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EDITED BY
Cui Liang,
Chinese Academy of Sciences (CAS), China

REVIEWED BY
Khaled Mohammed Geba,
Menoufia University, Egypt
Annam Pavan Kumar,
Central Institute of Fisheries Education (ICAR), India

*CORRESPONDENCE
Xuehui Wang
wxhscs@163.com

†These authors have contributed equally to this work

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DNA barcoding of marine fish species in the waters surrounding Hainan Island, northern South China Sea

Jinxia Zhang[1,2†], Lei Xu[2,3†], Feiyan Du[2,3], Quehui Tang[2,3], Lianggen Wang[2,3], Jiajia Ning[2,3], Delian Huang[2,3], Yafang Li[2,3], Shuangshuang Liu[2,3] and Xuehui Wang[2,4,5*]

[1]College of Marine Science, Shanghai Ocean University, Shanghai, China, [2]South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, China, [3]Guangdong Provincial Key Laboratory of Fishery Ecology and Environment, Guangzhou, China, [4]Key Laboratory of Marine Ranching, Ministry of Agriculture and Rural Affairs, Guangzhou, China, [5]Sanya Tropical Fisheries Research Institute, Sanya, China

Introduction: Hainan Island is encompassed within the shallow waters of the tropical continental shelf in China, which is recognized as a significant hotspot for fish biodiversity. Despite extensive research conducted on marine fish taxonomy surrounding Hainan Island, there remains a substantial gap between our current understanding and the actual fish diversity within this oceanic area.

Methods: In this study, we employed DNA barcoding and molecular identification approaches to explore the species diversity and distribution pattern of marine fish in both the northern and southern sea areas of Hainan Island in the northern South China Sea.

Results: A total of 186 sequences were obtained from the collected marine fish samples in the two sea areas surrounding Hainan Island. Through DNA identification, it was confirmed that all 186 sequences corresponded to typical fish species found in the northern South China Sea, all sequences represented a total of 56 species, 47 genera, 34 families, and 17 orders. The average Kimura 2-parameter (K2P) distances within species, genus, family, order and class were 0.15%, 6.53%, 13.17%, 16.95% and 24.81%, respectively.

Discussion: Our investigation in the northern sea areas of Hainan Islands identified a total of 33 distinct species, while the southern sea areas exhibited 29 distinct species, with only 5 species found to be shared between both regions. These findings clearly indicate a significant disparity in the species composition of fish communities between the northern and southern sea regions.

KEYWORDS

DNA barcode, COI, ABGD, GMYC, fish diversity

Introduction

The ocean is believed to contain an astonishing 21,800 distinct species of fish, exhibiting an extraordinary assortment of dimensions, hues, and configurations. Astonishingly, approximately between one-third and two-thirds of marine species remain unidentified through traditional methods of identification, even new marine species are being described at a rate of about 100-150 per year (Mora et al., 2008; Eschmeyer et al., 2010; Appeltans et al., 2012). Traditional methods of fish identification, classification, and naming predominantly rely on observable morphological diagnostic traits and measurable biological characteristics. Although modern taxonomic work employs other features such as geography, anatomy, physiology, isoenzymes, and behavior, morphological diagnosis remains the cornerstone of taxonomic treatment. In the face of the amazing diversity of fish in terms of body size, color, pattern, scale size, number and type of fin rays, traditional morphological classification and identification present some limitations. The above-mentioned diversity is also present in different stages of fish development. Morphological diagnostic characters used to discern taxa cannot be suitable for all developmental stages (Victor et al., 2009; Ward et al., 2009; Huang et al., 2022). In addition, morphological traits of marine fish can sometimes exhibit either significant intraspecific differences or minor interspecific differences Identification of marine fish requires not only proficiency in specialized disciplines like taxonomy and morphological identification, but also in marine ecology, developmental biology, biogeography, anatomy, and fisheries resources. Proficiency in these domains is imperative in order to precisely recognize and categorize marine piscine taxa. Relying solely on morphological diagnostic characters for identification poses challenges such as inaccuracy, difficulty, and time-consumption, particularly for non-specialists involved in marine ecosystem research (Lima-Filho et al., 2016; Struck et al., 2018).

DNA sequencing-based approaches offer a dependable, swift, and cost-effective diagnostic system that utilizes specific gene sequences known as “DNA barcodes” for organism identification. The fundamental basis of DNA barcoding rests upon the comparison of sequence similarity between two distinct organisms, serving to establish the degree of relatedness between them. In their seminal publication, Hebert et al. (2003) presented the notion of leveraging a standardized mitochondrial cytochrome c oxidase subunit I (COI) gene sequence, spanning approximately 658 base pairs in length, as a potent instrument for the identification of organisms across the entire animal kingdom. The basis for this approach stemmed from the observed sequence diversity among various taxa. The COI gene possesses several characteristics that render it reliable and efficient tool for species identification across a wide range of animal groups. Notably, it exhibits a predominantly haploid pattern of inheritance, primarily passed down from the mother. Furthermore, it is abundantly present in multiple copies within the mitochondria, displays a high substitution rate, and lacks introns and recombination, enhancing its utility as a marker for species identification. (Ballard and Whitlock, 2004; Bernt et al., 2013). In the following years, Hebert’s research group successfully established a global platform encompassing standardized datasets, universal technical protocols, and taxonomic identification systems for animals. This comprehensive framework served as a foundation to facilitate consistent and harmonized approaches in DNA barcoding research worldwide (Ratnasingham and Hebert, 2007; Ratnasingham and Hebert, 2013). Over the course of the last two decades, subsequent to the preliminary proposition of DNA barcoding for the purpose of identifying specimens, a plethora of investigations have undeniably exhibited the cost-efficient nature of this approach in identifying species.

