analysis (PCA), and Bayesian clustering are used to reveal genetic structure differences among geographic populations, between wild and cultured populations, and between parents and offspring (Figure 1) (Vaini et al., 2021; Yang et al., 2022).

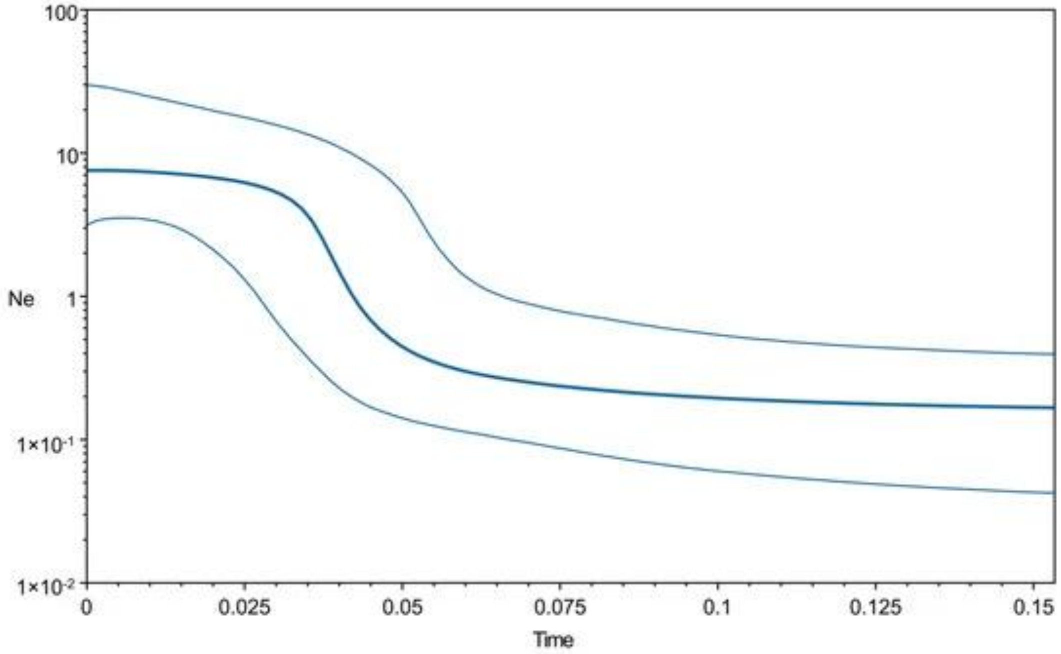

Figure 1 Bayesian skyline plot of the effective population sizes through time for *Epinephelus awoara* (Adopted from Yang et al., 2022)

Image caption: The y-axis is the product of effective population size (Ne) and generation length in a log scale while the x-axis is the time scale before present in units of million years ago (Adopted from Yang et al., 2022)

3.2 Population genetic structure and evolutionary mechanisms

Population genetic structure refers to the distribution patterns of genotypes and allele frequencies within and among populations and is a key aspect of genetic diversity research. In groupers, varying degrees of genetic differentiation may exist among geographic populations, among cultured populations from different hatcheries, and even among different families within the same farming system. Such differentiation can be quantified and visualized using methods such as FST, genetic distance, analysis of molecular variance (AMOVA), PCA, and clustering analysis, thereby revealing phylogenetic relationships, levels of gene flow, and potential boundaries of genetic units (Vaini et al., 2021; Yang et al., 2022). Therefore, population genetic structure analysis is not only fundamental for understanding patterns of population differentiation but also provides an important basis for germplasm conservation, broodstock management, and breeding utilization.

From the perspective of natural populations, the genetic structure of groupers results from the combined effects of gene flow, genetic drift, natural selection, and historical population dynamics. Traditional views suggest that marine fishes, due to their pelagic larval stages, should exhibit high connectivity and genetic homogeneity. However, extensive empirical studies have demonstrated that grouper populations are not completely randomly mixed. Studies on Nassau grouper, yellow grouper, orange-spotted grouper, and brown grouper indicate that their population structures are often influenced by straits, marginal seas, island barriers, ocean current systems, and dependence on specific spawning grounds, leading to significant regional differentiation (Vaini et al., 2021; Yang et al., 2022; Chen et al., 2025). In addition, Pleistocene glaciations, sea-level fluctuations, and repeated isolation-connection events in marginal seas have shaped the present-day genetic patterns of groupers over longer timescales. In other words, the observed population genetic structure reflects not only contemporary ecological processes but also the combined effects of historical geological events and modern ecological dynamics.

In aquaculture systems, anthropogenic factors play an even more significant role in shaping population genetic structure. Artificial breeding typically relies on a limited number of broodstock, and the effective population size is often smaller than the apparent number of parents. As a result, offspring populations are prone to founder effects and genetic drift, leading to rapid loss of rare alleles. Notably, such genetic erosion may not immediately manifest as a significant decline in heterozygosity but is often first reflected in reduced allelic richness and imbalanced family contributions. Therefore, relying on a single indicator to evaluate the genetic quality of cultured populations may underestimate potential risks. Moreover, directional selection for traits such as growth rate, feed efficiency, and disease resistance may lead to selective sweeps in specific genomic regions, forming distinct patterns of population differentiation (Wu et al., 2024). Without proper pedigree records and parentage verification, repeated use of closely related broodstock can exacerbate inbreeding and relatedness accumulation. Thus, molecular marker-based parentage analysis, pedigree reconstruction, and family contribution assessment are crucial for maintaining stable genetic structure in cultured populations (Weng et al., 2021).

