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DOI: https://doi.org/10.3897/arphapreprints.e72516

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Genetic characteristics of yellow seabream *Acanthopagrus latus* (Houttuyn, 1782) (Teleostei: Sparidae) after stock enhancement in southeastern China coastal waters

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Abstract

Yellowfin seabream is an important economic fish that is widely distributed in the East and South China seas. Many attempts to enhance stocks of yellowfin seabream have occurred in China, but a lack of genetic information for this species after stock release represents an obstacle to its management and conservation. To provide scientific guidance for sustainable germplasm resource development, we sequence the mitochondrial DNA (mtDNA) control region (CR) of 123 yellowfin seabream from 6 sample populations (Xiamen, Dongshan I, Dongshan II, Yangjiang, Fangchenggang, and Beibu Gulf). Populations of both wild and cultured yellowfin seabream have high genetic diversity, which we relate to their breeding habits and growth rate. A neighbor-joining tree of CR haplotypes reveals no specific phylogenetic structure corresponding to location of fish capture. Both neutral test and nucleotide mismatch distribution analyses suggest that yellowfin seabream have experienced population expansion events. Pleistocene glacial periods and recent stock releases have played important roles in the formation of present-day phylogeographical patterns. Our study provides baseline information which will assist future research on genetic structure, genetic diversity, and historical demography of yellowfin seabream after stock release in southeast China coastal waters. The use of exotic seeds should be avoided in stock breeding and release, and relevant follow-up surveys and genetic monitoring should be undertaken to clarify the genetic impact of exotic seed use on wild populations.

Keywords

Control region; genetic diversity; population structure; stock enhancement; Yellowfin seabream; mitochondrial DNA, demography, phylogeny

Introduction

Genetic diversity, the diversity of genes (Frankham et al. 2010), forms the basis of species survival and evolution (Huang et al. 2019) and is an important component of biodiversity (Jiang et al. 1997; Norse et al. 1986). Understanding genetic diversity assists with the study of fish evolutionary history, spatial distribution, and population historical demography (Huang et al. 2019; Zhou et al. 2019; Li

2015), and also provides reference opinions for the rational utilization and sustainable development of species germplasm resources (Pimm et al. 2014; Ward 2000). The most common method of studying genetic diversity, genetic structure, and historical population demography involves the use of molecular markers (Gao et al. 2007). Because mitochondrial DNA (mtDNA) is only inherited maternally, reorganization of genetic material does not occur during this process, and the speed of its evolution exceeds that of nuclear genes (Xu et al. 2014); mtDNA is often used to appraise both species diversity and in phylogenetic research (Li et al. 2018, 2019). The mtDNA control region (CR) is a non-coding sequence region which is subject to less natural selection pressure and has a faster mutation and evolution rate than other mtDNA coding sequences (Xiao et al. 2000); it has been widely used in marine fish genetic diversity research (Zhou et al. 2019; Huang et al. 2019).

The yellowfin seabream *Acanthopagrus latus* (Houttuyn, 1782) (Sparidae) is commercially important in China, and is an important species for enhancement releasing in sea areas. This species occurs widely throughout East and South China seas in warm, shallow, nearshore waters (Fan et al. 2011; Hong et al. 2003), is hermaphrodite, generally does not perform long-distance migrations, and has a wide salinity tolerance (Jiang et al. 2012; Iwatsuki 2013). Molecular research on this species has focused on microsatellite markers (Wu et al. 2019), gene expression (Zhu et al. 2019), and specific immune responses (Dehghani et al. 2020; Lin et al. 2020; Rezvan et al. 2020; Namjou et al. 2019). The effect of stocking and screening analysis of its markers was also examined by Lyu et al. (2019a, b). Huang et al. (2017) conducted phylogenetic analysis of sparid fishes at Hailing Island using mitochondrial CO1 gene, and Chen et al. (2015) used the mitochondrial CO1 gene to examine phylogenetic relationships of sparids in China.

To guide the sustainable development of yellowfin seabream germplasm resources, we sequence the mtDNA control region of fishes from 6 wild and farmed sample populations throughout this species' range, and identify genetic diversity and structure, analyze the spatial distribution of sample populations and their historical demography, and explore the influence of stocking on genetic characteristics.

Materials and methods

Sample Collection

From October to November 2019 we collected 123 yellowfin seabream individuals from bottom trawls and seafood markets. In October wild individuals were collected from Xiamen (XM), Dongshan I (DS I), and Fangchenggang (FC), and in November cultured individuals were collected from Dongshan II (DS II) and wild individuals from Yangjiang (YJ) and Beibu Gulf (BB) (Fig. 1, Table 1). Yellow seabream have pale yellow to yellow pelvic, anal, and caudal fins, and can be otherwise identified following Nakabo (2013). Back muscle tissue was cut from each tail, and then stored at −20°C in 95% absolute ethanol at the Laboratory of Marine Biology and Ecology, Third Institute of Oceanography, Ministry of Natural Resources.