The implementation of DNA barcoding has garnered significant recognition among various taxonomic groups, including but not limited to crustaceans (Costa et al., 2007; Matzen da Silva et al., 2011; Xu et al., 2018), insects (Hebert et al., 2004b; Janzen et al., 2005; Hajibabaei et al., 2006; Shashank et al., 2022), fish (Hubert et al., 2008; Ward et al., 2009; Francisco et al., 2022), birds (Hebert et al., 2004a; Kerr et al., 2009; Dimitriou et al., 2017) and mammals (Clare et al., 2007; Borisenko et al., 2008; Silva et al., 2021). The majority of DNA barcoding data generated from these studies were diligently documented in public databases, accompanied by voucher specimens featuring comprehensive collection records, specimen identifiers, and specimen images. This invaluable repository of information serves as a fundamental resource for subsequent studies, providing crucial foundational data for further investigations (Ratnasingham and Hebert, 2013). DNA barcoding offers a significant advantage as it proves to be effective across various organisms at different developmental stages, ranging from eggs to adults. This remarkable feature enables the identification of organisms during their larval stage, even in the absence of distinct morphological characteristics. By comparing the sequences of unidentified larval specimens with those of previously documented adults stored in the reference sequence library, it becomes possible to accurately determine their identities (Barber and Boyce, 2006; Caterino and Tishechkin, 2006; Kusbiyanto et al., 2020; Huang et al., 2022; Xu et al., 2022). These endeavors have made substantial contributions to the detection of previously unknown species and accelerated the identification of sibling species or cryptic species. Notably, DNA barcoding research has witnessed a concentrated focus on studying the diversity of marine fish. Various regions have been subject to intensive investigations in this field, including the Arctic Ocean (Mecklenburg et al., 2011), the South Pacific (Ward et al., 2005; Zemlak et al., 2009), the Atlantic (Lima-Filho et al., 2016; Oliveira et al., 2016), the Mediterranean (Bariche et al., 2015; Francisco et al., 2022), the East Pacific Ocean (Steinke et al., 2009; Mabragaña et al., 2011), the Indian Ocean (Lakra et al., 2011; Ahmed et al., 2021), and the West Pacific (Zhang and Hanner, 2011).

The fish species composition and faunal attributes in each marine area reflect the historical evolutionary outcomes that have been gradually shaped over extensive periods within distinct marine environments. These outcomes provide insights into the long-term processes and dynamics that have influenced the diversity and distribution of fish species in these areas (Briggs and Bowen, 2012). The northern South China Sea plays a vital role as both spawning and feeding habitats for commercially valuable fish populations, making it a significant marine fishing zone. The

spatial distribution of fish species is intricately influenced by various environmental factors, including salinity, temperature, and water depth. The northern South China Sea, due to the complex marine ecological environment created by the convergence of the South China Sea Warm Current and the Kuroshio South China Sea branches, harbors not only common tropical and subtropical fish species but also some oceanic species and coral reef fish (Shen and Heino, 2014; Gao et al., 2017). Based on a comprehensive review of literature and data amalgamation, the researchers inferred that the northern shelf region of the South China Sea is home to a diverse assemblage of 2025 fish species (Institute of Zoo and Chinese Academy of Sciences, 1962; Zhang and Hanner, 2012; Liu, 2013).

In this work, our aim was to examine the fish diversity and distribution in the two significant fisheries regions located in the northern South China Sea surrounding the Hainan Islands. To achieve this, we collected specimens from northern and southern areas on Hainan Island. We utilized DNA barcoding and DNA identification as practical and additional approaches to complement the traditional morphological method for identifying marine fish species in the northern South China Sea surrounding the Hainan Islands. Our study on species diversity in local fish faunas will have significant implications for the conservation and fish resource studies in the northern South China Sea.

Materials and methods

Study area

Hainan Island, an expansive landmass, is located within the northwestern expanse of the northern South China Sea. It is encompassed within the shallow waters of the tropical continental shelf in China. To the north of Hainan Island lies the Qiongzhou Strait, a narrow strait spanning a mere 20 kilometers. This strait acts as a separation between the island and mainland China. To the west of Hainan Island is situated the Beibu Gulf, which is a naturally occurring semi-enclosed gulf, enveloping a water area of roughly 128,000 square kilometers. The mean water depth in this gulf is approximately 39 meters, while certain regions exhibit a maximum depth of 100 meters. The southeastern sea region of Hainan Island showcases a slender continental shelf, which is succeeded by a sharp continental slope (Hu et al., 2003; Ma et al., 2010; Zhu, 2016). Hainan Island, being the second largest island within the Chinese territory, commands a significant expanse of sea region within its administrative purview, spanning an estimated 2 million square kilometers, constituting 42.3% of the nation's overall sea area. The coastline of Hainan Island extends for a total length of 1618 kilometers. Along this coastal expanse, a multifarious array of biotopes can be discerned, encompassing rock formations, coral formations, gravelly substrates, sandy substrates, and muddy substrates, contributing to the island's prodigious ecological heterogeneity (Liu, 2013; Zhang et al., 2020).

Sample collection

The piscine specimens were procured by means of bottom trawler nets by the commercial fishing vessel "Beiyu60011" during the time span of April to May 2017. The areas of collection were located respectively in the northern (111°15′E to 111°45′E, 19°45′N to 20°15′N) and southern (109°30′E to 110°00′E, 17°30′N to 18°00′N) sea area of Hainan Island (Figure 1). The identification and naming of species were conducted by conforming to the diagnostic morphological characteristics for marine fish as explicated in FishBase (http://www.fishbase.org) and FAO Fish Identification Sheets. A quantity of several specimens of each species identified through morphology were meticulously chosen, and their dorsal fins were meticulously excised and conserved in absolute ethanol at a temperature of -20°C. The aforementioned specimens shall be utilized in the subsequent investigation of DNA barcoding.