3.3 Relationship between genetic diversity and germplasm improvement

Genetic diversity is the foundation of germplasm improvement and genetic breeding. Abundant genetic variation provides the necessary basis for selecting desirable traits, estimating genetic parameters, and improving breeding values. In grouper breeding, whether through traditional mass selection, family-based selection, marker-assisted selection, or genomic selection, all approaches fundamentally rely on the available genetic variation within populations. If genetic diversity is insufficient, the reservoir of favorable alleles and opportunities for recombination are limited, leading to reduced breeding response and even stagnation in long-term improvement (Houston et al., 2020; Hsu et al., 2023; Wu et al., 2024). This is particularly important for complex economic traits such as growth rate, feed efficiency, disease resistance, and environmental tolerance, which are typically controlled by multiple genes. Sustained genetic gain can only be achieved when the base population maintains sufficient genetic variation.

On the other hand, genetic diversity assessment provides direct guidance for broodstock management and breeding population design. Using microsatellite and SNP markers for pedigree reconstruction, parentage identification, and kinship analysis enables accurate evaluation of parental contributions, control of inbreeding accumulation, balancing of family representation, and optimization of hybrid combinations (Weng et al., 2021). For aquaculture species like groupers, which rely heavily on artificial propagation and hatchery expansion, the absence of molecular-level pedigree management can result in repeated use of a few core broodstock, reducing effective population size and weakening the breeding foundation. In hybrid breeding, an appropriate level of genetic divergence is also a prerequisite for heterosis. If parental genetic backgrounds are too similar, heterosis may be limited; if the genetic distance is too large, risks such as unstable combining ability, developmental abnormalities, and increased segregation in offspring may arise (Xu et al., 2025). Therefore, genetic diversity analysis not only aids in broodstock selection but also helps establish theoretical relationships between genetic divergence and hybrid performance.

Furthermore, genetic diversity assessment provides important support for wild resource conservation and the renewal of cultured germplasm. By comparing genetic differences between wild and cultured populations, it is possible to identify whether genetic diversity loss, pedigree admixture, or localized genetic degeneration has occurred in aquaculture systems, thereby informing decisions on introducing wild germplasm or restoring local populations (Weng et al., 2021; Yang et al., 2022; Chen et al., 2025). At a broader scale, studies of population structure and historical dynamics help define management units, protect key spawning populations, and conserve unique genetic lineages—factors that are critical not only for natural resource conservation but also for maintaining a sufficiently broad genetic base for future breeding systems (Vaini et al., 2021). Therefore, grouper germplasm improvement must adhere to the principle of balancing “conservation and utilization”: on the one hand, maintaining sources of variation by increasing effective population size, introducing new germplasm, and preserving clear genetic structure; on the other hand, improving selection efficiency through molecular markers, whole-genome information, and trait association analyses. Only by advancing germplasm improvement on the premise of preserving genetic diversity can the grouper aquaculture industry achieve sustainable, stable, and high-quality development.

4 Genetic Diversity Assessment of Grouper Populations

4.1 Sample sources and experimental design

To evaluate the genetic diversity of typical marine-cultured grouper populations and their differences from adjacent wild populations, this study selected grouper populations from a representative aquaculture area along the South China Sea coast of China. Both cultured populations and nearby wild populations were included. Such a design is highly representative in grouper genetics, as key cultured species such as orange-spotted grouper (*Epinephelus coioides*) and giant grouper *(E. lanceolatus*) often coexist as both hatchery-produced populations and regional wild stocks, providing ideal materials for comparing genetic differences between “cultured domestication” and “wild retention” (Figure 2) (Tavakoli-Kolour et al., 2022; Chen et al., 2025). For sampling, cultured populations were collected from three large-scale aquaculture farms, with 30 individuals randomly sampled from each farm to capture genetic variation within different production units. Wild populations were obtained through nearshore fishing or traceability from regional fish markets, with approximately 40 individuals collected to represent the genetic background of adjacent natural populations.

