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Molecular Systematics and Phylogenetic Relationships of Wild Fish in Coastal Waters of Hainan Island

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Abstract The offshore waters of Hainan Island are a hot spot for tropical marine biodiversity in China. Wild fish resources are abundant but there is insufficient classification awareness. Traditional fish classification mainly relies on morphological characteristics and appears to be limited in the face of species diversity and hidden species problems. This study reviews the development history and main methods of molecular systems, sorts out the progress of fish molecular classification research at home and abroad, and its application cases in the tropical waters of the South China Sea. Focus on summarizing the current status of fish classification in Hainan offshore, comparing the applicability and polymorphic differences of commonly used molecular markers (such as COI, 16S, Cytb), and discussing the practice of building phylogenetic trees based on NJ, ML, and BI methods and evaluating reliability. Based on recent research results, the kinship pattern and population genetic structural characteristics of fish in Hainan near-shore areas are analyzed, and the discovery and significance of potential hidden species are revealed. From the perspective of geological evolution, the impact of environmental changes in Hainan Island and surrounding sea areas on fish lineage differentiation is discussed, and the ecological significance of the research results in marine biodiversity conservation and fishery resource management is expounded, and the research direction is expected to be carried out in the future. This study provides scientific reference for further in-depth research on molecular systems of fish in Hainan offshore fish and improving species classification system and protection strategies.

Keywords Offshore of Hainan Island; Fish classification; Phylogenetic; Kinship; Geographical evolution

1 Introduction

Hainan Island is located in the tropical shelf waters of the northern South China Sea and is an important hot spot for marine fish biodiversity. There are many species of tropical and subtropical fish in this area, however, there is still a big gap between traditional classification research based on morphological characteristics and actual fish diversity. The complex ecological environment offshore Hainan (such as the Qiongzhou Strait in the north, the deep water and land slope in the south, the semi-closed Beibu Gulf in the west, etc.) has nurtured a variety of fish communities (Chen et al., 2025). For a long time, Chinese fish classification has mainly relied on morphological diagnostic characteristics. However, since fish have a high degree of diversity in body shape, color, pattern, scales, fin strips, etc., the morphology varies significantly in different development stages, and simple morphology identification has limitations on accuracy and efficiency. Especially in tropical waters where species classification is complex, many close species have subtle morphological differences, and even hidden species (species with extremely similar morphology but genetically independent). These factors make it difficult to fully identify species and reveal true kinship by traditional methods alone (Wang et al., 2012).

With the development of molecular biology technology, DNA sequencing has become a powerful tool for fish classification and phylogenetic research. Molecular phylogenetics provides an objective basis for species identification and lineage reconstruction by analyzing the sequence differences of specific gene fragments. A large number of studies have shown that molecular means such as DNA barcode can efficiently discover unidentified species and accelerate the identification of sister species or hidden species (Xu et al., 2021). Therefore, introducing molecular systems into the classification of fish offshore fish in Hainan is of great significance to improving the regional species list and clarifying the evolutionary relationship of fish lineages.

This study first introduces the development history and core technical methods of molecular systems, summarizes the main progress of fish molecular classification research at home and abroad, and the application of these methods in tropical waters including the South China Sea; explains the current classification research status of fish offshore in Hainan, analyzes the limitations of traditional morphological classification and the results of existing molecular classification work, and introduces the target fish targeted by this research and its preliminary classification situation; compares the characteristics and polymorphic differences of several commonly used molecular markers, and explains the scientific basis for selecting molecular markers in this study. On this basis, the common methods of phylogenetic tree construction (NJ, ML, BI) and evaluation indicators of phylogenetic tree reliability (Bootstrap value, posterior probability) are introduced, and the correction suggestions for the current classification are proposed based on the research results. This study aims to systematically summarize and analyze the research progress on the system classification and evolutionary relationship between fish molecular system in Hainan Island in the past five years, explore the rules and shortcomings in existing research results, and provide reference for subsequent in-depth research.