DNA identification and genetic divergence

Genomic DNA extraction, amplification and sequencing of the COI gene, we followed the methodologies outlined in our previously

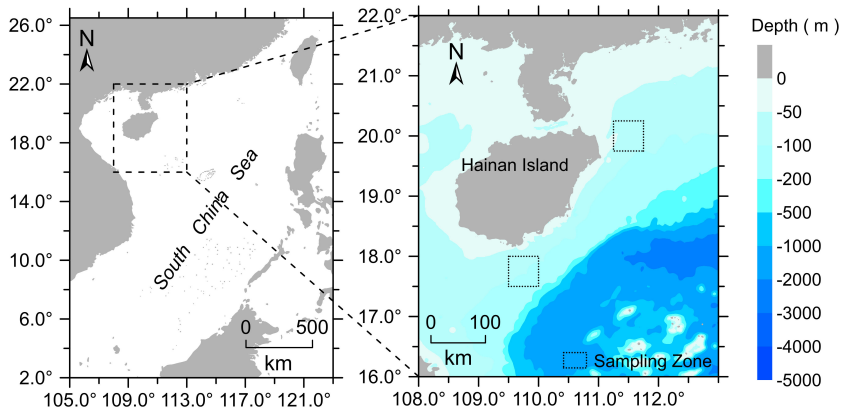

FIGURE 1
Map showing the two sampling areas in the sea area near Hainan Island.

published work (Xu et al., 2019; Xu et al., 2021). Genomic DNA was extracted from dorsal fin tissue using the TIANamp Marine Animals DNA Kit (TIANGEN, China) according to the manufacturer's protocol. The concentration of the DNA used as a template for PCR was adjusted to an A260 value of approximately 0.05 to 0.2. For PCR, we utilized the primers FishF1 and FishR1, which were specifically designed for fish analysis (Ward et al., 2005). Each 50μl PCR reaction consisted of the following components: 31.25μl dd H2O, 5μl PCR buffer, 5μl Coralload concentrate, 4μl of 25 μM $MgCl_2$, 1μl of 10 μM dNTPs, 0.5μl of 25 μM solution of each primer, 2.5μl DNA template, and 0.25μl of TopTaq DNA polymerase (QIAGEN, Germany). The PCR amplification conditions were as follows: an initial denaturation step at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 0.5 minutes, annealing at 54°C for 0.5 minutes, and extension at 72°C for 1 minute. The amplification process was concluded with a final extension step at 72°C for 7 minutes using a 2720 Thermal Cycler (Applied Biosystems, USA). The PCR products were visualized on 1% agarose gels, and the most intense bands were selected for sequencing. Bi-directional sequencing was performed on an ABI 3730XL automated sequencer, using both forward and reverse primers, in accordance with the manufacturer's instructions. We conducted a thorough BLAST search in GenBank, utilizing the BLASTn algorithm with the mega blast option, for the purpose of verifying the accuracy of our COI sequences. We compared the obtained sequences to find the highest match, ensuring a similarity of 98% to 100%. The sequences were aligned and analyzed using BioEdit (Hall, 1999). A sequence alignment of multiple sequences was executed through the utilization of the CLUSTALW algorithm while taking into account the nonexistence of insertions or deletions, also known as indels. Nevertheless, the initial 20 base pairs and the terminal 10 base pairs were excluded from the analysis on account of their usually show high level of non-informative nucleotides or sequence background. In order to compare our nomenclature, which was derived from the literature, with the names obtained from GenBank for each species, we included the COI sequences sourced from public databases into our analysis (Table S1). We employed two distinct tools, the Automatic Barcode Gap Discovery (ABGD) (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) and the Generalized Mixed Yule Coalescent (GMYC) model (The R Programming Language), to evaluate taxonomic units through DNA identification. These tools allowed us to estimate potential species boundaries using the COI dataset. The ABGD approach is designed to identify gaps in the DNA barcode data, which can be indicative of distinct species. It divides the sequences into putative species based on the magnitude of genetic divergence, utilizing a sliding window approach. By comparing intra- and inter-specific genetic distances, ABGD helps determine species boundaries (Puillandre et al., 2012). On the other hand, the GMYC model employs a different approach by utilizing the branching patterns of the genealogy. It assumes that gene trees within a single species follow a coalescent process, while between different species they diverge. The model estimates the transition point where the gene tree shifts from a coalescent to a divergent process, thus inferring species boundaries (Pons et al., 2006; Fujisawa and Barraclough, 2013). By utilizing both ABGD and the GMYC model, we aimed to enhance the accuracy and reliability of our taxonomic assessments. These tools provided complementary perspectives on species boundaries, allowing us to make more informed inferences based on the COI dataset. For detailed examination techniques and parameter configurations, we kindly refer the reader to our antecedently issued publications (Xu et al., 2019; Xu et al., 2022). The divergence of COI sequences was calculated using the Kimura 2-parameter (K2P) distance model in MEGA version 6 (Kumar et al., 2008; Tamura et al., 2013). We executed a phylogenetic analysis utilizing Bayesian Inference, incorporating all accessible sequences to demonstrate the interrelationships between pelagic and deep-sea species, as well as their distribution patterns surrounding Hainan Island. The full details of the analysis methods and parameters were described in our previously published papers (Xu et al., 2019; Xu et al., 2021). We also assessed the β-diversity of fish communities between northern and southern sea areas using Jaccard's dissimilarity index. (Koleff et al., 2003):

$$\beta_{jac} = \frac{b+c}{a+b+c} \tag{1}$$

where β_{jac} is Jaccard's dissimilarity index, a represents the number of species present in both sites, b indicates the number of species present in the first site but not in the second, and c represents the number of species present in the second site but not in the first. Further, we partition the β-diversity into turnover and nestedness components following equations (2) and (3), respectively.