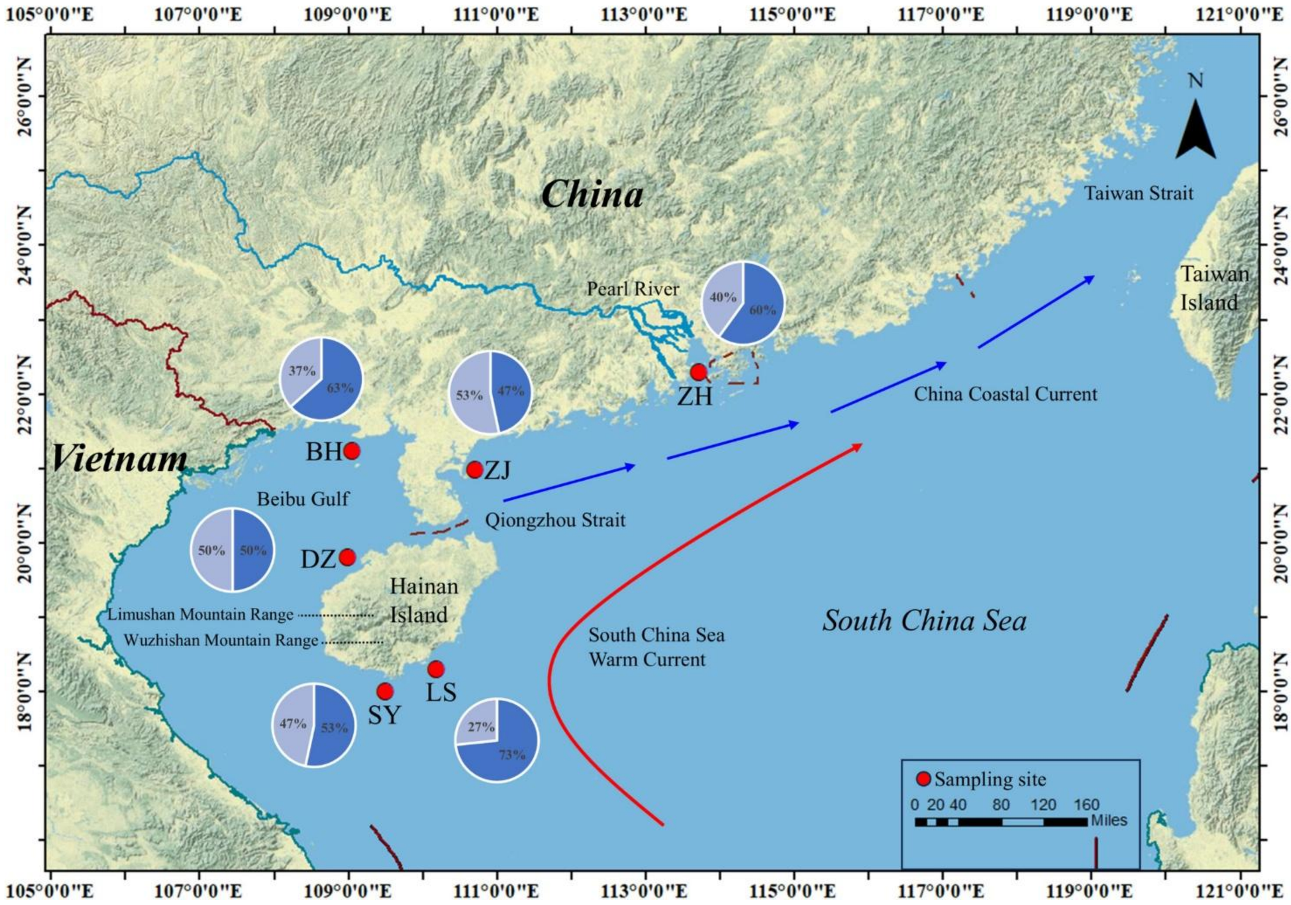

Figure 2 Map of the coast of Hainan Island and mainland China along the South China Sea illustrating the sampling locations of *Epinephelus* coioides (Adopted from Chen et al., 2025)

Image caption: Each color in the pie charts represents the frequencies of the Lineage A (orange) and Lineage B (gray) haplotypes in each population (Adopted from Chen et al., 2025)

All samples consisted of muscle or fin tissue, preserved in liquid nitrogen or absolute ethanol in the field and transported to the laboratory for DNA extraction and molecular analysis. Sampling procedures aimed to avoid repeated collection from the same families or closely related individuals. Information such as sample origin, collection site, farming history, broodstock source, and generation data was recorded to minimize sampling bias and improve interpretability. This design balanced spatial representativeness and genetic heterogeneity within aquaculture systems, providing a solid basis for comparing genetic differentiation between wild and cultured populations.

In terms of experimental design, a combined analysis using microsatellite markers (SSR) and selected SNP loci was adopted to achieve both high polymorphism detection and high-resolution population structure analysis. Previous studies indicate that grouper genetic analyses typically use 8-20 SSR loci supplemented by mitochondrial genes or SNP data derived from reduced-representation sequencing, enhancing the detection of within-population diversity, kinship, and differentiation patterns (Weng et al., 2021; Yang et al., 2022; Wu et al., 2024). In this study, DNA was extracted using the standard phenol-chloroform method, followed by PCR amplification of target fragments. SSR genotyping was performed using capillary electrophoresis platforms, while SNP loci were detected using high-throughput sequencing. Data analysis was conducted using software such as PopGen, Arlequin, and STRUCTURE to calculate indices including allele number, heterozygosity, polymorphism information content, inbreeding coefficient, and Hardy-Weinberg equilibrium. Further analyses such as FST, AMOVA, PCA, and clustering were used to characterize population differentiation. If known parent-offspring relationships were included, kinship and parentage analyses were also performed to estimate effective breeding population size and identify unequal reproductive contributions (Hassanien and Al-Rashada, 2020; Weng et al., 2021; Yang et al., 2022).

4.2 Molecular marker results and population structure analysis

The molecular marker results indicated clear differences in genetic diversity among grouper populations from different sources. Overall, wild populations exhibited higher allele numbers, allelic richness, and heterozygosity than cultured populations, suggesting greater genetic variation. In contrast, cultured populations showed allele loss, reduction of rare alleles, and imbalanced allele frequencies at certain loci, indicating genetic variation loss during artificial breeding and seed propagation. This pattern is consistent with previous studies showing that cultured populations are more susceptible to founder effects and genetic drift under domestication and limited broodstock conditions, resulting in a narrower genetic base.

From the perspective of heterozygosity and inbreeding parameters, wild populations generally showed higher expected heterozygosity (He) than cultured populations. In cultured populations, observed heterozygosity (Ho) at some loci was lower than expected, leading to elevated FIS values and suggesting heterozygote deficiency (Yang et al., 2022). This pattern may result from inbreeding, family structure, Wahlund effects, or the presence of mixed subpopulations. Notably, some hatchery studies have shown that even when offspring heterozygosity is similar to that of parents, rare alleles are significantly reduced and genetic bottleneck signals are detectable. This suggests that genetic drift may first manifest as a reduction in allelic richness rather than an immediate decline in heterozygosity (Wenne, 2023). Therefore, interpreting genetic status in cultured populations requires a multi-indicator approach rather than reliance on a single heterozygosity metric.

Population structure analyses further revealed that FST values among cultured populations were generally low, indicating frequent germplasm exchange or shared broodstock sources among farms. In contrast, FST values between cultured and wild populations were relatively high, indicating a certain degree of genetic differentiation. AMOVA results typically supported this pattern, showing that a significant proportion of genetic variation was attributable to differences among populations, particularly between “wild” and “cultured” groups. STRUCTURE, PCA, and PCoA analyses commonly separated samples into two main genetic clusters corresponding to wild and cultured origins, although some individuals exhibited admixture (Chen et al., 2025). Such admixture may reflect gene flow caused by stock enhancement, cross-regional seed transfer, introduction of wild broodstock, or historical germplasm exchange. Additionally, selection signals detected at certain SNP loci suggest that artificial selection has left functional genetic footprints in genomic regions associated with growth, stress resistance, or disease resistance (Wu et al., 2024).