2 Summary of Molecular Systems Research

2.1 Development history and core methods of molecular systems

Molecular systems originated in the mid-20th century and are a discipline that applies molecular biology technology to biological classification and evolutionary research. Early molecular classification studies used protein electrophoresis and isoenzyme analysis to compare differences between species. DNA sequencing technology emerged in the 1970s, first making breakthroughs in the development of viruses and microbial phylogenetics, and then quickly promoted to the field of animals and plants. Entering the 21st century, the development of high-throughput sequencing and genomics has promoted molecular phylogenetics into the era of "metagenome" and "transcriptionome", which can analyze hundreds or even thousands of genes at the same time, thereby building a more refined and reliable phylogenetic tree (Oliveira et al., 2018). The core methods of molecular phylogenetics include DNA/RNA extraction and purification, PCR amplification and sequencing of specific gene fragments, bioinformatics comparison, and phylogenetic tree construction. Commonly used phylogenetic reconstruction algorithms include distance-based adjacency method (NJ), maximum likelihood method (ML) based on optimal model, and Bayesian method (BI) based on Bayesian inference.

2.2 Research progress on fish molecular classification at home and abroad

Internationally, a lot of work has been carried out in the study of molecular classification and phylogenetic fish. DNA barcoding technology has been rapidly applied to global fish diversity surveys since Hebert proposed the standard mitochondrial *COI* gene as a species recognition tool. Fish DNA barcode databases in various major sea areas have been successively established. In addition to mitochondrial sequences, fish genome data have also begun to be used in phylogenetic analysis in recent years, allowing the traditional morphological classification framework to obtain a strong test of molecular evidence. In China, fish molecular classification research started relatively late but progressed rapidly. Chinese scholars have conducted DNA molecular identification and phylogenetic research on many fish in the Yangtze River, Pearl River and other river basins and near the sea. Through DNA classification methods, Chinese scientific researchers discovered some undistinguished population differences under traditional classification, such as the relative species with genetic differentiation between the Taiwan Strait and the South China Sea (Shen et al., 2016; Wang et al., 2022).

2.3 Application cases of related research in the South China Sea and tropical regions

Molecular systems have broad application prospects in fish research in the South China Sea and other tropical waters. As the center of biodiversity in the Western Pacific, the South China Sea has unique and complex fish species, which has attracted many scholars to conduct molecular analysis. For example, DNA barcode technology is used to investigate the fish diversity in the northern and southern seas of Hainan Island: Xu et al. (2023) collected fish samples from the northern and southern seas of Hainan Island, and identified 56 species of fish through COI barcodes, belonging to 17 orders and 34 families. The study found that the composition of fish populations in the northern and southern Hainan Island was significantly different, and only 5 species were shared by the north and south, indicating that there is significant spatial heterogeneity in the fish community structure in local waters (Xu et al., 2023). In terms of freshwater fish in tropical islands, Lin et al. (2022) used mitochondrial *Cytb*, *ND2* and *COI* multiple genes to analyze the genetic structure of *Opsariichthys hainanensis*, and found that the population of vegetation fish in Hainan Island and the Pearl River system on the mainland was significantly differentiated. Regardless of seawater fish or island freshwater fish, molecular phylogenetics has been successfully applied to analyzing population pattern and genealogical relationships. This provides valuable experience and reference for subsequent research on fish diversity in Hainan offshore.