$$\beta_{jtu} = \frac{2\min(b,c)}{a+2\min(b,c)} \tag{2}$$

$$\beta_{jne} = \frac{\max(b,c)-\min(b,c)}{a+b+c} \times \frac{a}{a+2\min(b,c)} \tag{3}$$

where β_{jtu} is the turnover component of Jaccard dissimilarity, β_{jne} is the nestedness-resultant component of Jaccard dissimilarity. a, b and c are the same as in equation (1). The taxonomic β-diversity and its components were calculated through the 'beta.pair' function of the R package 'betapart' (Baselga and Orme, 2012; Baselga et al., 2021).

Results

DNA identification

We performed an analysis on a dataset consisting of 242 sequences of the COI gene. Out of these sequences, 186 were obtained from 73 specimens collected in the southern sea areas of Hainan Island [available in Xu et al. (2021)], 113 specimens from the northern sea areas of Hainan Island (GenBank accession numbers: OR144930-OR145042). To ensure the accuracy of our nomenclature, we also incorporated 56 COI sequences from public databases, specifically selecting those sequences that showed the highest level of similarity to known species identities (see Table S1). The fragment's total length after sequence alignment is 602 bp. The

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average base composition for the COI sequence was as follows: A = 23.8%, C = 29.1%, G = 18. 4%, T = 28.8%, the transition/transversion (ti/tv) ratio was 1.19. Based on morphological diagnostic characteristics, a total of 56 morphological species were identified. Out of the total, 4 species only could be identified to the genus level. Additionally, 2 species were misidentified as cryptic species or sibling species based solely on morphological diagnostic characteristics (Table 1). The DNA identification process has confirmed that all 186 sequences analyzed correspond to typical fish species found in the South China Sea, encompass a total of 56 distinct species, belonging to 47 genera, 34 families, and 17 orders. DNA identification using the Automatic Barcode Gap Discovery (ABGD) method successfully detected a clear and distinct barcode gap in the COI alignment. The analysis indicated that out of the 186 sequences, they potentially represented 56 taxonomic units. The estimated number of putative clusters ranged from 50 to 58. The COI phylogeny analysis using the Generalized Mixed Yule Coalescent (GMYC) approach supported the hypothesis that the

TABLE 1 Summary of identification based on morphological taxonomy and species barcode using the BOLD-IDS and BLASTN search from GenBank.

Order	Family	Genus	Species (Identification based on barcode)	Similarity %	Morphological taxonomy	Sampling area
Acropomatiformes	Acropomatidae	Acropoma	*Acropoma japonicum*	99.84	*Acropoma japonicum*	N
Carangiformes	Carangidae	Alepes	*Alepes kleinii*	99.21	*Alepes kleinii*	N
Kurtiformes	Apogonidae	Apogon	*Apogon carinatus*	100	*Apogon carinatus*	S
Kurtiformes	Apogonidae	Apogon	*Apogon semilineatus*	99.8	*Apogon semilineatus*	N
Scombriformes	Ariommatidae	Ariomma	*Ariomma indica*	99.2	*Ariomma indica*	N
Pleuronectiformes	Bothidae	Arnoglossus	*Arnoglossus tapeinosoma*	99.78	*Arnoglossus tapeinosoma*	S
Perciformes	Scorpaenidae	Brachypterois	*Brachypterois serrulata*	99.8	*Brachypterois serrulata*	N
Perciformes	Malacanthidae	Branchiostegus	*Branchiostegus argentatus*	100	*Branchiostegus argentatus*	S
Carangiformes	Carangidae	Carangoides	*Carangoides equula*	100	*Carangoides equula*	S
Lophiiformes	Chaunacidae	Chaunax	*Chaunax abei*	99.77	*Chaunax fimbriatus*	S
Perciformes	Serranidae	Chelidoperca	*Chelidoperca margaritifera*	99.91	*Chelidoperca margaritifera*	S
Aulopiformes	Chlorophthalmidae	Chlorophthalmus	*Chlorophthalmus acutifrons*	100	*Chlorophthalmus acutifrons*	S
Perciformes	Percophidae	Chrionema	*Chrionema chlorotaenia*	99.84	*Chrionema chlorotaenia*	S
Scombriformes	Nomeidae	Cubiceps	*Cubiceps whiteleggii*	99.8	*Cubiceps whiteleggii*	S
Pleuronectiformes	Cynoglossidae	Cynoglossus	*Cynoglossus kopsii*	99.9	*Cynoglossus sinicus*	S
Pleuronectiformes	Cynoglossidae	Cynoglossus	*Cynoglossus macrolepidotus*	98.78	*Cynoglossus macrolepidotus*	N
Pleuronectiformes	Cynoglossidae	Cynoglossus	*Cynoglossus nigropinnatus*	100	*Cynoglossus* sp.	S
Myliobatiformes	Dasyatidae	Dasyatis	*Dasyatis zugei*	100	*Dasyatis zugei*	N
Carangiformes	Carangidae	Decapterus	*Decapterus maruadsi*	99.78	*Decapterus maruadsi*	N
Perciformes	Sparidae	Dentex	*Dentex tumifrons*	99.84	*Dentex tumifrons*	S
Acanthuriformes	Leiognathidae	Deveximentum	*Deveximentum megalolepis*	99.91	*Deveximentum megalolepis*	N
Anguilliformes	Synaphobranchidae	Dysomma	*Dysomma anguillare*	100	*Dysomma anguillare*	N
Perciformes	Serranidae	Epinephelus	*Epinephelus latifasciatus*	99.92	*Epinephelus latifasciatus*	S
Acanthuriformes	Leiognathidae	Equulites	*Equulites lineolatus*	99.28	*Equulites lineolatus*	N
Acanthuriformes	Leiognathidae	Equulites	*Equulites rivulatus*	99.76	*Equulites rivulatus*	S
Aulopiformes	Synodontidae	Harpadon	*Harpadon nehereus*	99	*Harpadon nehereus*	N