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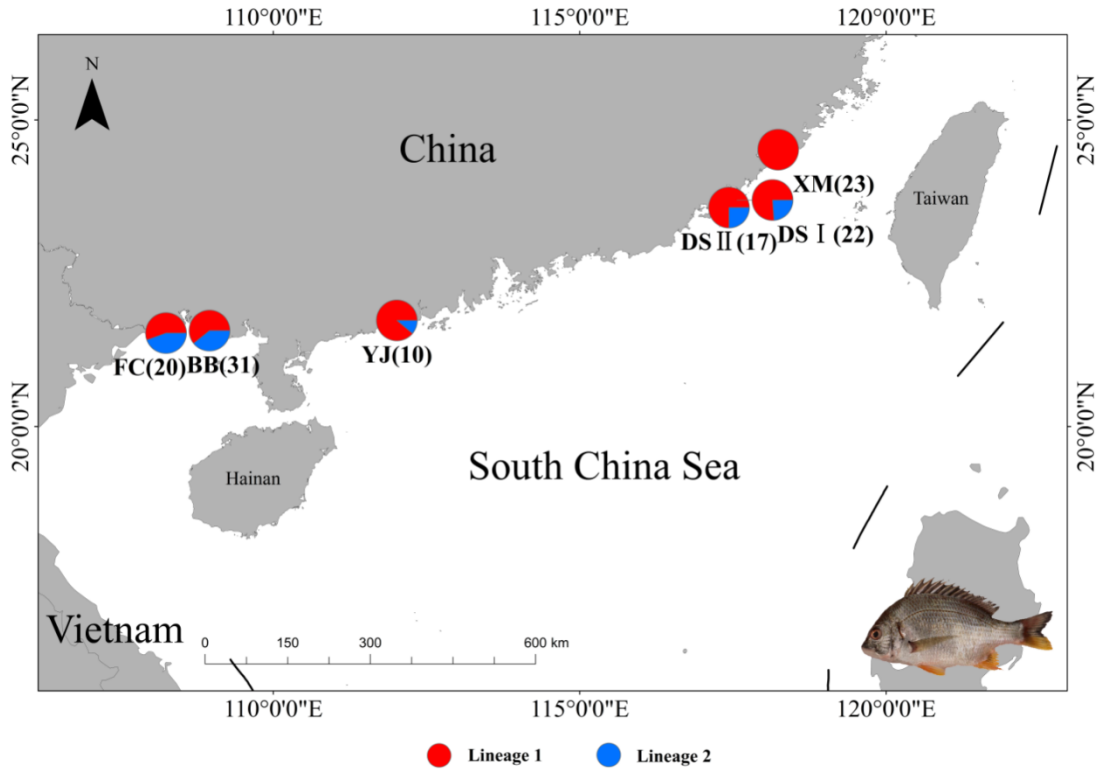

Figure 1 Yellowfin seabream sampling locations, South China Sea.

DNA extraction, PCR amplification and sequencing

A Transgen kit (Easy Pure® Marine Animal Genomic DNA Kit) was used to extract genomic DNA from muscle samples. PCR amplification was performed on DNA with qualified concentration detection, using the forward and reverse primers DL-S (5'-CCCACCACTAACTCCCAAAGC-3') and DL-R (5'-TTAACTTATGCAAGCGTCGAT-3') (Gao et al. 2019). The 25 μL PCR reaction system comprised 17.25 μL of deionized water, 2.5 μL of 10× PCR buffer, 2 μL of dNTP, 1 μL of each forward and reverse primer, 0.25 μL of Taq enzyme, and 1 μL of DNA template. PCR reaction conditions included denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 45 s, annealing at 50°C for 45 s, and extension at 72°C for 45 s; then a final extension at 72°C for 10 min. A 3 μL sample of the PCR product was separated on a 1.5% agarose gel by electrophoresis; samples that met sequence concentration standards were sent to Parsons Biotech Co., Ltd. for two-way sequencing. From these results, the haplotype sequence of yellowfin seabream CR was screened and submitted to GenBank (accession numbers MT312258-MT312358).

Data analysis

The original yellowfin seabream mtDNA CR sequence was edited and corrected manually using SeqMan in the DNASTAR software package, combined with PCR amplification of the same primers DL-S and DL-R. Genetic diversity indices (number of haplotypes, mutation sites, transitions and indels, haplotype diversity (h), nucleotide diversity (π) (Nei 1987), and mean number of pairwise differences (k) (Tajima 1983)) were calculated using ARLEQUIN version 3.0 (Excoffier et al. 2005). Blackhead seabream *Acanthopagrus schlegelii schlegelii* (Bleeker, 1854) was used as an outgroup in the construction of a neighbor-joining tree (NJ) (Saitou and Nei 1987) in MEGA 5.0, using

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yellowfin seabream haplotypes and the Kimura two-parameter (K2P) model. 1000 nonparametric self-expanding analyses were used for repeated tests, and the confidence of each branch of the phylogenetic tree was calculated (Tamura et al. 2011). An unrooted minimum spanning tree (MST) was constructed using the MINSPNET algorithm in ARLEQUIN version 3.0 (Excoffier et al. 2005) to determine relationships among haplotypes; the MST topological structure was drawn manually.