3 Classification Status of Wild Fish Offshore in Hainan

3.1 Limitations of traditional morphological classification

There are many species of fish offshore in Hainan, and traditionally, they are mainly classified and identified based on morphological characteristics. However, in practice, morphological classification faces many difficulties and limitations. On the one hand, different fish may have morphological phenomena such as "also-species" and "also-species" in terms of morphology, especially in tropical waters such as coral reefs. The body colors and markings of fish species are extremely diverse, and it is often difficult to distinguish close species in a timely and accurate manner based on external forms alone (Gravier-Bonnet et al., 2016). On the other hand, the larval and adult morphology of fish may vary greatly, and many fish lack reliable morphological characteristics in the early life history stages, resulting in traditional methods being unable to identify fish eggs and juvenile fish (Grealy et al., 2016). In addition, performing fine fish morphology classification requires long-term accumulation of experience from taxonomic experts, which has a high threshold for non-professional personnel. In an area off the coast of Hainan, where species are rich and relatively weak in research, the above problems are more prominent. In the past, the list of species records based solely on morphological records may have been omitted or misunderstood.

3.2 Review of existing molecular classification research

In view of the shortcomings of morphological identification, molecular classification research on fish in Hainan and adjacent waters has been gradually carried out and preliminary results have been achieved in recent years. Some studies have used DNA barcode technology to conduct species identification and classification status verification on fish in the sea area around Hainan Island. Du et al. (2021) used DNA taxonomy methods to evaluate the fish diversity throughout the South China Sea. In combination with COI sequence alignment, they found that some fish traditionally classified as widely distributed species actually had significant intraspecies genetic differentiation, suggesting that multiple undescribed new species may be implicit. There are also some molecular classification explorations in specific groups: for the South China Sea economic fish Grouper family and some species of the order Performin, researchers analyzed their phylogenetic relationships through Cytb and other sequences, and the results showed new insights into the family affiliation of certain species (Zeng et al., 2022).

4 Commonly Used Molecular Markers and Their Application in Fish Systemology

4.1 Introduction to commonly used molecular markers (such as COI, 16S, Cytb)

In molecular systems research, different gene markers are suitable for analysis at different classification levels due to their different evolutionary rates and functional characteristics. In fish studies, mitochondrial DNA has become the most widely used source of molecular markers due to its high mutation rate, maternal haploid inheritance and no recombination. The most commonly used markers include: *COI* gene, mitochondrial cytochrome oxidase *I* gene, with a total length of about 650 bp, and is internationally recognized as the standard DNA barcode sequence. *COI* has the advantages of high discrimination at the species level and strong versatility of amplification primers, and is widely used in species identification and classification research. For fish, *COI* fragments can usually clearly distinguish most of the relative species and are the preferred sequence for establishing species libraries. *16S rRNA* gene, mitochondrial small subunit ribosomal *RNA* gene, sequence conserved regions and variable regions interphase distribution. The 16S sequence evolution rate is slightly slower than *COI*, but it performs well in the classification above the department level and the analysis of older branches. In addition, 16S is often used in environmental DNA macrobarcode research because its fragments are short and facilitate high-throughput amplification. The *Cytb* gene, the mitochondrial cytochrome *b* gene, is one of the fastest-evolving coding genes in the mitochondrial genome.

Cytb sequence variation is abundant and is often used for phylogenetic analysis of population genetic structures and related species (Figure 1) (Jena et al., 2023).

