

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.

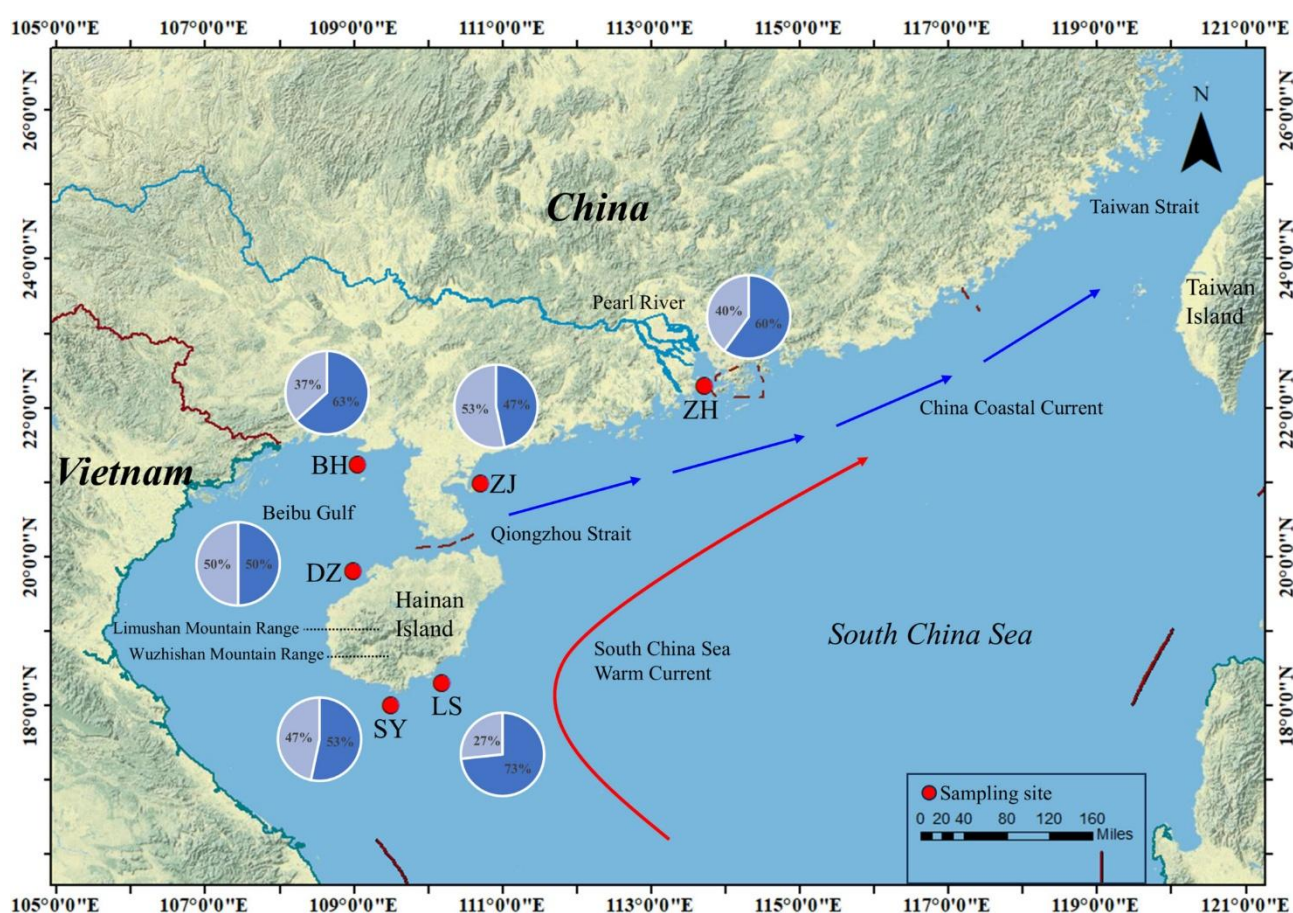


Figure 2 Map of the coast of Hainan Island and mainland China along the South China Sea illustrating the sampling locations of *Epinephelus coioides* (Adopted from Chen et al., 2025)

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.