4.3 Genetic diversity evaluation and germplasm conservation recommendations

Based on the comprehensive analysis of genetic diversity indices in this case, wild grouper populations in the study area still maintain relatively high genetic diversity, whereas cultured populations have shown varying degrees of genetic variation decline, reduced allelic richness, and increased differentiation from wild populations.

This pattern—"high variation in wild populations and gradual drift in cultured populations"—is consistent with general trends observed in multiple grouper studies, indicating that long-term artificial selection, limited broodstock usage, and patterns of germplasm exchange among hatcheries have significantly influenced the genetic structure of cultured populations (Chen et al., 2025). Without timely intervention, this trend may lead to further inbreeding accumulation, narrowing of the genetic base, and germplasm degradation, ultimately reducing production performance and future breeding potential.

Based on these findings, several measures should be implemented in aquaculture practice to protect and enhance genetic diversity. First, broodstock sources should be expanded by establishing base breeding populations from multiple genetically diverse and well-documented populations, while avoiding indiscriminate mixing of highly divergent management units to prevent disruption of local adaptation or new genetic instability (Yang et al., 2022). Second, standardized breeding management systems should be established, including pedigree recording, controlled reuse of broodstock, balanced family contributions, and rotational mating strategies to reduce inbreeding and maintain effective population size (Weng et al., 2021). In addition, molecular marker monitoring should be incorporated into routine germplasm management to continuously track key parameters such as allelic richness, He, FIS, and effective population size, and to detect genetic bottlenecks and germplasm degradation signals in a timely manner (Wenne, 2023).

Conservation of wild grouper resources is essential for maintaining overall germplasm quality. Studies have shown that although many wild populations still retain relatively high genetic diversity, some regional populations exhibit signs of historical contraction, low effective population size, and significant geographic differentiation (Vaini et al., 2021; Yang et al., 2022; Chen et al., 2025). Therefore, it is necessary to strengthen the protection of key spawning grounds, juvenile habitats, and locally unique populations, while restricting intensive fishing and habitat destruction. In stock enhancement programs, genetic data should be used to select appropriate source populations to avoid genetic homogenization or dilution of local genotypes in wild populations. Where feasible, long-term conservation strategies such as germplasm reserves, live conservation populations, cryopreserved sperm banks, and DNA repositories should be established to preserve important genetic resources. Ultimately, a dynamic management framework integrating "wild resource conservation—cultured germplasm optimization—genetic monitoring feedback—re-conservation" should be developed. Through this integrated cycle of conservation, utilization, and re-conservation, it is possible to maintain evolutionary potential and future breeding resources while ensuring production performance, thereby supporting the high-quality and sustainable development of the marine aquaculture industry (Hassanien and Al-Rashada, 2020; Weng et al., 2021).

5 Methods for Genetic Diversity Analysis in Groupers

5.1 Molecular marker technologies

Molecular marker technologies based on DNA variation are among the most widely used and informative approaches in grouper genetic diversity studies. Their core principle is to detect genetic variation in the nuclear or mitochondrial genome to reveal population genetic structure, diversity levels, kinship relationships, and germplasm origins (Hassanien and Al-Rashada, 2020). Compared with traditional phenotypic traits, molecular markers directly target genetic material and are less influenced by environmental factors, thus offering clear advantages in population identification, broodstock management, inbreeding monitoring, population differentiation analysis, and molecular breeding. Commonly used techniques include microsatellite markers (SSR), ISSR, single nucleotide polymorphisms (SNPs), and mitochondrial DNA markers. Each type differs in resolution, cost, and application scenarios, collectively forming the technical foundation for genetic diversity analysis in groupers.

Microsatellite markers (simple sequence repeats, SSR) are typical codominant markers characterized by high polymorphism, abundant information content, high resolution, and well-established protocols, and they have long been core tools in grouper population genetic studies (Hassanien and Al-Rashada, 2020). By analyzing allele number, frequency distribution, heterozygosity, and polymorphism information content at SSR loci, researchers can effectively assess genetic variation within populations, genetic differentiation among populations, and kinship among individuals. In important cultured species such as *Epinephelus* spp. and giant grouper, relatively stable SSR marker systems have been established and are widely used for population structure analysis, molecular fingerprinting, parentage identification, and broodstock management, particularly for detecting inbreeding accumulation and unequal reproductive contributions in cultured populations. In addition to SSR, ISSR and its derivative technologies are also applied in grouper genetic diversity analysis. ISSRseq combined with high-throughput sequencing can simultaneously generate large amounts of SNP data, making it especially suitable for materials lacking complete pedigree information or reference genomes (Hsu et al., 2023).

In recent years, SNPs have gradually become mainstream tools for grouper genetic diversity studies and precision breeding (Sherman et al., 2020; Hsu et al., 2023). As the most common type of genomic variation, SNPs are widely distributed, genetically stable, and suitable for automated and high-throughput detection. Using approaches such as RAD-seq, ISSRseq, genotyping-by-sequencing (GBS), or whole-genome resequencing, large numbers of SNP loci can be identified at the genome-wide scale, enabling fine-resolution analysis of population structure and further applications such as selection signal detection, genome-wide association studies, and candidate gene identification. For example, in leopard coral grouper, whole-genome resequencing identified more than 8.7 million SNPs, enabling high-resolution population clustering and identification of candidate genomic regions associated with growth traits (Wu et al., 2024). In addition, mitochondrial DNA markers, due to their maternal inheritance, lack of recombination, and relatively rapid evolutionary rate, have unique advantages in phylogenetics, phylogeography, DNA barcoding, and historical population dynamics analysis in groupers (Mainna et al., 2025). Therefore, combining nuclear and mitochondrial markers allows a more comprehensive characterization of genetic diversity patterns across different genetic levels.