(Continued)

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TABLE 1 Continued

Order	Family	Genus	Species (Identification based on barcode)	Similarity %	Morphological taxonomy	Sampling area
Perciformes	Hoplichthyidae	Hoplichthys	*Hoplichthys langsdorfii*	100	*Hoplichthys langsdorfii*	S
Tetraodontiformes	Tetraodontidae	Lagocephalus	*Lagocephalus gloveri*	100	*Lagocephalus gloveri*	S
Tetraodontiformes	Tetraodontidae	Lagocephalus	*Lagocephalus spadiceus*	99.78	*Lagocephalus spadiceus*	N
Acanthuriformes	Leiognathidae	Leiognathus	*Leiognathus ruconius*	99.84	*Leiognathus* sp.	N
Scombriformes	Trichiuridae	Lepturacanthus	*Lepturacanthus savala*	98.91	*Lepturacanthus savala*	N
Perciformes	Synanceiidae	Minous	*Minous quincarinatus*	99	*Minous quincarinatus*	N
Perciformes	Nemipteridae	Nemipterus	*Nemipterus bathybius*	100	*Nemipterus bathybius*	S/N
Perciformes	Nemipteridae	Nemipterus	*Nemipterus japonicus*	99.77	*Nemipterus japonicus*	N
Perciformes	Nemipteridae	Nemipterus	*Nemipterus virgatus*	99.91	*Nemipterus virgatus*	N
Siluriformes	Ariidae	Netuma	*Netuma thalassina*	98.97	*Netuma thalassina*	N
Holocentriformes	Holocentridae	Ostichthys	*Ostichthys japonicus*	99.84	*Ostichthys* sp.	S
Zeiformes	Parazenidae	Parazen	*Parazen pacificus*	99.8	*Parazen pacificus*	S
Perciformes	Sciaenidae	Pennahia	*Pennahia pawak*	99.9	*Pennahia pawak*	N
Perciformes	Peristediidae	Peristedion	*Peristedion liorhynchus*	100	*Peristedion liorhynchus*	S
Acanthuriformes	Leiognathidae	Photopectoralis	*Photopectoralis bindus*	99.77	*Photopectoralis bindus*	N
Perciformes	Priacanthidae	Priacanthus	*Priacanthus macracanthus*	99.91	*Priacanthus macracanthus*	S/N
Perciformes	Priacanthidae	Priacanthus	*Priacanthus tayenus*	98.97	*Priacanthus tayenus*	N
Perciformes	Scorpaenidae	Pterois	*Pterois russelii*	99.84	*Pterois russelii*	S
Scombriformes	Scombridae	Rastrelliger	*Rastrelliger kanagurta*	99.84	*Rastrelliger kanagurta*	N
Anguilliformes	Congridae	Rhynchoconger	*Rhynchoconger ectenurus*	95.18	*Rhynchoconger ectenurus*	S
Aulopiformes	Synodontidae	Saurida	*Saurida undosquamis*	99.8	*Saurida undosquamis*	S/N
Acropomatiformes	Acropomatidae	Synagrops	*Synagrops japonicus*	98.91	*Synagrops japonicus*	S
Aulopiformes	Synodontidae	Synodus	*Synodus macrops*	99	*Synodus* sp.	N
Scombriformes	Trichiuridae	Tentoriceps	*Tentoriceps cristatus*	100	*Tentoriceps cristatus*	S
Tetraodontiformes	Monacanthidae	Thamnaconus	*Thamnaconus hypargyreus*	99.77	*Thamnaconus hypargyreus*	S/N
Aulopiformes	Synodontidae	Trachinocephalus	*Trachinocephalus myops*	99.91	*Trachinocephalus myops*	S
Carangiformes	Carangidae	Trachurus	*Trachurus japonicus*	98.97	*Trachurus japonicus*	S/N
Scombriformes	Trichiuridae	Trichiurus	*Trichiurus brevis*	98.91	*Trichiurus brevis*	N
Scombriformes	Trichiuridae	Trichiurus	*Trichiurus japonicus*	99	*Trichiurus japonicus*	N
Mulliformes	Mullidae	Upeneus	*Upeneus japonicus*	99.8	*Upeneus japonicus*	N

N: Northern sea area; S: Southern sea area.

examined individuals belonged to 56 distinct taxonomic units. The confidence interval for the estimated number of taxonomic units ranged from 55 to 59. The probability of the null hypothesis, which posited the existence of only a single species (likelihood = 1967.19, likelihood ratio test = 371.35, p =1.13×10^{-9}), was significantly diminished in comparison to the probability of the model that permitted for multiple species (likelihood = 2152.86) (Figure 2).

Genetic divergence and distribution pattern

When analyzing the Kimura 2-parameter (K2P) distances in our sequence dataset, we observed a significant discrepancy of 1.23% to 2.7% between intraspecific and interspecific distances (Figure 3). This divergence provides compelling evidence for the differentiation and distinguishability of species within our dataset.

