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Spatial Genetic Structure and Diversity of Large Yellow Croaker (*Larimichthys crocea*) from the Southern Yellow Sea and North-Central East China Sea: Implications for Conservation and Stock Enhancement

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Abstract: As a wild resource, the large yellow croaker *Larimichthys crocea* has been seriously threatened since the mid-1980s. Owing to the implementation of protection measures, such as the establishment of a protection zone, fishing prohibitions, restocking programs and successful mariculture, its resources have gradually recovered year by year. Limited by the low spatial resolution and incomplete spatial coverage of sampling stations, the spatial genetic structure and diversity of large yellow croakers from the southern Yellow Sea and north-central East China Sea remains unclear. In order to evaluate the genetic diversity status of this wild stock, 22 wild sites were collected from the southern Yellow Sea and north-central East China Sea and analyzed by investigating genetic variability and its population structure using mitochondrial COI sequence in this study. Among the 662 sequences, a total of 71 different haplotypes were defined. The haplotype diversity (h) and nucleotide diversity (π) values were 0.644~1.000 and 0.00220~0.00473 respectively. The highest h and π occurred in the southern Yellow Sea (YS). AMOVA analysis showed no genetic differentiation among those 22 sites after Bonferroni correction. By comparing with previous studies, the croaker has maintained relatively steady genetic diversity in recent years. Our result also suggested that the croakers in the South Yellow Sea and north-central East China Sea belonged to the same group. Thus, they can be released as a management unit without regard for heterogenicity among those in the sea area. The YS populations can serve as parents for released fish fries in the South Yellow Sea and north-central East China Sea.

Keywords: *Larimichthys crocea*; mitochondrial COI; genetic diversity; population; conservation

Citation: Zhang, F.; Jiang, Y.; Ma, C.; Chen, W.; Cheng, J.; Ma, L. Spatial Genetic Structure and Diversity of Large Yellow Croaker (*Larimichthys crocea*) from the Southern Yellow Sea and North-Central East China Sea: Implications for Conservation and Stock Enhancement. *Water* **2023**, *15*, 338. https://doi.org/10.3390/w15020338

Academic Editors: Xiutang Yuan, Wei Huang and Chuanxin Qin

Received: 6 September 2022
Revised: 19 December 2022
Accepted: 26 December 2022
Published: 13 January 2023

1. Introduction

The large yellow croaker *Larimichthys crocea*, a type of warmly-clustered migratory fish, belongs to the order Perciformes, family Sciaenidae, genus *Larimichthys*. It is widely distributed from the south of China, Leizhou Peninsula, to the north, Shandong Peninsula, and extends to the outer sea near Korea in the southwest [1]. The large yellow croaker (abbreviated as the croaker) once ranked first out of the four major marine products in China. Its production reached approximately 200,000 tons in the mid-1970s, and it ranked among the top three commercial fish species in Mainland China [2,3]. Before the 1970s, the croaker had an obvious fishing season, but due to overfishing and environmental deterioration, it was nearly exhausted as a natural resource in the mid-1980s. Although the fishery department has implemented several protection measures, such as establishing a protection zone or executing fishing prohibition measures, the croaker's numbers still declined severely.

Stock enhancement, defined as the release of hatchery-reared fish into wild populations, is an increasingly popular management strategy used to supplement the populations of many species that are declining in the wild. Hatchery programs for enhancing threatened populations of the larger yellow croaker release tens of millions of juvenile hatchery fish into the coastal waters of China every year [4]. Generally, the primary focus of these stock enhancement programs is to boost the biomass of the target species, and ideally this should be achieved with few adverse effects on the local gene pool. However, numerous studies have shown that hatchery practices can have deleterious effects on the genetic makeup of released stock [5–7], and the released stock might hinder the recovery of natural populations [8–10]. The protection of genetic diversity and adaptive potential in wild populations must be a primary concern in any stock enhancement program. In theory, genotype introductions from genetically similar populations are likely to have smaller negative effects than those from genetically dissimilar populations. Therefore, it is necessary to have a good understanding of the spatial genetic structure and diversity of the wild pupations to be enhanced in stock enhancement programs [11,12].

Three putative geographic stocks of the large yellow croaker were initially identified in the coastal waters of China based on morphological characteristics, i.e., the Daiquyang, Min-Yuedong and Naozhou stocks [3]. The Daiquyang stock, extending from the southern Yellow to the central East China Sea, is the most important stock in terms of annual catch [13]. Previous investigations on the genetic structure and diversity of this stock were mainly performed over small spatial scales [7,14–16] or were restricted to a few specific geographical locations [17–20]. Limited by low spatial resolution and incomplete spatial coverage of sampling stations, the spatial genetic structure and diversity of large yellow croaker stocks from the southern Yellow Sea and north-central East China Sea remain unclear.