5.2 Phenotypic and morphological analysis methods

Phenotypic and morphological analyses are traditional approaches in genetic diversity research, primarily based on measuring and comparing external morphological traits, growth characteristics, and color patterns to indirectly reflect genetic differences among populations. In grouper studies, commonly used indicators include body length, body height, body weight, head length, eye diameter, fin ray counts, scale counts, and body coloration patterns (Figure 3) (Hassanien and Al-Rashada, 2020; Mainna et al., 2025). These traits have a genetic basis but are also closely influenced by environmental conditions, nutritional status, developmental stage, and culture practices. Therefore, they remain valuable in germplasm description, preliminary species identification, and evaluation of production traits. Particularly in baseline resource surveys and germplasm inventories, morphological methods are indispensable as initial screening tools due to their simplicity, low cost, and intuitive results.

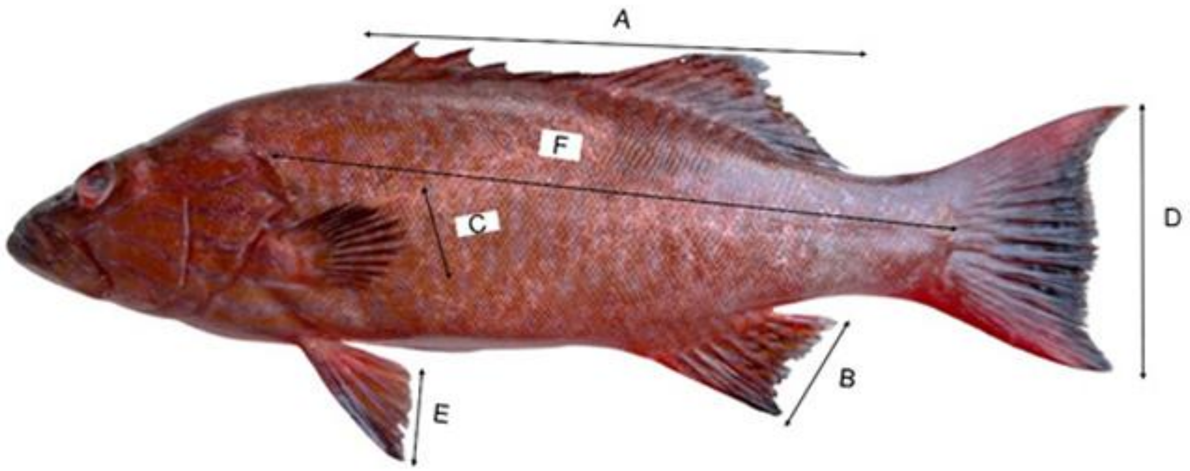

Figure 3 Meristic counts used in this study (Adopted from Mainna et al., 2025)

Image caption: Spines and rays: (A) Dorsal fin, (B) Anal fin, (C) Pectoral fin, (D) Caudal fin, (E) Pelvic fin; Scale counts: (F) Lateral line (Adopted from Mainna et al., 2025)

In practical applications, phenotypic and morphological analyses are usually combined with multivariate statistical methods. By standardizing multiple morphological traits, methods such as principal component analysis (PCA), discriminant analysis (DA), cluster analysis, and diversity indices can be applied to reveal phenotypic differences and classification relationships among populations (Sarif et al., 2020; Tian et al., 2024; Verma et al., 2024). These approaches not only provide preliminary grouping for molecular analyses but also help identify candidate germplasm with desirable traits such as superior growth, body shape, or coloration from a production perspective. Thus, phenotypic and morphological analyses have strong practical significance in grouper germplasm evaluation, particularly when aligned with breeding objectives and production needs.

However, morphological traits in groupers also have notable limitations. First, groupers exhibit strong morphological plasticity at different developmental stages, especially during the juvenile phase, when body coloration and patterns are highly variable and often similar among species, leading to potential misidentification and taxonomic confusion (Mainna et al., 2025). Second, environmental factors such as water temperature, salinity, feeding conditions, stocking density, and habitat can significantly influence morphological and growth traits, reducing the reliability of genetic inference based solely on phenotype (Hassanien and Al-Rashada, 2020). As a result, integrated “morphology-molecular” approaches have been increasingly developed, combining morphometric measurements, color phenotypes, or growth indicators with DNA barcoding, SSR, or SNP data. These combined approaches show higher reliability in species identification, germplasm evaluation, and cryptic species detection (Mainna et al., 2025). This trend indicates that although phenotypic and morphological analyses alone are insufficient for precise genetic evaluation, they remain important complementary tools in grouper germplasm research, supporting validation and interpretation of molecular results.

5.3 High-throughput sequencing and genomic technologies

With rapid advances in sequencing technologies and bioinformatics, high-throughput sequencing (next-generation sequencing, NGS) has become one of the most advanced and scalable approaches in grouper genetic diversity research. Compared with traditional molecular markers based on a limited number of loci, NGS enables the acquisition of large-scale genome-wide genetic data within a relatively short time, allowing high-resolution analysis of population genetic structure, selection signals, population history, effective population size, and genotype-phenotype relationships (Wu et al., 2024; Lu et al., 2025). This technological advancement has shifted grouper genetic diversity analysis from “marker-based” to “genome-wide” approaches, significantly improving the depth and accuracy of germplasm evaluation.