Neutrality tests and mismatch distribution analyses were used to identify historical demographic patterns (Fu 1997; Tajima 1989); the *D* test of Tajima (1989) and *Fs* test of Fu (1997) were used to test for neutrality. Both mismatch analyses and neutrality tests were performed in ARLEQUIN 3.0 (Excoffier et al. 1992). Pairwise genetic divergences between sample populations were tested using the conventional population index F_S (Excoffier et al. 1992). In ARLEQUIN, 1000 permutations were used to test the significance of each F_S pairwise comparison. For multiple comparisons, a sequential Bonferroni procedure to adjust the *P* value (Rice 1989) was performed. To examine sample population hierarchical structure and geographical patterns, an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was used. Historical demographic expansions were further tested by nucleotide mismatch distribution; the values of the age expansion parameter (τ) were transformed to estimates of 'real time since expansion' using the formula: $\tau = 2\ \mu kt$ (Rogers and Harpending 1992), where t is an expected date when changes occurred, μ is the substitution rate of CR, and k is fragment length.

Results

Genetic diversity

A total of 123 sequences were obtained from the six sample populations. After manual alignment, target fragment lengths ranged 548–550 bp; 1 sequence was 548 bp in length; 549 bp sequences were dominant (115); and 7 sequences were 550 bp long. There were 107 variable sites among sequences, 66 parsim-informative sites, 41 singleton variable sites, and 5 insertions/deletions. Base contents comprised A (34.66%), T (32.07%), G (13.81%), and C (19.46%); the A+T content (66.73%) was higher than the G+C content (33.27%), indicating an AT preference.

The 123 sequences defined 100 CR haplotypes. Within each sample population the number of haplotypes ranged 9–28, of which 11 (11%) were shared by 2 or more sample populations, and 89 (89%) were unique to a single sample population. For given locations, XM had shared haplotypes (Hap_45, Hap_52, and Hap_81) with DS I and DS II; DS I had shared haplotypes (Hap_9, Hap_45, Hap_48, and Hap_81) with XM, DS II and YJ; DSII had shared haplotypes (Hap_5, Hap_12, Hap_40, Hap_45, Hap_48, and Hap_52) with XM, DS I, YJ, and BB; YJ had shared haplotypes (Hap_5, Hap_9, and Hap_12) with DS I and DS II; and FC had shared haplotypes (Hap_20, Hap_21, and Hap_29) with BB; no haplotype was shared by all six populations (Table 2).

The six combined populations had high haplotype (0.9959 ± 0.0018) and low nucleotide (0.0207 ± 0.0105) diversity. Among sample populations, wild individuals from DS I had the highest diversity (0.9957 ± 0.0153), and wild individuals from YJ had the lowest (0.9778 ± 0.0540) (Table 1).

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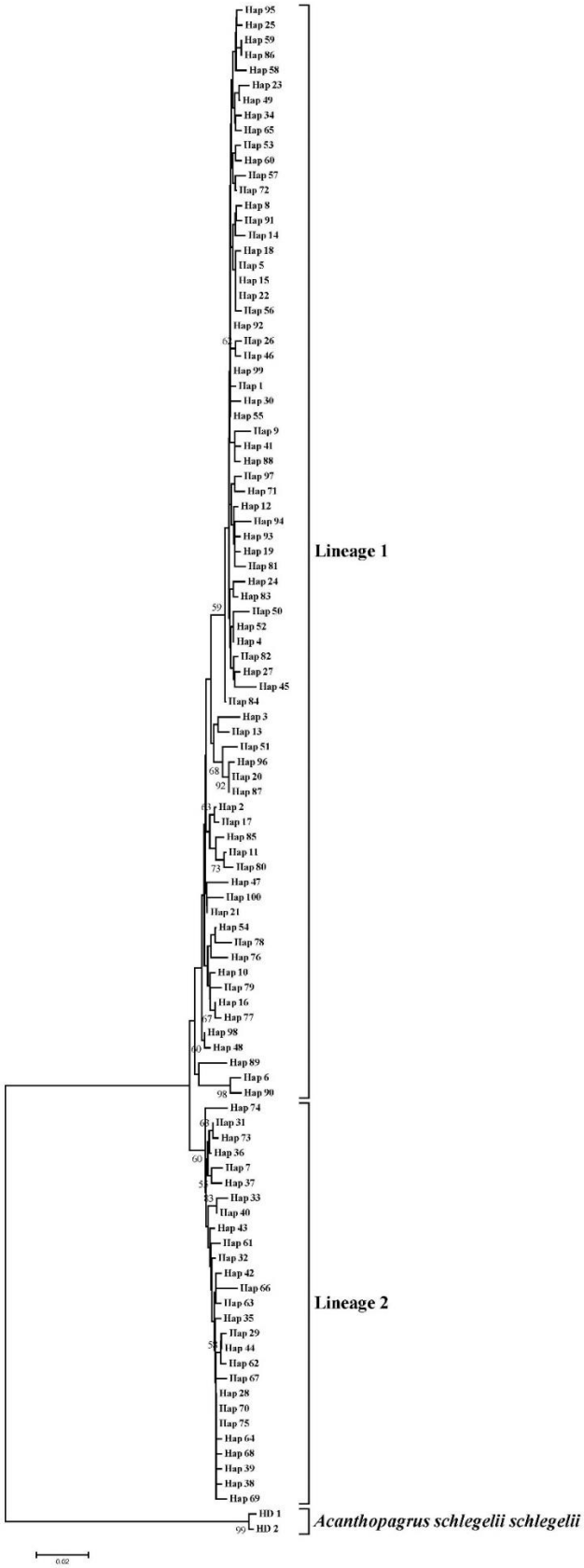

Figure 2. Neighbor-joining tree of CR haplotypes for yellowfin seabream. Only values with bootstrap support >50 (1000 replicates) are shown.