It was reported that nucleotide diversity in mitochondrial COI is a good marker for the evaluation of the conservation status of species populations since its sequence includes both conserved and variant sites [21]. In the present study, we expanded the previous investigation on the genetic diversity and structure of larger yellow croaker by collecting specimens from 22 sampling stations distributed throughout the southern Yellow Sea and north-central East China Sea using COI sequence. These findings reported here can provide baseline data on the population genetic structure of larger yellow croakers and serve as a benchmark for genetic management of hatchery-reared stocks of the targeted species regarding stock enhancement in the southern Yellow Sea and north-central East China Sea.

2. Materials and Methods

2.1. Sample Collection

The croaker specimens were obtained from two sources: fishery-independent bottom trawl surveys and fishery-dependent samplings (catch provided by commercial fisheries) conducted in the southern Yellow Sea and north-central East China Sea during the period between May and September 2015. A total of 662 samples were collected from 22 stations distributed in the continental waters between 27° N and 34° N. In the southern Yellow Sea (YS), three sites (i.e., YS-1, -2 and -3) were sampled. In the inshore region of the northern East China Sea (INE), nine sites were sampled including INE-1, INE-2, INE-3, INE-4, INE-5, INE-6, INE-7, INE-8 and INE-9. Three sites near the offshore region of the northern East China Sea (ONE), ONE-1, ONE-2 and ONE-3, were sampled. We also sampled seven sites in the inshore region of the central East China Sea (ICE) including ICE-1, ICE-2, ICE-3, ICE-4, ICE-5, ICE-6 and ICE-7. The sites and numbers are shown in Figure 1 and Table 1. Muscle tissues were taken from under the dorsal fin of the fish, preserved in 100% ethanol, and then stored at 4 °C until the DNA was extracted.