Whole-genome resequencing (WGR) is currently one of the most information-rich methods in population genomics. By sequencing multiple individuals and aligning them to a reference genome, millions of SNPs can be identified, enabling analyses of population differentiation, linkage disequilibrium, selective sweeps, runs of homozygosity, and candidate functional genes. For example, in a study of 326 leopard grouper individuals, WGR identified eight genetic groups, characterized growth-related selection regions, and established a haplotype reference database to support low-depth sequencing and genotype imputation, thereby reducing costs while maintaining high resolution (Wu et al., 2024). In addition to WGR, reduced-representation sequencing methods such as RAD-seq and GBS are widely used in grouper population genetics. These approaches can generate thousands to tens of thousands of SNPs without requiring a complete reference genome, making them suitable for non-model species and for analyzing population structure and adaptive variation (Sherman et al., 2020; Martchenko and Shafer, 2023).

High-throughput sequencing is also widely used for developing molecular marker resources and supporting breeding tools. For example, pyrosequencing has been used to develop numerous polymorphic SSR loci in giant grouper, facilitating parentage analysis, individual identification, and population genetic studies. ISSRseq has been applied in tomato grouper to generate genome-wide SNP data for analyzing genetic diversity, constructing kinship networks, and identifying high-growth populations (Hsu et al., 2023). In addition, transcriptome sequencing (RNA-seq), although primarily used for gene expression analysis, can complement population genomic data in elucidating the mechanisms of economically important traits, identifying candidate functional genes, and interpreting selection signals.

6 Current Status of Genetic Diversity Research in Groupers

6.1 Comparison of genetic diversity among different grouper species

At present, based on microsatellite markers, mitochondrial DNA, and other molecular marker technologies, researchers have systematically evaluated the genetic diversity of multiple grouper species (*Epinephelus* spp. and related taxa). Overall, most grouper species exhibit moderate to high levels of genetic variation; however, significant differences exist among species. These differences are closely related to their evolutionary history, distribution range, ecological niches, and population dynamics (Hassanien and Al-Rashada, 2020). Therefore, comparative studies of genetic diversity among different grouper species not only help to elucidate their evolutionary divergence but also provide important references for germplasm conservation and the selection of superior breeding stocks. Existing studies suggest that species with wider distribution ranges and more complex ecological environments generally possess higher genetic diversity.

For example, yellow grouper (*Epinephelus awoara*) exhibits high haplotype diversity (h = 0.968) and a rich number of microsatellite alleles (13-20 alleles per locus), indicating strong genetic variation potential (Yang et al., 2022). Giant grouper (*E. lanceolatus*) shows an average of approximately 5.9 alleles per locus and expected heterozygosity (He) ranging from 0.62 to 0.80, suggesting a relatively high level of nuclear genetic diversity suitable for fine-scale population analysis and molecular breeding studies. Although brown grouper (E. marginatus) maintains relatively high heterozygosity across multiple regions, its effective population size is relatively low, indicating potential risks to its genetic stability (Vaini et al., 2021).

In contrast, some grouper species with narrower ecological niches or more restricted distributions tend to exhibit lower levels of genetic diversity and are more prone to pronounced population differentiation. In certain island or localized populations, limited gene flow can lead to the accumulation of genetic drift and the formation of unique lineages (Hassanien and Al-Rashada, 2020; Yang et al., 2022). In addition, different marker systems may vary in their ability to detect genetic variation; for example, studies on Plectropomus species have reported discrepancies in diversity estimates across different technical platforms. With the increasing study of hybrid groupers, their offspring often show relatively high diversity in some indices; however, their long-term stability and genetic security still require further evaluation.

6.2 Genetic differences between wild and cultured populations

Wild grouper populations generally exhibit higher genetic diversity, as they have long been subjected to natural environmental conditions, including ocean currents, habitat heterogeneity, and natural selection (Yang et al., 2022). Gene flow among wild populations helps maintain genetic connectivity, and although geographic differentiation may occur at large spatial scales, they collectively form a relatively stable genetic resource pool. Therefore, wild populations are not only critical for conservation but also serve as key genetic sources for artificial breeding and broodstock renewal.

In contrast, cultured populations typically show lower genetic diversity, a conclusion consistently supported by multiple studies. Research on giant grouper in the South China Sea indicates that cultured populations have significantly lower allelic richness and heterozygosity compared to wild populations. At the same time, analyses such as FST, AMOVA, and STRUCTURE reveal significant genetic differentiation between the two groups. Similarly, in orange-spotted grouper, clear genetic differences exist between cultured populations and multiple wild populations, while variation among wild populations is relatively small (Wang et al., 2011), indicating that genetic changes occur more rapidly within aquaculture systems.

The decline in genetic diversity in cultured populations is mainly attributed to founder effects, genetic drift, and the accumulation of inbreeding. In aquaculture, limited broodstock numbers and repeated use reduce effective population size and lead to unequal family contributions (Hsu et al., 2023). In addition, artificial selection for economic traits can impose directional selection on specific genomic regions, thereby altering population structure. Although introducing new broodstock or hybrid breeding may partially increase genetic diversity, these practices may also blur the boundaries between wild and cultured populations and increase the risk of genetic contamination. Therefore, enhanced genetic monitoring and structural assessment are necessary.

6.3 Analysis of genetic bottlenecks and inbreeding effects

A genetic bottleneck refers to the loss of genetic diversity resulting from a sharp reduction in population size, while inbreeding effects arise under conditions of small population size or closed breeding systems. In grouper aquaculture, these issues are relatively common, particularly during artificial seed production and juvenile propagation (Hsu et al., 2023). Without effective management, genetic bottlenecks and inbreeding can reduce population adaptability and breeding potential, negatively impacting industry development.