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147 *Genetic structure*

148 The genetic distance between BB and DS sample populations was the largest (0.026), while that
149 between YJ and DS sample populations was the lowest (0.013) (Table 3). Based on the 100 mtDNA
150 CR haplotypes from the 6 sample populations, an NJ tree depicting 2 large lineages (with low
151 confidence) is apparent; lineage 1 contains 73 haplotypes (92 individuals) and lineage 2 contains 27
152 haplotypes (31 individuals). There is no apparent pedigree structure corresponding to capture
153 location (Fig. 2). The genetic distance between the two lineages is 0.032; of the 73 haplotypes in
154 lineage 1, all 20 from the XM sample population are included, as are 16 from DS I, 12 from DS II,
155 8 from YJ, 10 from FC, and 17 from BB sample populations. Lineage 2 comprised remainding
156 haplotypes, with no specific internal topological structure.
157
158 An unrooted MST was constructed based on the 100 mtDNA CR haplotypes (Fig. 3). All sequences
159 exhibited multiple primary haplotypes, with other haplotypes radially distributed around them,
160 without obvious phylogenetic structure corresponding to different sample populations.
161
162 Pairwise F_S values estimated from mtDNA CR sequences ranged −0.024-0.456 (Table 4). Because
163 F_S values between western (FC, BB) and eastern (XM, DS I, DS II, YJ) Qiongzhou Strait
164 populations are large, with exact P values being extremely significantly different, genetic
165 differentiation between sample populations either side of this strait is significant. Differences in
166 pairwise F_S values among XM, DS I, DS II and YJ were small, and exact P values were not
167 significant, as were those for FC and BB, indicating less genetic differentiation among these sample
168 populations.
169
170 AMOVA analyses conducted with one, two, and four gene pools were performed to identify
171 population genetic structure (Table 5). All sample populations were first analyzed as a single gene
172 pool; genetic differences mainly existed within sample populations and accounted for 78.75% of all
173 variation; genetic differences among sample populations accounted for 21.25% of all variation.
174 Sample populations were then grouped into two and four gene pools based on location ((XM, DSI,
175 DSII), (YJ, FC, BB) and (XM, DSI), (DSII), (YJ), (FC, BB)), or the result of pairwise F_S values
176 (XM, DSI, DSII, YJ), (FC, BB), and also into wild (XM, DSI, YJ, FC, BB) and cultured (DSII)
177 sample populations. Genetic differences occurred mainly within sample populations with very
178 significant genetic differentiation ($P < 0.01$); genetic differentiation among gene-pool groups was
179 small and not statistically significant ($P > 0.05$); and genetic differentiation among sample
180 populations within gene-pool groups was small and nonsignificant ($P > 0.05$) based on geographical
181 distribution and pairwise F_S values; in wild and cultured gene pools the genetic differentiation
182 among sample populations within gene-pool groups was large and very significant ($P < 0.01$).
183
184

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Table 3 Genetic distance among yellowfin seabream sample populations. XM (Xiamen), DS I (Dongshan I), DS II (Dongshan II), YJ (Yangjiang), FC (Fangchenggang), BB (Beibu Gulf)

	XM	DS1	DS2	YJ	BB	FC
XM	0.016					
DS1	0.017	0.018				
DS2	0.014	0.016	0.013			
YJ	0.014	0.015	0.013	0.013		
BB	0.025	0.026	0.026	0.025	0.015	
FC	0.022	0.022	0.022	0.021	0.018	0.020

Table 4. Pairwise F_S (below diagonal) and exact P values (above diagonal) among different yellowfin seabream sample populations: XM (Xiamen), DS I (Dongshan I), DS II (Dongshan II), YJ (Yangjiang), FC (Fangchenggang), BB (Beibu Gulf)

	XM	DS I	DS II	YJ	FC	BB
XM	-	0.667	0.676	0.297	0.000	0.000
DS I	−0.008	-	0.126	0.829	0.000	0.000
DS II	−0.012	0.016	-	0.171	0.000	0.000
YJ	0.002	−0.024	0.022	-	0.000	0.000
FC	0.203*	0.169*	0.242*	0.221*	-	0.099
BB	0.425*	0.376*	0.456*	0.456*	0.045	-

*Significant at $P < 0.01$.

Table 5. AMOVA results of yellowfin seabream sample populations based on mtDNA CR sequences.