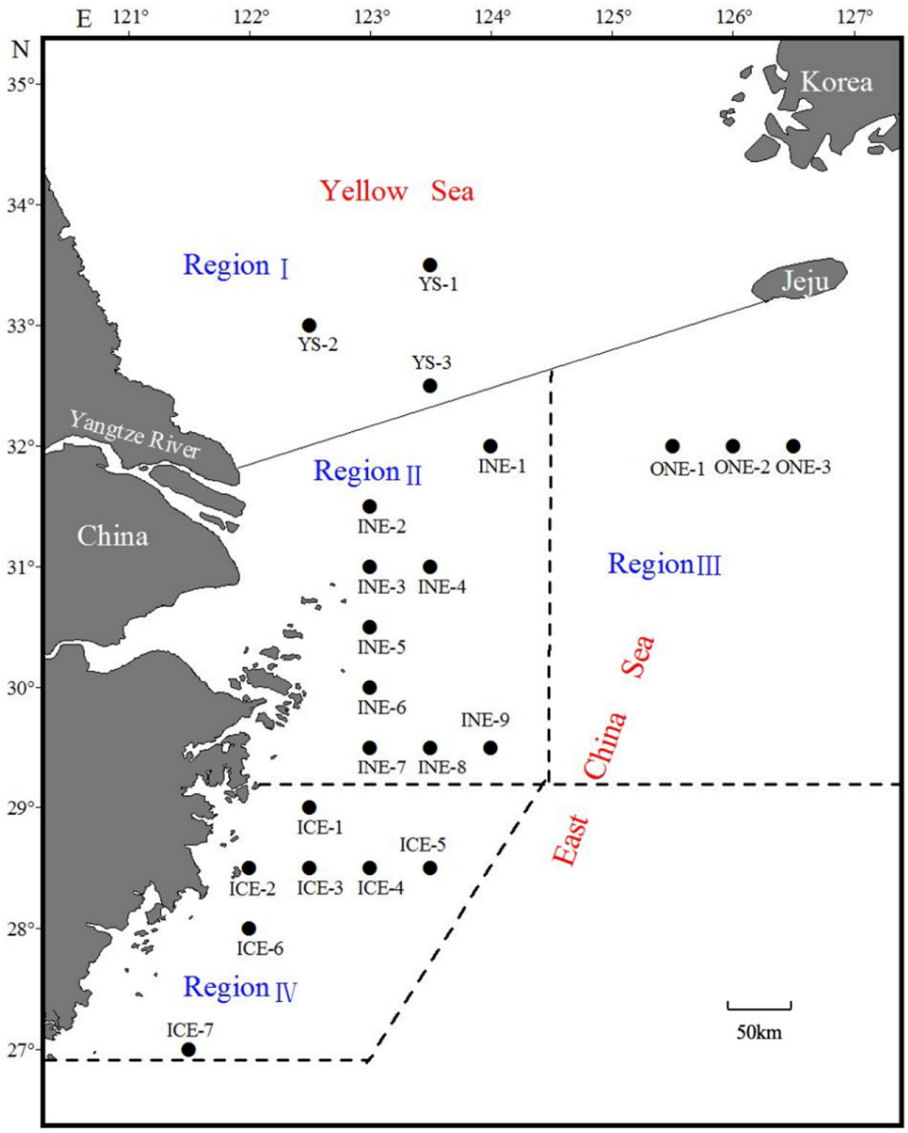

Figure 1. Sampling sites of the large yellow croaker.

2.2. DNA Extraction, PCR Amplification and Sequencing

DNA was extracted using a DNA extraction kit following the protocol of the manufacturer (Tiangen Biotech, Beijing, China). Twenty-five milligrams of tissue from each sample was used for DNA extraction. A pair of degenerate primers was used to amplify partial mitochondrial COI sequence, the forward primer CFF (5′-TCRACYAAYCAYAAAGAYATYGGCAC-3′) and the reverse primer CFR (5′-ACTTCWGGGTGRCCRAAGAATCA-3′). Polymerase chain reaction (PCR) amplification was carried out in a reaction mix of 25 μL (10 mM Tris-HCl, 50 mM KCl, 0.1% TritonX-100, 2.5 mM $MgCl_2$, 0.1 mM of each dNTPs and 1 μM of each primer) with one unit of *TaKaRa Ex Taq*®DNA polymerase and 50 ng genomic DNA. The thermal cycle followed: initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 45 s, primer annealing at 52 °C for 45 s and extension at 72 °C for 55 s, with the final extension lasting 6 min at 72 °C. The amplified fragments were checked on 1.5% agarose gels. The amplified products were sequenced at Shanghai Jieli Biotechnology Co., Ltd. (Shanghai, China).

Table 1. Sampling sites and summary statistics of COI sequences in the 22 sites of the large yellow croaker.

Sea Area	Sites	No. of Samples	No. of Polymorphic Sites	No. of Haplotypes	Haplotype Diversity, h	Nucleotide Diversity, π
Region I: southern Yellow Sea (YS)	YS-1	43	19	17	0.907	0.00443
	YS-2	35	19	15	0.892	0.00443
	YS-3	19	13	10	0.895	0.00358
Region II: inshore region of northern East China Sea (INE)	INE-1	26	13	10	0.738	0.00298
	INE-2	33	16	15	0.917	0.00473
	INE-3	26	13	10	0.895	0.00358
	INE-4	31	14	13	0.892	0.00309
	INE-5	91	28	27	0.858	0.00407
	INE-6	20	9	9	0.795	0.00225
	INE-7	32	22	18	0.993	0.00437
	INE-8	36	23	18	0.916	0.00440
	INE-9	35	16	14	0.832	0.00327
Region III: offshore region of northern East China Sea (ONE)	ONE-1	28	14	11	0.839	0.00367
	ONE-2	5	4	5	1.000	0.00308
	ONE-3	7	4	5	0.857	0.00220
Region IV: inshore region of central East China Sea (ICE)	ICE-1	35	25	21	0.948	0.00444
	ICE-2	9	4	5	0.861	0.00256
	ICE-3	69	28	25	0.852	0.00325
	ICE-4	36	20	17	0.875	0.00335
	ICE-5	22	11	10	0.866	0.00367
	ICE-6	10	5	4	0.644	0.00282
	ICE-7	14	9	6	0.813	0.00338
	Total	662			0.872	0.00373

2.3. Sequence Alignment and Data Analysis

Sequences were edited by software DNAstar version 7.1 (DNAstar, Madison, WI, USA) and manually revised. The identification of variable and parsimonious sites was conducted using software MEGA 5.1 [22]. The software DnaSP (version 4.1) was employed to identify haplotypes, calculate haplotype diversity (h) and nucleotide diversity (π) [23,24].

A median-joining network of phylogenetic relationships among haplotypes was constructed using software Network version 5.0 [25]. The Nei's distances within and between the populations were calculated using software MEGA 5.1. Based on that, an unweighted pair group mean analysis (UPGMA) tree was constructed [21]. Furthermore, analysis of molecular variance (AMOVA) was performed to evaluate the structure of genetic variation based on K2P distance using software Arlequin version 3.01 [26]. The significance level of the test was assessed using 1000 permutations of each pairwise comparison. Fixation index (Fst) value was carried out to assess the genetic differentiation among different geographical populations. Significance values for all multiple tests were corrected by sequential Bonferroni procedure [27].