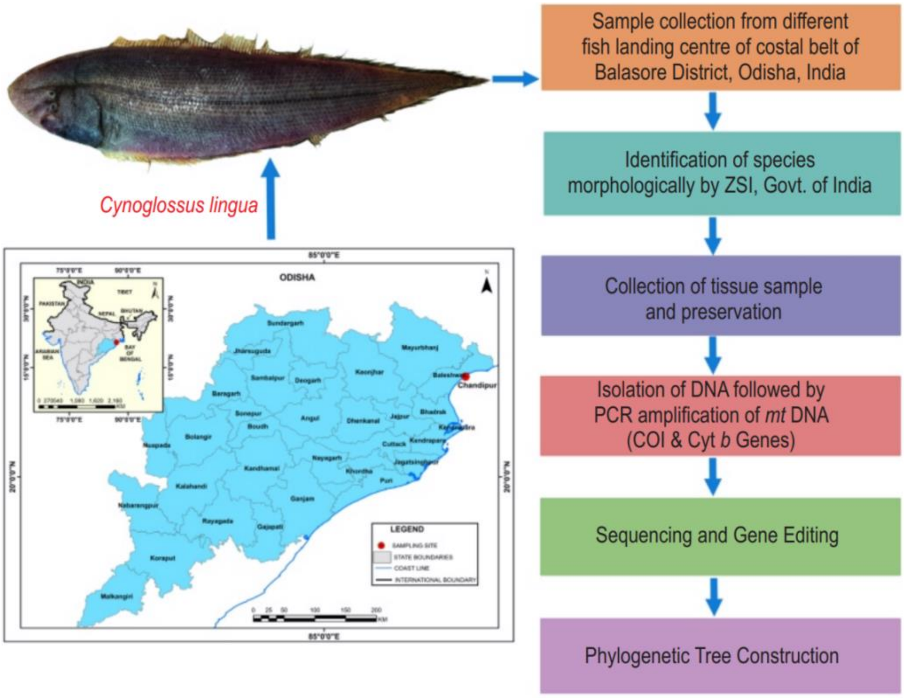

Figure 1 Workflow of phylogenetic analysis of *Cynoglossus lingua* based on *COI* and *Cytb* genes (Adopted from Jena et al., 2023).

4.2 Comparison of label applicability and polymorphism

Choosing the right molecular marker is critical to obtaining reliable classification and phylogenetic results. Different genes have different recognition abilities and polymorphisms at different classification levels due to their different degree of evolutionary conservatism. When targeting species identification, labels are required to differ significantly between species and small intraspecies. A large number of studies and practices have shown that *COI* genes meet this requirement in most fish species, and their interspecies regret distances are usually much higher than those intraspecies, making them an ideal species identification marker. Comparison of data from freshwater fish in Hainan Island shows that the interspecies genetic distance threshold of COI is about 0.69%, and the discrimination accuracy can reach 94.96%, which is significantly better than the *12S* and *16S* genes. The *16S rRNA* gene has a slightly slow evolution rate and relatively low interspecies, which may not be sufficiently distinguished in some close species, but as a supplementary marker, it can improve the robustness of the identification. The degree of variation of the *Cytb* gene is comparable to or higher than that of COI, and is especially suitable for analyzing intraspecies lineages and population structures. However, high variation also means that sequence alignment and model selection need to be more cautious, otherwise deep relationship judgments may be affected by synonymous replacement saturation (Silva-Santos et al., 2018). Therefore, when reconstructing phylogenetic relationships, it is often preferable to use multiple markers in combination: including slower evolution 16S, etc. to stabilize higher-order relationships, and also including COI, Cytb, etc. to capture low-order differentiation. Nuclear genes such as RAG1 are slowly evolving, and are suitable for exploring long-term evolution of family-level genes, but they lack the ability to distinguish close relatives, and because diploid genes may have allelic sequences, the analysis is relatively complicated.

4.3 Scientific basis for selected molecular markers

Based on the above comparison, we selected mitochondrial *COI* and *Cytb* genes as main molecular markers in this study, and verified some species with the *16S* gene. The *COI* gene has mature databases and extensive application experience in fish species identification globally. Previous environmental DNA studies on Hainan fish have proved that COI sequences have the highest coverage in public databases and the accuracy of species annotation is significantly better than the 12S and 16S sequences (Ahmad et al., 2024). The *Cytb* gene has been widely proven to be effective in fish system geography and population studies. Its higher nucleotide polymorphism is conducive to distinguishing recent differentiation events and is very suitable for exploring the genetic structure and lineage differentiation of populations near Hainan Island. Again, we consider that a single mitochondrial gene may have maternal bias and other limitations, so we also introduced another mitochondrial marker 16S for cross-validation. On the one hand, 16S has rich reference data in some taxa, and on the other hand, its conservatism helps to test the robustness of the relationship constructed by COI and Cytb.