Source of variation	Sum of squares	Percentage variation	*F*-Statistics	*P*
***One gene pool* (XM, DSI, DSII, YJ, FC, BB)**				
Among populations	150.102	21.25	0.212	0.000 ± 0.000
Within populations	546.744	78.75		
***Two gene pool* (XM, DSI, DSII), (YJ, FC, BB)**				
Among groups	88.654	18.25	0.182	0.109 ± 0.009
Among populations within groups	61.448	8.58	0.105	0.000 ± 0.000
Within populations	546.744	73.17	0.268	0.000 ± 0.000
***Two gene pool* (DSII), (XM, DSI, YJ, FC, BB)**				
Among groups	23.413	−2.71	−0.027	0322 ± 0.016
Among populations within groups	126.688	22.44	0.219	0.000 ± 0.000
Within populations	546.744	80.26	0.197	0.000 ± 0.000
***Two gene pool* (XM, DSI, DSII, YJ), (FC, BB)**				
Among groups	126.487	29.84	0.298	0.058 ± 0.006
Among populations within groups	23.615	0.95	0.014	0.148 ± 0.011
Within populations	546.744	69.21	0.308	0.000 ± 0.000
***Four gene pool* (XM, DSI), (DSII), (YJ), (FC, BB)**				
Among groups	134.978	22.47	0.225	0.055 ± 0.007
Among populations within groups	15.123	2	0.026	0.125 ± 0.008
Within populations	546.744	75.53	0.245	0.000 ± 0.000

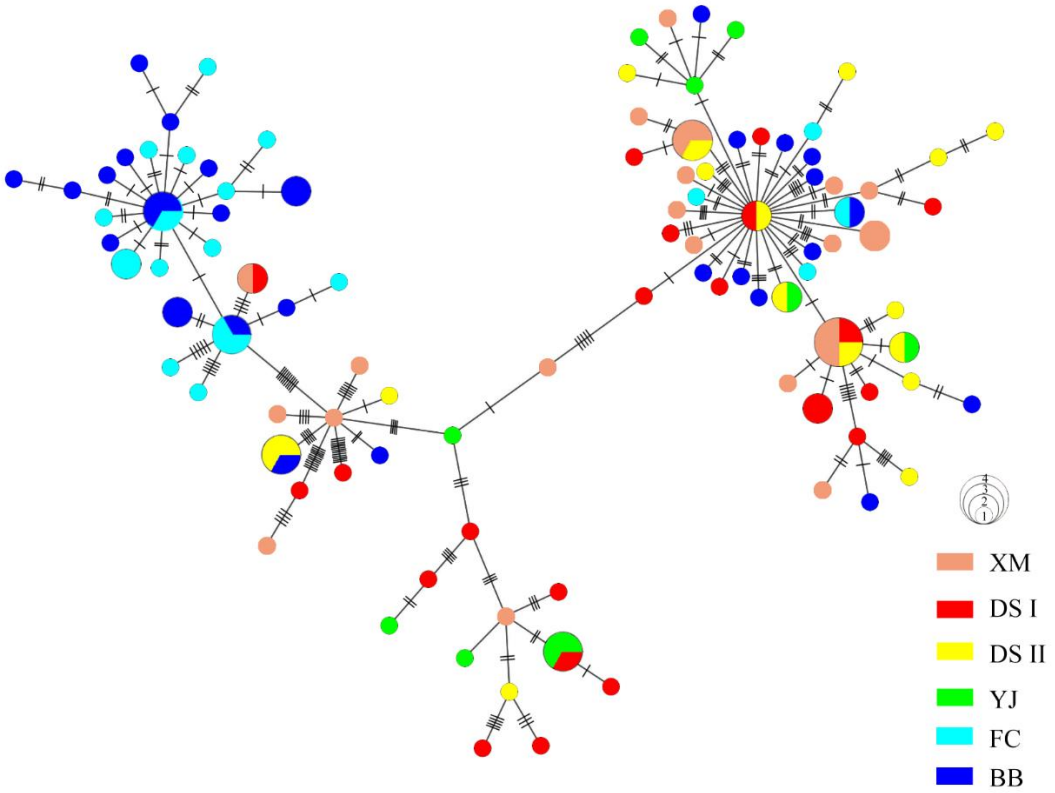

Figure 3. Minimum spanning tree showing phylogenetic relationships among yellowfin seabream sample populations.

Historical demography

While two haplotype lineages occurred in the combined sample populations, no significant geographical structure was apparent. This indicates that genetic variation may not have reached equilibrium following population differentiation. Because sample populations were obviously differentiated, we assessed them for historical dynamics for each haplotype lineage. The nucleotide mismatch distribution in all sequences was bimodal (Fig. 4), with one peak corresponding to the difference between sequences within each lineage, and the other to the difference between sequences in the two lineages. Pedigree structure is more complicated, so analysis of nucleotide mismatch distribution based on all sequences overestimates τ. To improve parameter estimates, we performed a neutral test and nucleotide mismatch distribution analysis on the two haplotype lineages. Although the mismatch between the nucleotide unpaired distribution in the two haplotype lineages and the expected distribution under a population expansion model is not ideal, SSD and Harpending's Raggedness index tests were not significant ($P > 0.05$). As such there is no significant deviation from the expected distribution under the lineage expansion model, which we can then use to analyze historical lineage dynamics (Table 6). The unpaired nucleotide distribution of haplotype lineage 1 is bimodal (Fig. 4), indicating that branches within the lineage exist, with one peak corresponding to the difference between all individuals, and the other to the small branch difference between them, as is the case for lineage 2. Neutral tests on each haplotype lineage (Table 6) produced significantly negative ($P = 0.000$) *Fs* values for both lineages, indicating that yellowfin seabream has experienced