3. Results

3.1. Characteristics of COI Sequence and Genetic Diversity of the Large Yellow Croaker

The fragment length of the mtDNA COI gene was 520 bp, which was amplified from 662 individuals of the croaker. The average nucleotide frequencies among all sequences were 28.3% T, 29.8% C, 22.4% A and 19.4% G, respectively. The A/T content (50.7%) was nearly equivalent with the C/G content (49.2%). Sixty-five variable sites were found, and no insertion or deletion was detected.

Among the 662 sequences, a total of 71 different haplotypes were defined. The haplotype H5 was the dominant haplotype shared by 207 individuals (31.3% of all individuals)

and was present in all sites. A low percentage of unique haplotypes (13 of 71 haplotypes) which were only present in one individual was found. The average nucleotide diversity (π) of all specimens was 0.00373, ranging from 0.00220 (ONE-3) to 0.00473 (INE-2). The haplotype diversity (h) ranged from 0.644 (ICE-6) to 1.000 (ONE-2) with an average of 0.872 (Table 1). The genetic diversity of all sites was reanalyzed after being divided into four groups according to the sampling sea area. The h and π values were both highest in the southern Yellow Sea (YS) and lowest in the offshore region of the northern East China Sea (ONE) (Table 2).

3.2. Genetic Differentiation and Phylogeny of the Large Yellow Croaker

Pairwise genetic distances within sites ranged from 0.0022 to 0.0048 and those between sites ranged from 0.0021 to 0.0046 (Table 2). The molecular variance analysis (AMOVA) showed that the total genetic variation mainly occurred within-sites (99.68%) and only 0.32% occurred among-colonies (Table 3). AMOVA analysis showed all the Fst values showed no significant genetic differentiation after Bonferroni correction ($p > 0.00022$, adjusted 0.05 level by Bonferroni procedure). The results indicated there was no genetic differentiation among those sites. The haplotype network was color-coded for samples collected from different sea regions, which were labeled with four different colors, red for YS, blue for INE, pink for ICE and green for ONE (Figure 2). A star-like topology was observed (Figure 2). Haplotype 5 was the ancestral haplotype of this topology. It was present in the center and closely related to most of the haplotypes. The populations mingled together, and no distinguishable clades could be identified. No clade was separated from each other, which showed that the croaker from 22 sites might be a panmictic population. The phylogenetic reconstruction corroborated the patterns. No obvious clade was found, and 22 sites scattered irregularly in the tree (Figure 3).