Under aquaculture conditions, genetic bottlenecks often occur during the initial stages of artificial breeding. When broodstock numbers are limited or derived from a single source, offspring inherit only a restricted portion of genetic variation, leading to rapid allele loss. Studies have shown that offspring populations of kelp grouper and tomato grouper exhibit bottleneck signals, closely associated with the contributions of a small number of broodstock (Hsu et al., 2023). Inbreeding effects manifest as an increased probability of homozygosity for deleterious recessive alleles, often resulting in reduced growth, lower survival rates, and decreased disease resistance in production systems.

It is noteworthy that genetic bottlenecks and inbreeding are not limited to cultured populations but are also observed in some wild populations. For example, yellow grouper shows high haplotype diversity but low nucleotide diversity, indicating historical population contraction (Yang et al., 2022), while brown grouper exhibits low effective population sizes globally (Vaini et al., 2021). To mitigate these issues, it is necessary to expand broodstock sources, optimize mating designs, and establish pedigree management systems, combined with molecular marker-based kinship analysis and population renewal strategies. Furthermore, strengthening habitat protection and resource management for wild populations is essential for maintaining the long-term genetic stability of grouper germplasm resources.

7 Existing Issues and Future Development Trends

7.1 Current limitations in research

Although significant progress has been made in recent years in grouper genetic diversity research—particularly in molecular marker development, population structure analysis, and germplasm evaluation—there are still notable limitations overall. First, in terms of sampling and study design, most research focuses on a limited number of economically important species or specific key aquaculture regions. Sample sizes are often relatively small and geographic coverage is insufficient, making it difficult to comprehensively reveal large-scale spatial genetic patterns and their temporal dynamics in grouper germplasm resources (Hassanien and Al-Rashada, 2020; Tavakoli-Kolour et al., 2022; Yang et al., 2022). This is particularly problematic for grouper species with strong connectivity across marine regions and complex historical population dynamics, where limited sampling may fail to accurately identify true genetic boundaries and evolutionary units.

In terms of methodology and analytical frameworks, there is a lack of standardization across studies regarding marker types, number of loci, and evaluation indices, resulting in limited comparability among findings. Some studies rely on a single marker system, such as mtDNA or a small number of SSR loci, which can provide preliminary insights but are insufficient for integrating multiple layers of information, including neutral variation, adaptive variation, and selection signals. This limitation reduces the ability to resolve fine-scale population structure and local adaptive differentiation (Hassanien and Al-Rashada, 2020; Saha et al., 2021; Tavakoli-Kolour et al., 2022). In addition, inconsistencies in statistical models, parameter settings, and quality control procedures further affect the robustness and reproducibility of results.

Species misidentification and unclear sample origins remain significant issues. Groupers are highly diverse and morphologically similar, and samples from markets, fisheries, or hatcheries often involve mixed species or inconsistent naming. Without molecular-level species identification, genetic structure and diversity assessments may be based on incorrect taxonomic assumptions, leading to biased conclusions (Hassanien and Al-Rashada, 2020; Tavakoli-Kolour et al., 2022). Meanwhile, genetic monitoring of cultured populations lacks systematic and continuous implementation. Most studies are based on single-time sampling and static analyses, making it difficult to detect ongoing processes such as inbreeding accumulation, allele loss, and genetic drift in a timely manner (Sonesson et al., 2023; Ybanez and Gonzales, 2023). Overall, current research on grouper genetic diversity remains largely descriptive, with insufficient integration of dynamic processes, environmental drivers, and anthropogenic effects.

7.2 Challenges in germplasm conservation and utilization

Grouper germplasm conservation and utilization are currently facing dual pressures from the decline of wild resources and the rapid expansion of aquaculture. Declines in wild populations have become a major issue in many regions. In areas such as the South China Sea and the Persian Gulf, overfishing, habitat degradation, and marine environmental changes have led to reduced population sizes and habitat deterioration. Additionally, groupers generally have relatively slow growth rates, late sexual maturity, and, in some species, spawning aggregation behavior. These life-history traits increase their vulnerability to genetic diversity loss and resource depletion (Tavakoli-Kolour et al., 2022; Yang et al., 2022; Ai et al., 2025). The decline of wild populations not only weakens the stability of natural germplasm reservoirs but also limits access to valuable genetic resources and locally adapted genetic units.

Within aquaculture systems, germplasm management remains insufficiently standardized, and genetic risk management often lags behind breeding and industry expansion. Many cultured strains and hybrid combinations are developed primarily for growth performance, stress resistance, and economic returns, while systematic evaluations of their genetic background, long-term stability, and potential impacts on wild populations are still lacking (Yang et al., 2021; Yang et al., 2023; Wu et al., 2024). Particularly with the widespread use of hybrid groupers, the absence of germplasm purity identification, pedigree management, and risk warning systems may lead to disturbances in wild population genetic structures through introduction, stock enhancement, or escape. At the same time, stock enhancement programs lacking proper genetic planning may increase genetic homogenization due to limited source populations or mismatches with local wild populations.

From a management perspective, national-level systems, information platforms, and commercial frameworks for aquatic genetic resources (AqGR) remain relatively underdeveloped. Many regions lack clear regulations for the introduction of cultured species, the spread of exotic species, hybrid management, and genetic risk assessment, making it difficult to achieve a balance between utilization and conservation (Sonesson et al., 2023). Furthermore, ex situ conservation systems—such as germplasm banks, live conservation populations, and cryopreservation platforms—are still in early stages of development, facing challenges in scale, quality control, and integration into long-term conservation strategies (Li, 2022). Therefore, achieving efficient utilization while maintaining genetic diversity remains a key issue in both research and industry practice.