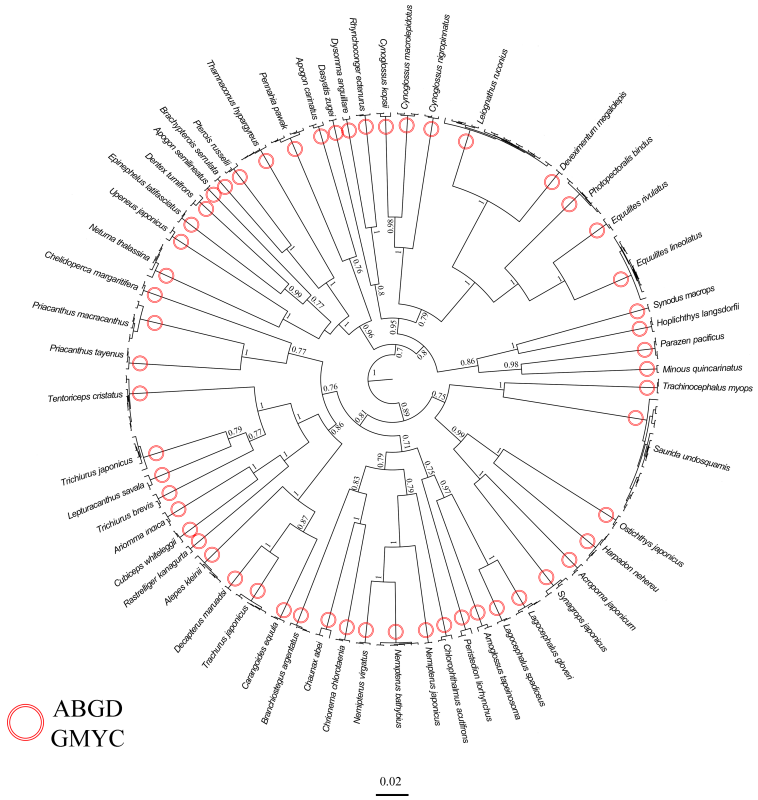

FIGURE 2
Results of DNA-based classification from ABGD and GMYC on COI data set. The phylogenies are the ultrametric trees obtained from BEAST, with the scale bars proportional to substitution rates; support values are Bayesian Posterior; support values below 0.7 and for short branches are not shown. DNA identification from ABGD and GMYC are shown on the branches.

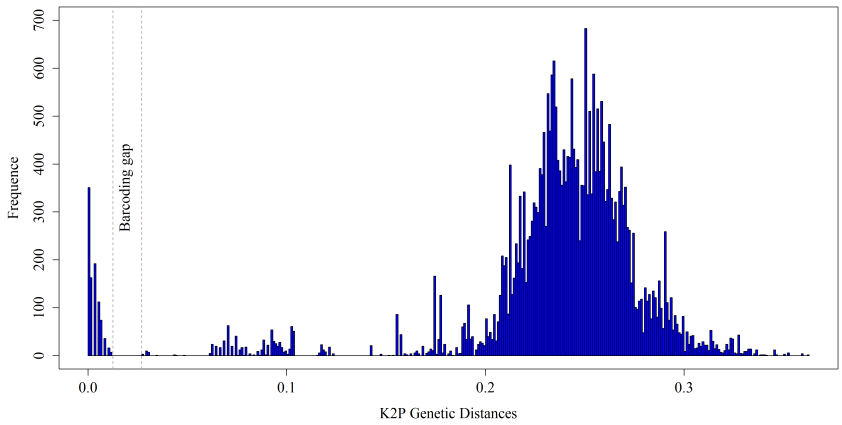

FIGURE 3
The frequency distributions of intraspecific and interspecific pairwise genetic K2P distance based on all samples. The area between dashed line display the 'barcoding gap' between the intra- and interspecific distance.

In our study, the uncorrected Kimura 2-parameter (K2P) pairwise distances within species were consistently below 1%, with an average of 0.15% and a range between 0 and 0.78%. The average divergence among congeners was recorded at 6.53%. These divergences varied across species, with *Equulites* exhibiting the lowest divergence of 1.65%, while *Priacanthus* displayed the highest divergence of 10.27%. The average confamilial divergence was 13.17%, ranging from a minimum of 10.16% in Scorpaenidae to a maximum of 15.53% in Serranidae. The average divergence within orders was calculated to be 16.95%, with Acanthuriformes having the lowest divergence of 12.12% and Perciformes exhibiting the highest divergence of 22.57% (Table 2). In the northern sea areas of

TABLE 2 Summary of pairwise mitochondrial cytochrome c oxidase I (COI) barcode nucleotide divergences within various taxonomic levels, using K2P distances (%).

Comparisons within	Minimum	Mean distance	Maximum	s.e.
Species	0	0.15	0.78	± 0.09
Genera	1.65	6.53	10.27	± 0.77
Families	10.16	13.17	15.53	± 1.21
Orders	12.12	16.95	22.57	± 1.33
Classes	21.03	24.81	29.41	± 2.03

Hainan Islands, a total of 33 distinct species were found. In contrast, the southern sea areas revealed 29 distinct species, while only 5 species were found to inhabit both regions (Figure S1). The Jaccard dissimilarity index was 0.9, which measured the β diversity of fish community between northern and southern sea areas. Taxonomic turnover component showed a value of 0.85, while taxonomic nestedness component presented a value of 0.05 (Figure 4). We observed a significant disparity between the taxonomic turnover and nestedness components, with the turnover component contributing significantly to taxonomic β-diversity.