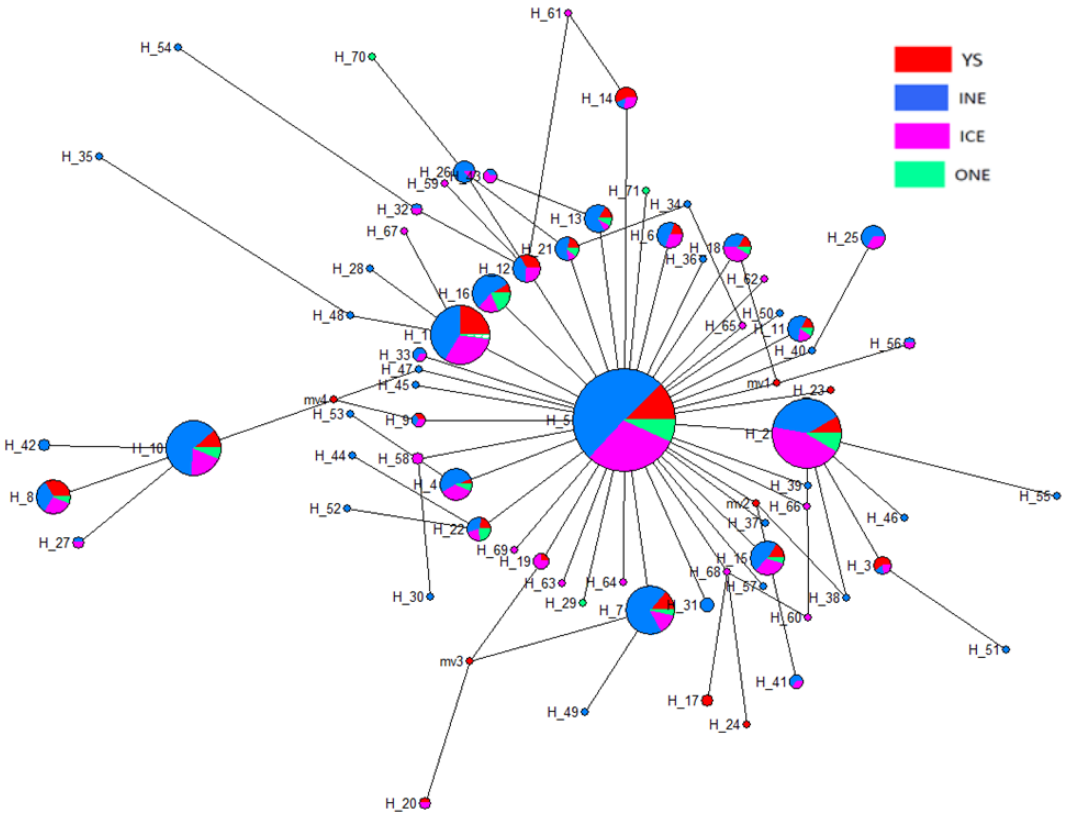

Figure 2. Haplotype network of the large yellow croaker based on the mtDNA COI region (71 haplotypes). The dots between the nodes indicate the number of mutations. The area of each circle is proportional to the corresponding haplotypes.

Table 2. Summary statistics of COI sequences in four groups of the large yellow croaker.

Locality	YS-1	YS-2	YS-3	INE-1	INE-2	INE-3	INE-4	INE-5	INE-6	INE-7	INE-8	INE-9	ICE-1	ICE-2	ICE-3	ICE-4	ICE-5	ICE-6	ICE-7	ONE-1	ONE-2	ONE-3
YS-1	0.0041	−0.0016	−0.0110	−0.0088	0.0051	−0.0071	0.0101	0.0089	−0.0111	−0.0095	−0.0082	−0.0061	−0.0141	0.0052	0.0023	−0.0061	−0.0128	−0.0325	0.0062	−0.0003	−0.0051	−0.0323
YS-2	0.0043	0.0045	0.0301	0.0110	0.0031	0.0085	0.0417	0.0017	0.0245	−0.0018	0.0022	0.0004	0.0033	0.0469	0.0264	0.0206	−0.0075	−0.0462	0.0386	0.0053	0.0035	−0.0139
YS-3	0.0038	0.0042	0.0036	−0.0166	0.0125	0.0015	0.0024	0.0265	−0.0124	−0.0001	−0.0069	0.0147	−0.0148	−0.0421	−0.0061	−0.0231	−0.0112	−0.0056	−0.0286	−0.0013	0.0269	−0.0525
INE-1	0.0035	0.0038	0.0032	0.0030	−0.0025	−0.0108	0.0036	0.0057	−0.0075	−0.0103	−0.0165	−0.0121	−0.0200	0.0114	−0.0141	−0.0158	−0.0102	−0.0220	−0.0007	−0.0145	0.0240	−0.0462
INE-2	0.0044	0.0046	0.0042	0.0039	0.0048	0.0191	0.0538	−0.0099	0.0378	0.0116	0.0071	0.0123	0.0032	0.0342	0.0242	0.0195	−0.0180	−0.0511	0.0079	−0.0125	0.0310	0.0000
INE-3	0.0038	0.0041	0.0036	0.0033	0.0043	0.0036	0.0082	0.0128	−0.0129	−0.0149	−0.0136	−0.0180	−0.0185	0.0319	0.0081	0.0008	0.0069	−0.0158	0.0373	0.0002	−0.0101	−0.0291
INE-4	0.0036	0.0039	0.0033	0.0031	0.0042	0.0034	0.0031	0.0486	−0.0160	0.0062	0.0027	0.0109	0.0007	0.0049	−0.0002	−0.0087	0.0299	0.0328	0.0187	0.0266	−0.0349	−0.0499
INE-5	0.0041	0.0043	0.0040	0.0036	0.0044	0.0039	0.0038	0.0041	0.0300	0.0131	0.0133	0.0047	0.0073	0.0553	0.0246	0.0222	−0.0074	−0.0495	0.0297	−0.0101	0.0189	0.0069
INE-6	0.0031	0.0035	0.0029	0.0026	0.0037	0.0029	0.0026	0.0033	0.0023	−0.0043	−0.0076	−0.0079	−0.0152	0.0170	−0.0114	−0.0204	0.0147	0.0233	0.0201	0.0086	−0.0085	−0.0380