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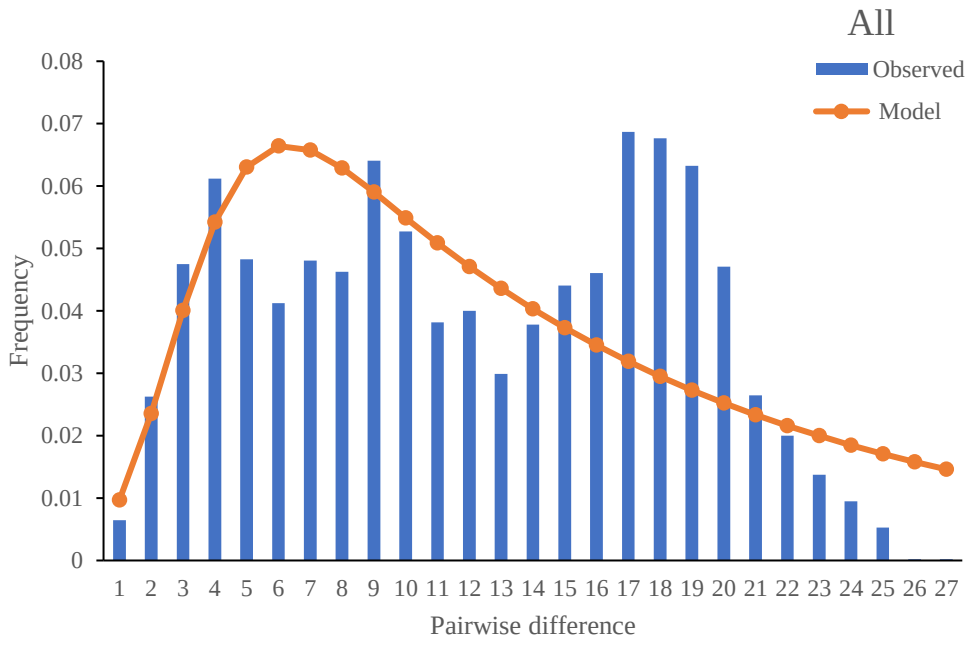
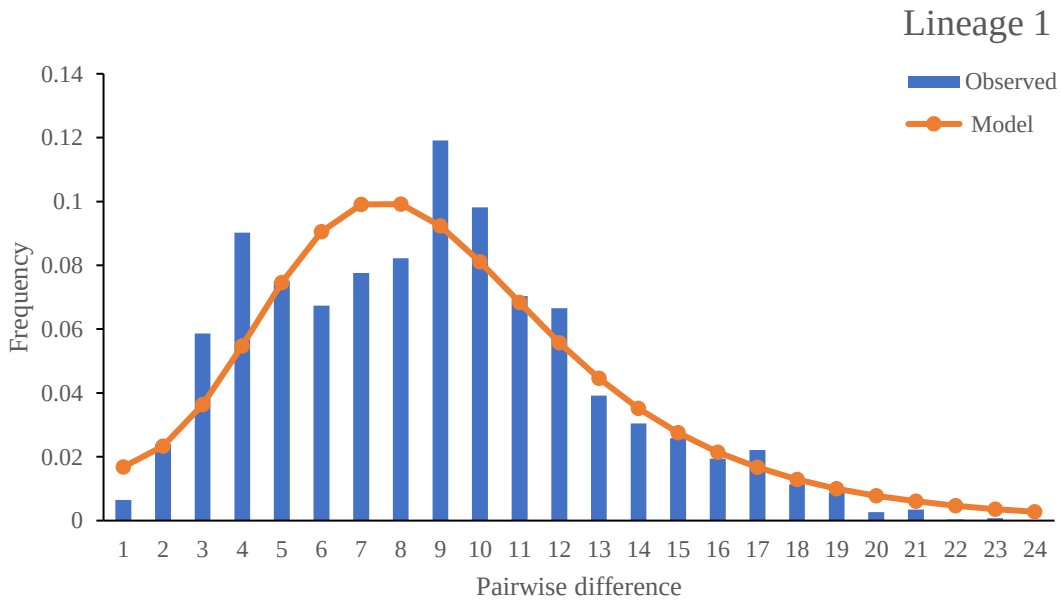
population expansion events. The *D* test on both lineages is also negative, and both are significant ($P < 0.05$).

Observed τ values provide an estimated time for population expansion, with values for haplotype lineage 1 being 6.227 (95% CI: 2.582–20.242) and for lineage 2, 3.748 (95% CI: 2.598–4.826). According to the divergence rate and τ value of 5%–10%/MY, the timing of expansion of lineage 1 occurred between 95,500 and 47,800 YBP (years before present), and that for lineage 2 between 57,500 and 28,700 YBP. Both expansions occurred in the late Pleistocene.