Discussion

the Indo-West Pacific Ocean context places paramount importance on the South China Sea (SCS) as a hub of marine biodiversity on a worldwide scale. The SCS is renowned for its remarkable and distinctive marine ecosystems that encompass a diverse range of species, a significant proportion of which are exclusive to this region (Barber et al., 2000). The copious amounts of biodiversity present within the South China Sea are ascribed to several factors, such as its geographical positioning, propitious environmental circumstances, and intricate oceanographic and ecological mechanisms. The SCS functions as a pivotal abode for a myriad of marine creatures, encompassing piscine, invertebrate, and marine mammal species, furnishing them with sustenance sources, propagation locales, and asylum (Institute of Zoo and Chinese Academy of Sciences, 1962; Morton and Blackmore, 2001; Ma et al., 2008). In recent times, there has been a notable surge in research efforts aimed at assessing fish diversity in the South China Sea using COI barcodes. Wang et al. (2012b) carried out a meticulous scientific investigation, wherein they meticulously scrutinized 222 DNA barcode sequences, with each of them denoting 95 distinct fish species. Their research focused on two significant fisheries regions within the South China Sea, namely the Nansha Islands and the Beibu Gulf. Similarly, Zhang and Hanner (2012) conducted a study with the aim of identifying fish species in the South China Sea through the use of DNA barcoding. They were able to analyze a more extensive dataset, which consisted of 1336 DNA barcode sequences. This allowed them to identify as

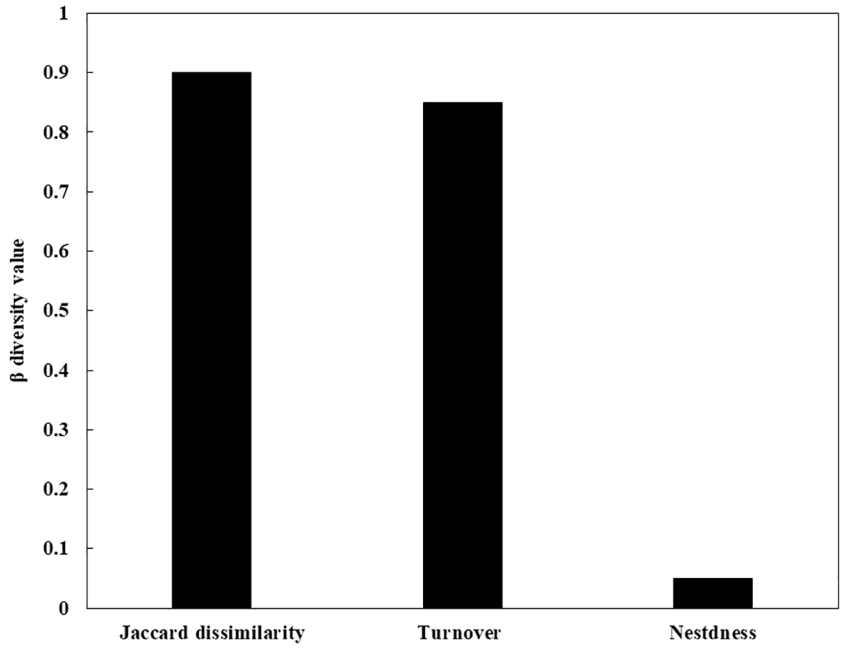

FIGURE 4
The Jaccard dissimilarity index of the two fish communities between southern and northern sea areas and its turnover and nestedness component.

many as 242 different fish species in the region. The aforementioned investigations have illuminated the efficacy of COI barcodes as a sturdy instrument for appraising piscine diversity in the expanse of the South China Sea. By scrutinizing genetic data conserved within the COI gene, scholars have successfully differentiated among multiple species of fish with precision. This category of inquiry may manifest as incomparable in endowing discernment into the manifoldness and ecological fluctuations of the maritime milieu in the South China Sea.

In the present study, a total of 56 morphospecies were identified from the southern and northern sea areas surrounding Hainan Island in northern South China Sea. Additionally, we achieved successful amplification of the COI barcoding gene for all 56 marine fish species included in our study. However, due to taxonomic ambiguity within certain genera and species, four taxa could only be identified at the genus level while two others were misidentified as sibling species. The outcomes of two distinct methodologies employed to deduce putative species boundaries based on our COI dataset were congruent. Both Automatic Barcode Gap Discovery (ABGD) and Generalized Mixed Yule Coalescent (GMYC) model indicated the presence of 56 taxonomic units in our COI dataset. Most of the sequences we obtained were identified through molecular analysis using public databases such as BOLD and Genbank. The DNA barcoding technique successfully distinguished all fish species examined, providing a more accurate representation of species diversity in the South China Sea compared to relying solely on morphological characteristics. When utilizing the COI gene as a DNA barcode for animal species, the intraspecific Kimura 2-parameter (K2P) distances typically fall below 1% and seldom exceed 2% (Hebert et al., 2003). However, it is worth noting that certain arthropod groups, such as crustaceans, may exhibit comparatively higher distances (Costa et al., 2007; Xu et al., 2022). In our fish dataset, the average intraspecific Kimura 2-parameter (K2P) pairwise distance was calculated to be 0.15%, with a range spanning from 0% to 0.78%. On the other hand, the average divergences among congeneric species were 6.53%, ranging from 1.65% to 10.27%. Indicating varying levels of genetic divergence among different taxa. Some taxa exhibited deeper divergences than others. The average intraspecific divergence observed in this study was lower compared to previous studies conducted in other areas of the South China Sea, such as the Nansha Islands (0.25%) and Dongsha Islands (0.33%). However, the average divergence among congeneric species in this study was slightly higher than that of the two aforementioned areas (4.56% and 3.43%) (Xu et al., 2019; Xu et al., 2021). The average intraspecific and congeneric distances observed in fishes from the northern South China Sea surrounding the Hainan Islands are slightly lower compared to the distances recorded in other geographical regions in previous studies. For instance, previous studies have reported average intraspecific distances of 0.52% and average congeneric distances of 6.86% in Mediterranean marine fishes (Francisco et al., 2022), 0.30% and 6.60% in the Indian Ocean marine fishes (Lakra et al., 2011), 0.39% and 9.93% in Pacific Ocean marine fishes (Ward et al., 2005), and 1.06% and 15.34% in Indo-Pacific coral-reef fishes (Hubert et al., 2012). In our study, the average congeneric divergence in *Priacanthus* was 10.27%, considerably larger than 1.65% in the genus *Equulites*. Higher congeneric divergence was reported in *Platycephalus*, reaching up to 15.55%, and in *Caracanthus*, reaching 12.1% (Ward et al., 2005; Hubert et al., 2012). These differences between genera may reflect variations in the average age of species divergence within congeneric groups. Within a genus, some species may be older than others, and certain genera may have diverged earlier compared to others. Biological mechanisms, dispersal capacity, water dynamics (including ocean currents), geographic distance, and historical events are all factors that can play important roles in shaping the population genetic structures of marine species (Elliott and Ward, 1995; Nelson et al., 2000; Monaghan et al., 2009).