INE-7	0.0042	0.0044	0.0040	0.0037	0.0046	0.0039	0.0038	0.0043	0.0033	0.0044	−0.0088	−0.0142	−0.0136	0.0034	0.0039	−0.0007	−0.0060	−0.0324	0.0093	0.0072	−0.0142	−0.0417
INE-8	0.0042	0.0045	0.0040	0.0037	0.0046	0.0040	0.0038	0.0043	0.0033	0.0044	0.0044	−0.0096	−0.0125	0.0069	0.0025	−0.0029	−0.0082	−0.0258	0.0107	−0.0001	−0.0156	−0.0524
INE-9	0.0037	0.0039	0.0035	0.0031	0.0041	0.0034	0.0032	0.0037	0.0028	0.0038	0.0038	0.0033	−0.0179	0.0503	0.0018	0.0030	0.0033	−0.0236	0.0401	−0.0036	−0.0185	−0.0242
ICE-1	0.0042	0.0045	0.0040	0.0037	0.0046	0.0040	0.0038	0.0043	0.0033	0.0044	0.0044	0.0038	0.0045	0.0064	−0.0070	−0.0113	−0.0071	−0.0269	0.0052	−0.0090	−0.0289	−0.0390
ICE-2	0.0034	0.0038	0.0030	0.0028	0.0039	0.0032	0.0029	0.0036	0.0024	0.0036	0.0036	0.0031	0.0036	0.0026	0.0008	−0.0203	0.0004	0.0448	−0.0517	0.0283	0.0802	−0.0424
ICE-3	0.0037	0.0039	0.0034	0.0031	0.0041	0.0034	0.0032	0.0038	0.0027	0.0038	0.0038	0.0033	0.0038	0.0030	0.0033	−0.0120	−0.0002	−0.0099	−0.0006	0.0016	−0.0056	−0.0420
ICE-4	0.0037	0.0040	0.0034	0.0031	0.0041	0.0035	0.0032	0.0038	0.0028	0.0039	0.0039	0.0033	0.0039	0.0029	0.0033	0.0034	−0.0052	−0.0118	−0.0158	−0.0056	−0.0064	−0.0433
ICE-5	0.0038	0.0040	0.0036	0.0033	0.0042	0.0037	0.0035	0.0039	0.0030	0.0040	0.0040	0.0035	0.0041	0.0032	0.0035	0.0035	0.0037	−0.0599	−0.0167	−0.0177	0.0343	−0.0306
ICE-6	0.0034	0.0035	0.0032	0.0029	0.0037	0.0032	0.0031	0.0033	0.0026	0.0035	0.0036	0.0030	0.0036	0.0028	0.0030	0.0031	0.0031	0.0028	−0.0015	−0.0424	0.0467	0.0016
ICE-7	0.0038	0.0041	0.0034	0.0032	0.0041	0.0036	0.0033	0.0039	0.0029	0.0039	0.0040	0.0035	0.0040	0.0029	0.0033	0.0033	0.0035	0.0031	0.0034	0.0034	0.0452	−0.0297
ONE-1	0.0039	0.0041	0.0036	0.0033	0.0042	0.0036	0.0035	0.0039	0.0030	0.0041	0.0041	0.0035	0.0040	0.0033	0.0035	0.0035	0.0036	0.0031	0.0036	0.0037	0.0070	−0.0182
ONE-2	0.0036	0.0039	0.0035	0.0031	0.0042	0.0033	0.0030	0.0038	0.0026	0.0038	0.0038	0.0031	0.0038	0.0030	0.0032	0.0032	0.0036	0.0031	0.0034	0.0035	0.0031	0.0107
ONE-3	0.0031	0.0034	0.0028	0.0025	0.0036	0.0029	0.0026	0.0033	0.0021	0.0033	0.0032	0.0027	0.0033	0.0023	0.0027	0.0027	0.0029	0.0025	0.0028	0.0030	0.0026	0.0022