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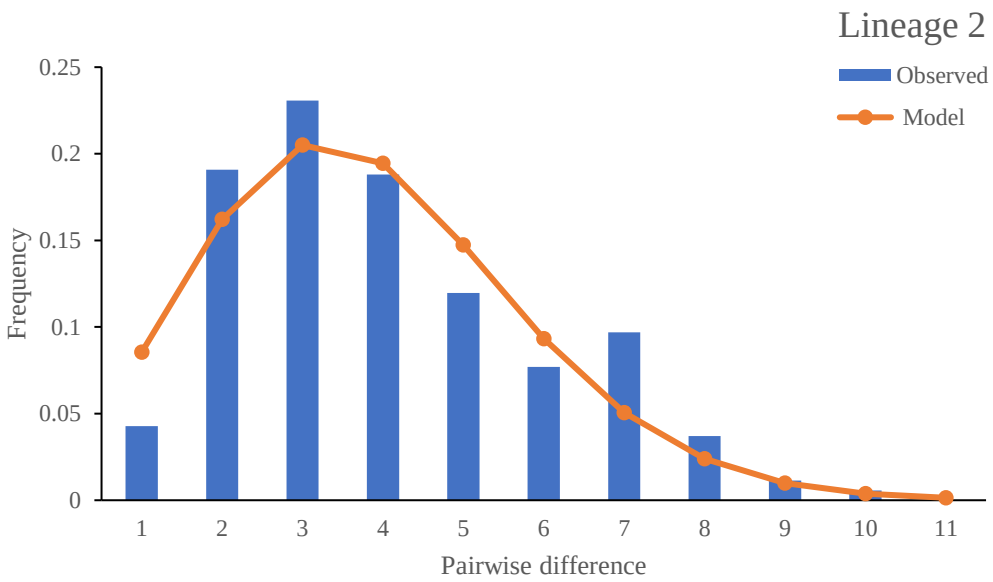

Fig. 4. Mismatch distributions of yellowfin seabream control region haplotypes.

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Discussion

Yellowfin seabream is a warm, shallow-dwelling, largely non-migratory nearshore, salinity tolerant species that occurs widely throughout East and South China seas (Jiang et al. 2012; Iwatsuki 2013). Using the mtDNA CR, we report genetic diversity, current genetic structure, and historic dynamics of this species from 123 fish collected from six sample populations: wild individuals from Xiamen, Dongshan I, Yangjiang, Fangchenggang, and Beibu Gulf, and cultured individuals from Dongshan II. Results of molecular phylogeography provide a scientific basis for division of protection units and the formulation of resource protection measures.

Genetic diversity

Nucleotide diversity is an important index of population genetic diversity, which together with haplotype diversity reflects the degree of variation in mtDNA. The impact of releasing cultured fish on the genetic diversity of wild fish has been intensively debated. If genetic diversity of released fish is significantly lower than that of wild fish, then the large-scale release of stocked fish is likely to reduce overall genetic diversity of a species. The genetic diversity of released cultured fish is determined largely by that of parental stock. Should there be too few parents, should parental genetic diversity differ significantly from that of wild fish, or should there be an imbalance in the contribution of gametes of different parents to offspring, then genetic differences between released and wild populations will increase (Bert 2007). In such instances the genetic diversity of the released population will be lower than that of the wild population.

Because fitness is closely related to genetic diversity, the fitness of released fish will be strongly influenced by the genetic diversity of parental stock (Bert 2007). Reduced genetic diversity can lead to increased expression of harmful recessive genes and a decline in certain traits, leading to changes in population fitness, reduced survival rates, fecundity, and growth, and lowered adaptive capacity (Asahida et al. 2004). If fish with low fitness are released into natural waters, crossbreeding between them and wild populations might greatly increase the frequency and expression of harmful recessive genes in wild populations, and even lead to long-term degradation of a species. To avoid releasing fish with low genetic diversity and poor fitness, parents of comparable or greater genetic diversity than wild fish should be selected for breeding, and the number of parents involved in breeding should be increased to as many as possible.

We report 100 haplotypes from 6 yellowfin seabream sample populations, with 89 of them exclusive to a single sample, and with no dominant haplotype shared by all samples. The short-distances this species migrates might explain the lack of shared and exclusive haplotypes and the high genetic diversity in different geographical locations, which is comparable in wild and cultured populations. The genetic diversity of wild fish from Xiamen in 2019 was slightly lower than it was in 2008 and higher than it was in 2002, while that of wild fish from Yangjiang in 2019 was slightly lower than it was in 2008, and that of wild fish in Beibu Gulf in 2019 was higher than it was in 2002 (Xia et al. 2008). We infer that levels of genetic diversity in yellowfin seabream have remained at highs before and after stocking, and that there have been no major fluctuations during this period.

High levels of genetic diversity are a likely function of the breeding habits of this species, which

spawns in batches and produces 300,000–2.38 million (average 1.36 million) eggs, with relative fecundity 740–5757 spawn/g (averaging 2511 spawn/g). The natural growth rate of this species is also no less than that of other fish (Shi et al. 2012), which ensures plenty of supplementary populations in natural waters. Although artificial propagation of yellowfin seabream was first achieved in 1981, most fry continue to be sourced from the wild (Jiang et al. 2012). Each year, from the end of November, wild-caught fry become available on the market, through until the end of December, there will be a supply of seedlings in succession. Therefore, a sufficient number of parents can be procured in the release population of this species, avoiding reductions in genetic diversity caused by fewer parents and more offspring, to ensure maintenance of high genetic diversity. Yellowfin seabream can survive in brackish water, but they generally do not migrate long distances; it is a hermaphroditic male premature species, which can maintain a certain male-female ratio during its life history (Buxton and Garratt 1990; Li and Ou 2000), possibly explaining the high level of genetic diversity after years of stock enhancement.