Our findings indicate a significant disparity in the species composition of fish communities between the northern and southern sea regions. Within the northern sea areas of the Hainan Islands, we observed a total of 33 distinct species. Conversely, the southern sea areas exhibited 29 distinct species, with only 5 species found to inhabit both regions. The northern sea areas of the Hainan Islands are situated on the northern continental slope of the South China Sea. The topography of this region exhibits a distinct pattern of alternating steep and gradual slopes, which descend in a stair-like manner from northwest to southeast. Both depth and temperature exert significant influences on the species composition of demersal fish in the northern continental slope of the South China Sea. *Acropoma japonicum*, *Trachurus japonicas*, *Leiognathus ruconius* were recognized as common species in the northern continental slope of South China Sea, occupied a very higher proportion of fish community. The northern continental slope of the South China Sea is characterized by the direct influence of coastal currents and runoff from the Hanjiang River and Pearl River, particularly along the northern margin of the continental shelf. This area serves as a noteworthy fishing ground due to its remarkable primary productivity. Additionally, the eastern portion of the slope is influenced by a branch of the north-flowing, warm Kuroshio Current Complex. These oceanographic conditions, along with the unique habitats present on the northern continental slope of the South China Sea, contribute to the abundance and diversity of living resources. Moreover, this region serves as an important hunting, spawning, and breeding ground for fish (Qiu et al., 2010; Wang et al., 2012a). Recent study on benthic fish diversity and community structure in the northern South China Sea have revealed that there were significant differences in species composition based on depths, but no significant differences were found between seasons and temperatures, as well as in the composition at the genus and family levels (Zhang et al., 2022).

The topography of the continental shelf in the northern sea areas of the Hainan Islands, particularly the eastern part of the Leizhou Peninsula, is relatively flat, with depths shallower than 200 meters. The isobaths in this region run parallel to the coastline. In contrast, the southeast coasts of Hainan Island run alongside the continental slope, which reaches a maximum depth of 1900 meters (Li et al., 2014). The fish community in the southern sea areas of the Hainan Islands is dominated by demersal fish and reef fish. The waters adjacent to the southernmost part of Hainan Island and the southern and eastern coasts of Taiwan have experienced an increase in tropical species of scleractinian coral and reef

communities. This expansion has provided favorable conditions for the livelihood and reproduction of reef fish species (Liu, 2013; Du et al., 2020). The differentiation in fish community and distribution between the northern and southern sea areas of Hainan Island can be traced back to the historical process of separation between Hainan Island and the mainland. According to a prevailing theory, it is believed that Hainan Island was once connected to Guangdong province on the mainland of China. However, during the middle Holocene period, a significant geological event occurred, resulting in the formation of the Qiongzhou Strait. This event took place approximately 8.5 thousand calendar years ago before the present (cal. ka BP). During this period, there was a rise in sea levels, which led to the inundation of the low-lying land that previously connected Hainan Island and Guangdong. As the sea levels continued to rise, the water eventually breached the land bridge, and the Qiongzhou Strait began to open up gradually from west to east. This geological process was a result of natural environmental changes and had a significant impact on the separation of Hainan Island from the mainland (Yao et al., 2009). The diverse habitats and historical events in the northern and southern waters of Hainan Island have led to distinct fish community structures.

Conclusion

In summary, our study demonstrates the reliability, speed, and efficiency of DNA barcoding as a precise tool for species identification of marine fish. In comparison to traditional taxonomical methods, DNA barcoding proves particularly valuable for rapid surveys of marine fish in the northern and southern sea areas of Hainan Island in the northern South China Sea. Morphological classification alone often resulted in misidentifications, with some species being identified only at the genus level or incorrectly identified as different species or closely related sibling species. Both ABGD and GMYC model indicated the presence of 56 taxonomic units in our COI dataset. A significant disparity exists in the species composition of fish communities between the northern and southern sea areas of Hainan Island in the northern South China Sea. The dominant process shaping the β diversity pattern of the marine fish community is species turnover/replacement. Such fundamental diversity datasets play a crucial role in ongoing and future monitoring programs aimed at evaluating fisheries resources and biodiversity in the sea areas surrounding Hainan Island in the northern South China Sea. They provide essential information for understanding and managing the marine ecosystem in the region.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: National Center for Biotechnology Information (NCBI) BioProject database under accession numbers: OR144930- OR145042. The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JZ: Formal analysis, Data curation, Writing - original draft. LX: Conceptualization, Methodology, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. QT, LW, JN, DH, YL and SL: Methodology, Formal analysis. XW and FD: Funding acquisition, Project administration, Resources. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1249073/full#supplementary-material

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