Table 3. Analysis of molecular variance (AMOVA) of mtDNA COI sequence of wild croaker in China.

Source of Variation	Sum of Squares	Variance Components	Percentage of Vatiation (%)	*p* Value
Among groups	3.704	0.00150	0.15419	0.39003
Among populations within groups	18.250	0.00160	0.16444	0.24829
Within populations	619.574	0.96808	99.68137	0.16031
Total	641.527	0.97118	100	

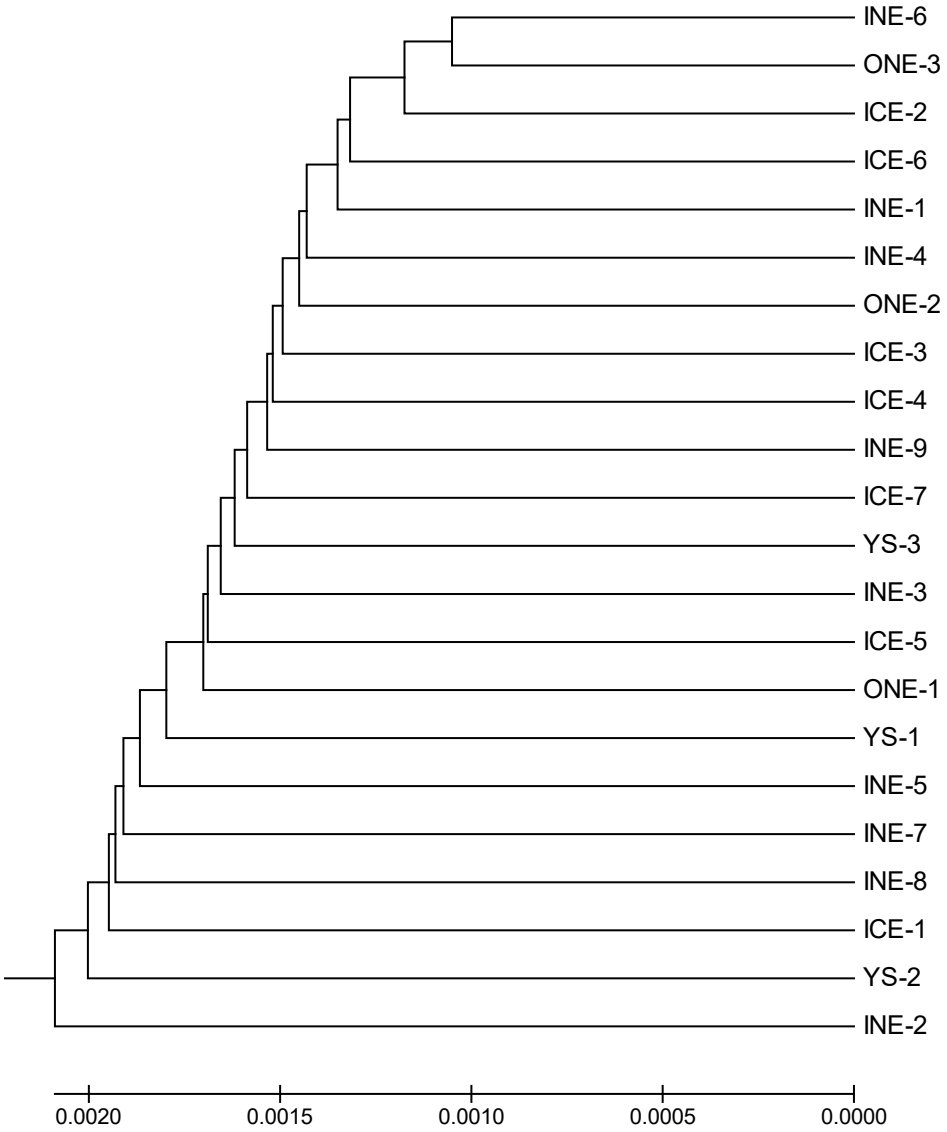

Figure 3. The UPGMA phylogenetic tree based on mtDNA COI sequences of the large yellow croaker. Note: YS: southern Yellow Sea; INE: inshore region of northern East China Sea; ONE: offshore region of northern East China Sea; ICE: inshore region of central East China Sea.

4. Discussion

Throughout the world, coastal and marine stocks are declining, and many stocks are fully exploited. These declines have many causes, from mismanagement to habitat degradation. China is taking a holistic approach to meeting the challenges of its fish-related industries – for example, aquaculture, restocking and translocation [13]. However, wild resources of the croaker have collapsed over the past few decades, due to heavy exploitation of spawning and over-wintering aggregations, poor stock management, habitat pollution

and changes in climate [3,7]. Although the wild biomass or stock size from the mid-1980s onwards is unknown, no spawning or over-wintering aggregations occurred anywhere within the geographic range of the croaker [1,28–30]. In order to restore wild resources of this species, the Chinese government has conducted artificial releases over the past two decades. The released fish were able to survive in natural conditions, feed and grow, spawn and migrate, and at last begin to rebuild the population in the area of the Zhejiang sea until 2008 [13]. In this study, 662 individuals were collected from 22 sites, which suggested that the resources of the croaker had been obviously restored after implementation of release regulations over the course of more than four decades.

As a warm water near-shore migratory fish, the large yellow croaker goes through feeding migration, breeding migration and overwintering migration every year, which increases the difficulty in population sample collection. It is important for the sampled time to avoid collection of the same breeding population. The samples in this study were collected between May and September 2015, reflecting spawning and breeding times of the croaker, in order to avoid mixing population habitats. The collection sites mostly covered the spawning areas of the croaker in the north and central areas of the East China Sea and the southern Yellow Sea, which ensure the samples came from different breeding groups of Daiquyang stock. The genetic diversity of the croaker was investigated based on mitochondrial COI gene sequences. We found 71 haplotypes of the large yellow croaker, which was higher than the number in the study by Wang et al. [31] (haplotype number of 40) and Chen et al. [16] (haplotype number of 34). This may be largely due to the difference in the sample number or that of the length of the COI sequence used; the sampling number in this study was much larger than those in the study of Wang et al. [31] (86 individuals) and Chen et al. [16] (140 individuals).