7.3 Prospects for emerging technologies in genetic diversity assessment

With the rapid advancement of biotechnology, sequencing technologies, and data science, new technologies offer unprecedented opportunities for grouper genetic diversity assessment and germplasm management. First, continuous improvements in whole-genome resequencing, chromosome-level genome assembly, and high-density SNP genotyping enable researchers to analyze genetic variation, selection signals, population structure, and quantitative trait loci (QTLs) at the genome-wide level, significantly enhancing the resolution and functional interpretation of genetic diversity assessments (Yang et al., 2021; Yang et al., 2023; Wu et al., 2024). Meanwhile, SNP genotyping platforms based on multiplex PCR capture or targeted enrichment provide cost-effective, high-throughput solutions for breeding and genetic monitoring (Shan et al., 2022; Wu et al., 2024).

Beyond individual-level genotyping, the development of environmental DNA (eDNA) and metabarcoding technologies offers efficient and non-invasive approaches for wild resource surveys and population monitoring. Compared with traditional capture-based methods, eDNA can identify target species and haplotype information from DNA traces in water samples, making it particularly suitable for continuous monitoring in restoration areas, protected zones, and critical habitats of wild populations (Li, 2022; Ai et al., 2025). In terms of germplasm conservation, the establishment of standardized germplasm banks and high-throughput cryopreservation systems also holds great promise. By developing live conservation populations, cryopreserved sperm banks, DNA repositories, and integrated database platforms, it is possible to systematically preserve allelic diversity and provide long-term support for resource restoration, broodstock renewal, and breeding innovation (Li, 2022).

In the future, grouper genetic resource management will increasingly rely on bioinformatics, big data analytics, artificial intelligence-assisted phenotyping, and genome-based decision support systems. By integrating genomic, transcriptomic, phenotypic, and environmental data, researchers can more comprehensively elucidate the genetic mechanisms underlying important economic traits, thereby supporting molecular-assisted breeding and genomic selection (Yang et al., 2021; Wu et al., 2024). At the same time, the development of national or regional aquatic genetic resource information systems that integrate genetic monitoring, germplasm conservation, breeding records, and risk warning mechanisms is expected to significantly enhance the standardization and intelligence of germplasm management (Sonesson et al., 2023; Wenne, 2023). Overall, future grouper genetic diversity assessment will move toward “genome-wide analysis, dynamic monitoring, data platform integration, and intelligent management.”

8 Conclusions and Recommendations

Studies on the genetic diversity of groupers indicate that most wild populations still maintain moderate to high levels of genetic variation, although significant differences exist among species and regions. For example, yellow grouper and brown grouper exhibit high haplotype or microsatellite diversity, while also showing clear population structure and, in some cases, historical declines in effective population size. These patterns are largely influenced by marine environmental barriers, hermaphroditic reproductive traits, and historical population fluctuations. Research on orange-spotted grouper and giant grouper consistently shows that cultured populations exhibit reduced allelic richness, lower heterozygosity, and significant genetic differentiation compared to wild populations, mainly due to founder effects, genetic drift, and artificial selection. Overall, in the aquaculture sector, the management of aquatic genetic resources (AqGR) has lagged behind production development. Many cultured populations lack systematic genetic evaluation, and genetic monitoring has not yet been routinely implemented. Meanwhile, rapid advances in molecular and genomic technologies—such as microsatellites, SNPs, reduced-representation sequencing, and whole-genome resequencing—have made it possible to assess genetic diversity, monitor inbreeding, and conduct molecular-assisted breeding even in non-model aquaculture species. Existing evidence suggests that cultured grouper germplasm is facing ongoing risks of genetic deterioration, but also has the potential for sustainable management through the application of advanced technologies.

The conservation of grouper germplasm resources should be based on systematic genetic evaluation, with genetic management incorporated into seed production systems. For major cultured species such as orange-spotted grouper and giant grouper, molecular markers (e.g., microsatellites or SNPs) should be regularly used at broodstock, seed, and grow-out stages to monitor allelic richness, heterozygosity, FST, and effective population size, with wild populations serving as references. Broodstock populations should be maintained at sufficiently large sizes with high genetic diversity and, where feasible, derived from multiple genetically compatible wild populations, while avoiding indiscriminate mixing of highly divergent management units to preserve local adaptation. In seed production, molecular marker-based parentage and kinship analyses should be applied to balance family contributions, reduce inbreeding, and prevent unintended domestication effects in stock enhancement programs. At the policy and management level, it is necessary to strengthen capacity for aquatic genetic resource management, promote cost-effective genotyping technologies, incorporate genetic risk assessment into species introduction and stock enhancement planning, and integrate these efforts with marine protected area development and fisheries management measures. Incorporating these strategies into national aquaculture development plans will help maintain genetic diversity in both wild and cultured populations and provide a solid foundation for future breeding improvement.

Future research on grouper genetic diversity should increasingly rely on high-throughput genomic technologies and integrate genetic variation with production traits and adaptive potential. Whole-genome resequencing and reduced-representation methods such as RAD-seq and ddRAD can provide high-density SNP data for fine-scale population structure analysis, detection of selection signals, and genomic selection of traits such as growth, disease resistance, and environmental adaptability—approaches that have already proven successful in species such as salmonids. At the same time, low-cost, species-specific genotyping tools (e.g., targeted SNP panels) suitable for small and medium-sized aquaculture enterprises should be developed, and the effects of different detection strategies (e.g., sequencing depth and marker density) on genetic diversity and kinship assessment should be systematically evaluated. At the ecological level, integrating population genomics with marine environmental factors and tagging technologies can further optimize the delineation of management units, particularly for heavily exploited species such as Nassau grouper and brown grouper. In addition, incorporating genetic data into stock enhancement evaluation, studies of interactions between cultured and wild populations, and emerging biotechnologies (e.g., genomic selection, surrogate broodstock technology, and gene editing) will help develop breeding and conservation strategies that balance production efficiency with genetic security. Ultimately, such integrated approaches will ensure the long-term health and sustainable utilization of grouper germplasm resources.

Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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