5 Progress in Research on the Kinship and Genetic Structure of Fish in Hainan Offshore

5.1 Phylogenetic relationship of fish in Hainan based on molecular data

Molecular systems research provides a new perspective for understanding the kinship of fish offshore in Hainan. Overall, Hainan offshore fish belong to part of the Western Pacific tropical-subtropical system, and its phylogenetic relationship reflects the historical evolution of regional biogeography. Based on the preliminary molecular phylogenetic tree, it can be seen that many fish in Hainan waters are closely related to populations in the South China Sea and other regions of the Indo-Western Pacific, showing the characteristics of the pan-Indian-Pacific system (Luo et al., 2025). For example, coral reef fish such as *Dambesaidae* and *Floycephala*, mixed branches with corresponding groups in the South China Sea and Southeast Asia adjacent waters on the systemic trees, indicating that these species have experienced frequent regional diffusion and genetic exchange in geological history, and Hainan is not an isolated evolutionary unit (Xu et al., 2023). However, molecular phylogenetic trees also reveal some previously unnoticed details of kinship. Some Hainan offshore fish were previously thought to be closer to southern China coastal populations based on geographical sources, but genetic data suggest that they are more closely related to populations in the further south (such as Southeast Asia or the South Pacific). This suggests that we should have a more open understanding of the genealogical relationships of Hainan fish, and we cannot infer the systematic relationship based on geographical proximity. For some small fish that are locally distributed near Hainan, molecular evidence suggests that they may represent unique evolutionary branches, alienated from other known species, and have a unique evolutionary status. Such discoveries are very important for understanding the uniqueness of Hainan's marine biodiversity.

5.2 Population structure, gene flow and genetic diversity characteristics

In addition to analyzing systematic relationships among species, molecular data can also be used to study population genetic structures and gene flow dynamics within Hainan offshore fish species. Research in recent years has shown that there is significant genetic differentiation among different geographical populations of some offshore fish in Hainan. Mitochondrial DNA analysis of freshwater fish Hainan phlegm showed that the FST differentiation index between groups in Hainan Island and Pearl River water system population was as high as 0.511, and showed obvious isolation and distance-related patterns. This means that although Hainan saphenous morphology is similar, groups on and off the island have long been isolated, with extremely limited genetic communication, and have formed an independent lineage (Lin et al., 2022). Similarly, among marine fish, some nearshore settled fish (such as local reef species) may form their respective genetic groups in different sea areas of Hainan Island (Beibu Gulf, Xisha Sea, etc.). On the other hand, for marine fish with strong migration ability or larvae in the plankton period, their population genetic structure is often more diffuse. Some distant migratory fish species do not show significant genetic differentiation between Hainan and surrounding waters, and molecular mutations mainly exist between individuals rather than between regions. This suggests that these species may conduct extensive gene flows through ocean currents, etc., thereby maintaining the genetic continuity and homogeneity of the population. In this group of fish, the level of genetic diversity is usually high, often with abundant haplotype types and larger effective population sizes. A study on a variety of freshwater fishes in South China pointed out that freshwater fishes in the southern continent and Hainan Island generally have high haplotype diversity, which is believed to be related to the warm climate and diverse environment in the region (Lin et al., 2023).