The average haplotype diversity (h) and nucleotide diversity (π) among all samples were 0.872 and 0.00373, respectively. The haplotype diversity and nucleotide diversity in the southern Yellow Sea (YS) were the highest (i.e., h = 0.897 and π = 0.00411), which was in accordance with the findings of Chen et al., in which the sampled sites were partly covered in the southern Yellow Sea and north-central East China Sea [16]. This may be because large-scale artificial seedling was released in the East China Sea area in order to try to restore the wild resources. Therefore, gene communication occurs between the breeding individuals and wild populations, which might reduce the genetic diversity of wild populations. In addition, over-fishing of the croaker between 1950s and 1970s in the East China Sea led to a sharp reduction in scale [3]. Thus, the bottleneck effect has caused a rapid decrease in diversity. On the other hand, the fishing intensity in the Yellow Sea is lower than that in the East China Sea. Therefore, genetic diversity in the southern Yellow Sea is higher than that in the East China Sea. Moreover, the h and π of the croaker in the southern Yellow Sea (YS) (h = 0.897 and π = 0.00411) in this study were slightly lower than that found by Chen et al. [17] (h = 0.915 and π = 0.00412). This shows that the genetic diversity of the croaker might decrease, but only a little, because of several years of restocking programs., This is to say that the croaker has maintained a relatively steady genetic diversity in recent years. It is suggested that restocking programs might be feasible for the stock's recovery, since the genetic diversity maintained a steady status. Moreover, since the croaker proliferation and release program has been in place for over two decades, there may be breeding of wild–hybrids or farmed generations, and wild resources may have been threatened. However, the croaker resource is recovering year by year, and the genetic diversity is also in a stable state, indicating that protective measures are proving effective. In terms of the decreasing trends in the effective population sizes, the genetic diversities of the croaker still remain at a low level [32]. Therefore, it is vital to make policy related to the management and conservation of this population in the future.

The AMOVA analysis indicated no genetic differentiation among sites after Bonferroni correction (p >0.00022) and a high percentage of variation within sites (99.68%). In this study, the four regions of YS, INE, ONE and ICE cover the Lvsiyang, Daiquyang, Damuyang, southwest North Korea, Maotouyang, and Dongtouyang spawning populations in the

south Yellow Sea and north-central East China Sea [33]. There was no genetic differentiation among 22 sites, which may be due to the fact of feeding, spawning and overwintering migrations as well as the limited physical barriers within the marine environment. Moreover, the haplotype network and phylogenetic tree also support the contention that the South Yellow Sea–East China Sea stock is a panmictic population. Moreover, artificial release might also promote gene communication among sea populations. Thus, our results also suggest that the croaker in the southern Yellow Sea and the north-central East China Sea belonged to the same group. Thus, they can be released as a management unit without regard for heterogenicity among those sea areas. In order to replenish the croaker stock, the Yellow Sea populations with high genetic diversity can serve as parents for released fish fries in the southern Yellow Sea and north-central East China Sea. The released juveniles would recruit to the adult population and contribute genes to the next generation. This should facilitate the retention and conservation of pre-existing genetic heterogeneity within and among populations [12].

5. Conclusions

In this study, we amplified and characterized the mtDNA COI gene sequence of the croaker from the southern Yellow Sea and north-central East China Sea. This study will provide useful background for the management, conservation and wild stock recovery of this important commercial fish species. The genetic diversity might have still maintained a relatively stable level through many years of restocking programs, which play a certain role in the conservation and recovery of croaker resources. Moreover, it is suggested that the released fish fries should be the first generation of wild stock from the southern Yellow Sea population with a higher genetic diversity in order to promote genetic resources and hybrid germplasm of the south Yellow Sea and north-central East China Sea stock. In addition, stock enhancement of the croaker should be monitored regularly to determine whether it may affect the genetic structure and diversity.

Author Contributions: Conceptualization, F.Z., Y.J., J.C. and L.M.; methodology, F.Z. and L.M.; resources, Y.J. and J.C.; formal analysis, F.Z., W.C. and C.M.; writing—original draft preparation, F.Z.; writing—review and editing, F.Z. and Y.J.; supervision, L.M. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the National Key Research and Development Project (2020YFD0900804), the National Infrastructure of Fishery Germplasm Resources and Central Public-interest Scientific Institution Basal Research Fund (2021JC0102).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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