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