5.3 Discovery and significance of potential hidden species in Hainan fish

Using molecular systems approaches, an important gain is the discovery of hidden species that are not identified in traditional classifications. The existence of hidden species is of great significance for evaluating biodiversity and developing conservation strategies. In the offshore fish study in Hainan, we have shown signs of potential hidden species. Some morphology is identified as individuals with the same species, but their mitochondrial sequence differences are far beyond the general intraspecies level and are located in different branches on the phylogeny tree (Wang et al., 2020). This suggests that these individuals may actually represent two or even multiple independent lineages. Similar findings have been common in fish DNA barcode studies in other regions, and they often contribute to the publication of new species or revisions of existing classifications. Specifically, we suspect that some small reef-dwelling fish have hidden species in the nearshore coral reef ecosystem of Hainan Island. For example, several species from the Dambesidae and the Serbaceae family, their different geographical groups or different marking types may correspond to significant genetic differentiation. For example, the COI sequence of a detected fetus fish cannot be found in the public database for its COI sequence, and the known species closest to its morphology have a significant genetic distance (Shan et al., 2021). The discovery of hidden species not only increases the length of the species list, but more importantly reveals the fine process of biological evolution. In a biogeographically special location like Hainan, hidden species may arise from historical geographical isolation (such as population isolation caused by sea level changes) or ecological differentiation (potential reproductive isolation caused by adaptation to different habitats).

6 The Influence of Geographical Evolution on the Formation of Fish Lineages

6.1 Geological history and evolutionary background of Hainan Island and surrounding waters

The geological evolution of Hainan Island has a complex and changeable history, which has had a profound impact on the formation of regional biozones. From the perspective of earth structure, Hainan Island was connected to the South China continent at the end of the Paleozoic period, and then gradually became a continental island with the cracks in the South China Sea. Qiongzhou Strait is a narrow strait between Hainan Island and Leizhou Peninsula. It is about 70 kilometers long, 30 kilometers at the narrowest point, an average water depth of only about 40 meters, and a deepest point of about 120 meters. Such a shallow strait means that Hainan Island and the mainland may have been connected to form land bridges multiple times when sea levels fall during the Quaternary Ice Age (Lin et al., 2023).

The South China Sea basin itself was formed during the Oligocene-Midocene and then experienced multiple changes in climate and sea conditions. The repeated ice-interglacial cycles in the Pleistocene caused drastic sea level fluctuations, which in addition to land bridges, also led to significant changes in the area of the South China Sea and the marine environment. During the ice age, the area of the South China Sea was reduced and some shallow seas became land or brackish water environments, which may provide shelter for some marine species. Some studies have pointed out that there may be a late Pleistocene marine glacial shelter near the southwest of Hainan Island, where some warm water species survive and spread outward after the end of the glacial period (Yan et al., 2021). At the same time, Hainan Island's own topography has also shaped its inland water system pattern: the central Wuzhishan-Pagu Ridge Mountain separates the island's river water system into several major directions. The independent evolution of these river systems further influenced the lineage differentiation of freshwater fish on the island.

6.2 Geographical isolation and lineage differentiation patterns revealed by molecular systems

Through molecular systems research, we can correspond to the lineage differentiation of Hainan fish with geographical evolution events and reveal the laws. A typical pattern is the land bridge isolation effect: during the formation of land bridges during the glacial period, marine environmental segmentation leads to the isolation of species populations, thereby accumulating genetic differences. For example, the fish, which is mainly distributed in rivers, isolates the Hainan river from the Pearl River system because the land bridge separates the Hainan river from the Pearl River system, and the genealogy inside and outside the island evolves independently, forming a significantly differentiated genetic branch. This isolation model is reflected in multiple freshwater species, indicating that the repeated generation of land bridges is an important driving force for the differentiation of the lineage between islands and continents. However, not all species strictly follow the land bridge isolation model. Molecular evidence reveals that the lineage differentiation of some species earlier than the last land bridge. For example, the major branches of the big-eyed big-eyed big-hide fish differentiated on Hainan Island are estimated to be about 1.05 million to 160,000 years ago, which is earlier than the land bridge in the last ice age (Chen and Jang-Liaw, 2023). Therefore, its geographical genealogy pattern does not match the simple island-land binary caused by the isolation of the Qiongzhou Strait. In addition, for some marine fish, the Qiongzhou Strait is not an absolute biological obstacle. Inshore fish with strong swimming power may also migrate through offshore waters during the existence of the straits, so there is no obvious differentiation of island land (Yan et al., 2021).

