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.

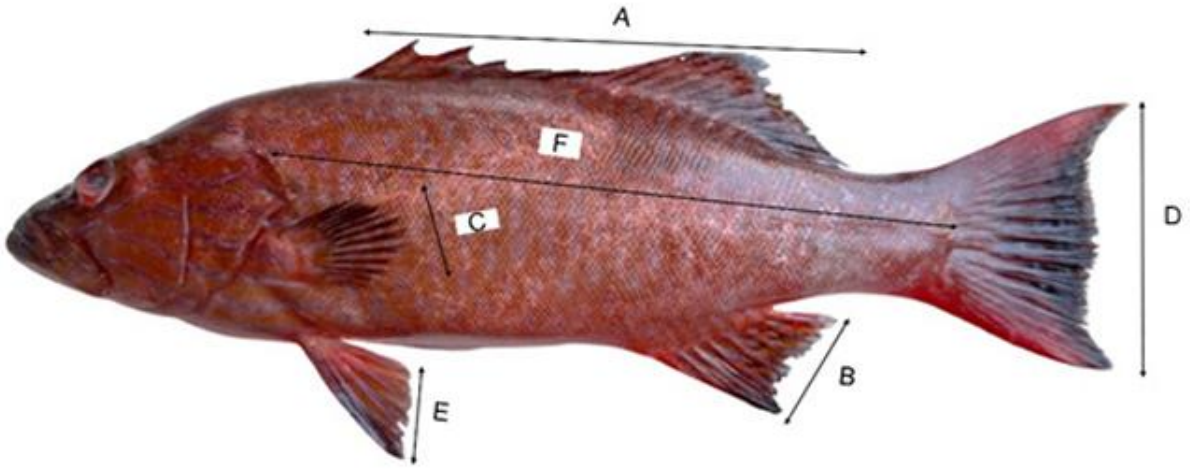

Figure 3 Meristic counts used in this study (Adopted from Mainna et al., 2025)

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)