6.3 Coupled analysis of marine environment changes and species formation mechanisms

Species formation is often the result of the combined action of geographical isolation and environmental selection. In the history of differentiation of offshore fish in Hainan, the profound impact of changes in the marine environment is naturally indispensable. Molecular systems science provides us with clues to explore the coupling relationship between these effects and species formation mechanisms. Sea level lift driven by climate warm and cold cycles not only affects isolation patterns through land bridges, but also changes the scope of coastal habitats (Sun et al., 2013). When the climate warms up and sea levels rise, these differentiated lineages may re-contact, and ecological interactions such as competition, hybridization or substitution occur.

Secondly, changes in the current pattern may affect the direction and intensity of gene flow. During the ice and interglacial periods, the circulation patterns and intensity of the South China Sea differed significantly, with different levels of Kuroshio invasion and the status of water exchange with the Beibu Gulf (Hu et al., 2018). This will affect the diffusion path of young slapped fish. The differences in environmental selection pressures in different regions can also promote lineage differentiation, i.e. ecological species formation. Offshore Hainan, the coastal ecosystems are diverse (mangroves, seagrass beds, coral reefs, sand mud base, etc.), and fish experience different selection pressures in different habitats, and ecologically adaptable genetic differentiation may occur over time. Some reef fish adapt to coral habitats with high structural complexity, while nearshore sandy benthic fish evolved into different adaptation characteristics (Figure 2) (Wang et al., 2022). If sea level changes or other factors expand the intertwined distribution areas of the two habitats, the two groups may each embark on different evolutionary paths and eventually accumulate to a point where it is difficult to hybridize.

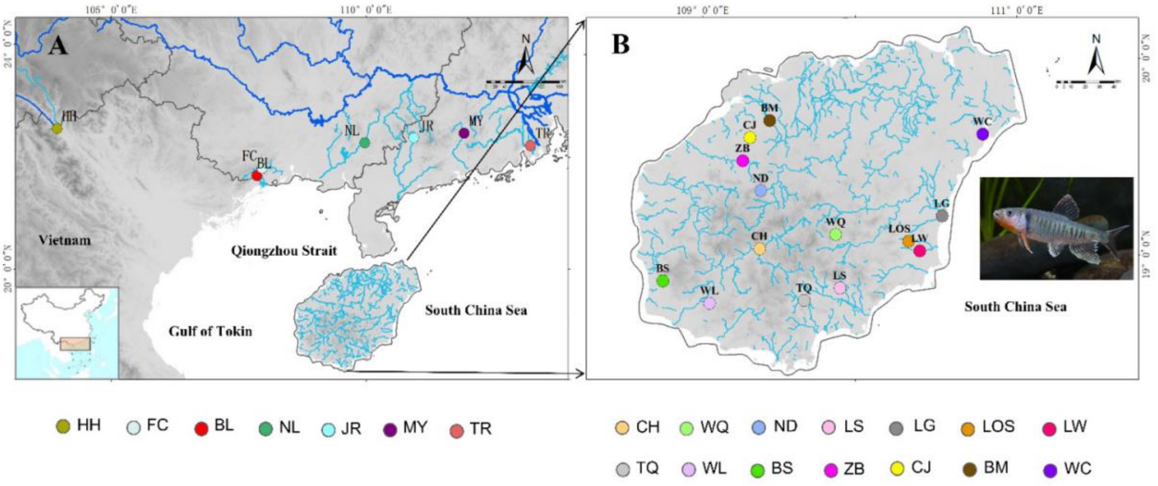

Figure 2 Maps of the study region in mainland China (A) and Hainan Island (B) indicate sites where 21 sampling localities of *O. hainanensis* were collected (circles) (Adopted from Wang et al., 2022)

7 The Ecological and Protection Significance of Research Results

The molecular systems research results collected by this research institute have greatly enriched our understanding of the diversity of fish offshore in Hainan Island. Given the hidden species and misidentified heteronomial species that traditional methods may miss, molecular evidence helps confirm species identity and reveal new species clues. We found that some fish that were previously recorded as a single species actually contained multiple lineages with significant genetic divergence, suggesting that species diversity was underestimated. Once these lineages are confirmed as new species by further study, the actual number of species in offshore fish in Hainan will increase. Even for taxa that have not been found, molecular classification has established an "identity card" for each fish species to ensure the accuracy and reliability of the species list. Secondly, the clarification of phylogenetic relations allows us to have a deeper understanding of the evolutionary background of Hainan's fish diversity. Now, through the phylogenetic tree, we have clarified which species are close to each other and which groups are derived from common ancestors. This revelation of lineage diversity is an important aspect of diversity perception-focusing not only on species counts, but also on evolutionary differences and uniqueness among species. Third, molecular analysis also provides information on fish diversity function and genetic diversity. For example, species with high genetic diversity tend to be more ecologically adaptable, while species with unique lineages are of great value to maintaining evolutionary potential.

Sustainable use of fishery resources and marine ecological conservation need to be based on scientific species and population boundaries. The new classification and kinship information provided by the Institute of Molecular Systems has direct guiding significance for fishery management and species protection. By clarifying the true identity and distribution of species, we can more accurately assess the status of fishery resources and develop management measures. With the results of molecular identification, management departments can formulate differentiated quotas and protection strategies for different lineages. Second, understanding the population structure and gene flow characteristics of fish can help demarcate the snap-in. Molecular markers reveal that certain populations of Hainan offshore fish are geographically independent of each other (the FST is obvious), so these populations should be regarded as independent management units, avoiding simply distributing resources or seedlings from one area to another. For example, if the populations in the closed bay and offshore populations around Hainan Island are significantly differentiated, they should not be combined and evaluated, otherwise the local recession trend may be concealed. Furthermore, the study of phylogenetic relationships can point to evolutionary unique species that need special protection. Some species that are isolated on the phylogenetic tree (such as endemic species of a family that is only one genus) represent an irreplaceable evolutionary heritage and deserve priority protection. This consideration has been adopted in the international EDGE species protection index, and we can also identify and pay attention to the "evolutionary treasure" species in the Hainan sea area.

The research progress summarized in this study not only provides current understanding, but also reveals many issues that need to be explored in depth, and points out the direction of future research. In terms of data depth and breadth, genomic level research will be a future trend. Most of the current analysis is still based on mitochondria and a few nuclear genes, but the whole genome and high-throughput SNP data will provide richer information and higher resolution. Using ddRAD, whole genome sequencing and other means, more refined genetic structures and adaptive variants can be analyzed at the population level. As sequencing costs decrease, these technologies should be introduced into the study of important species offshore Hainan to make up for the shortcomings of traditional markers. Secondly, the research on interdisciplinary integration is worth strengthening. The conclusions of molecular systems need to be comprehensively explained in combination with morphological, biogeography and ecological evidence. The identification of some hidden species depends on morphological reexamination, and the driving forces of some lineage differentiation require ecological experiments and modeling verification. Future research should strengthen cooperation between taxonomic experts and molecular biologists, carry out integrated taxonomic research, combine morphological, DNA and ecological data, and make authoritative classification revisions to controversial groups. In terms of macro-structure, it is recommended to conduct regional comparative research. Comparative analysis of the molecular phylogenetic results of fish offshore in Hainan with adjacent areas (such as Beibu Gulf, Taiwan Strait, Nansha Islands, etc.) can test which models are universal and which are unique to Hainan. This helps deepen our understanding of the mechanisms of biogeographic pattern formation in the South China Sea.

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Conflict of Interest Disclosure

The authors affirm 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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