Genetic structure

The genetic structure of a species reflects geographical and ecological adaptive differentiation during evolution. Genetic structure enables inferences to be made regarding the historical dynamics of a population, such as effective population size, changes in geographic distribution, gene flow, and genetic differentiation, protection and rational development and utilization are of great significance (Crandall et al. 1999).

Genetic distance can indicate population genetic differentiation; the smaller the genetic distance the closer a genetic relationship. We use a conventional population index Fs as a parameter to measure the degree of genetic differentiation in alleles between sample populations. When $Fs < 0.05$, genetic differentiation is not significant; when $0.05 < Fs < 0.15$, genetic differentiation is moderate; when $0.15 < Fs < 0.25$, genetic differentiation is significant; and when $Fs > 0.25$, genetic differentiation is extremely significant (Wright 1984). mtDNA CR results indicate the genetic distance between four sample populations (XM, DS I, DS II, YJ) east of Qiongzhou Strait is relatively small, with an Fs value < 0.05 (indicating they are closely related and not significantly genetically differentiated). However, the genetic distance between the two sample populations west of Qiongzhou Strait (FC, BB) and these eastern sample populations is slightly greater; Fs values between the BB sample population and each of the four eastern sample populations all exceed 0.25, and Fs values between the FC sample population and the four eastern sample populations all fall between 0.15 and 0.25. Genetic differentiation of the BB and FC sample populations from the four eastern sample populations is great, consistent with findings of Liu et al. (2004), in that large genetic differences exist between populations east and west of Qiongzhou Strait. It is essential to avoid use of exotic seeds in the breeding of released seedlings from the perspective of responsible release, and at the same time, it is important to conduct follow-up surveys and genetic monitoring in waters where fish are released to understand the effects of using exotic seeds on wild populations.

Historical demography

The distribution of yellowfin seabream sample populations is consistent with model 2 of Grant and Bowen (1998), with high haplotype diversity and low nucleotide diversity indicative of populations

affected by environmental change, having experienced a period of rapid expansion, and with the number of populations then increasing sharply. Pleistocene glacial–interglacial periods resulted in changes in climate, sea level, salinity, temperature and ocean current patterns, which significantly impacted the distribution and population of many marine species (Avise 2000; Liu et al. 2006; Han et al. 2012; Gao et al. 2014). Rapid population expansion can result in accumulation of considerable genetic variation and reduce the effect of genetic drift, resulting in more haplotypes being retained (Avise et al. 1984). The distribution of yellowfin seabream was probably severely affected by glacial periods, during which time it was likely restricted to refugia, but after the last glacial period, with increased sea levels, populations expanded to recolonize many marine environments. The unpaired distribution of nucleotides and neutral test of populations indicate a significant deviation from neutrality, suggesting that this species experienced an expansion event, while genetic differences between populations are not significant. This systematic geographical pattern may be a product of mixing cultured fish fry with wild populations.

Conclusions

We relate high levels of genetic diversity in yellowfin seabream to the breeding habits and growth rate of this species. No specific phylogenetic structure corresponds with the locations at which fished were collected. Pleistocene glacial periods and enhancement activities in recent years have played important roles in shaping present-day yellowfin seabream phylogeographical patterns. We provide basic information to support future research on genetic structure, genetic diversity, and historical demography of yellowfin seabream after stock release into southeastern China coastal waters. Subsequent studies should avoid use of exotic seeds in the breeding of released seeds. Genetic monitoring of fishes in waters where stock are released would also enable the effects of using exotic seeds on wild populations to be investigated.

Acknowledgements

This study could not have been achieved without the assistance of Mr Yanping Wang and Dr Weiwen Li in the collection of yellowfin seabream individuals. We are very grateful to Mr Ran Zhang for assisting us with molecular and data analysis. We thank reviewers for their constructive comments on an earlier version of this manuscript, which improved it greatly, and Steve O'Shea, PhD, from Liwen Bianji (Edanz) (www.liwenbianji.cn/), for editing the English text of a draft of this manuscript. This research was supported by the Natural Science Foundation of Fujian Province of China (2019J05146), Scientific Research Foundation of TIO, MNR (2019017, 2019018), and Science and Technology Project of Guangdong Province, China (2019B121201001). No author has any conflict of interest to declare, including the implementation of research experiments and writing of this manuscript.

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503 **Figure legends**

504 Figure 1. XM (Xiamen), DS I (Dongshan I), DS II (Dongshan II), YJ (Yangjiang), FC
505 (Fangchenggang), BB (Beibu Gulf). Numbers in parentheses indicate the numbers of individuals
506 from each sample population (n = 123).
507
508 Figure 3. XM (Xiamen), DS I (Dongshan I), DS II (Dongshan II), YJ (Yangjiang), FC
509 (Fangchenggang), BB (Beibu Gulf)
510
511

Genetic characteristics of *Acanthopagrus latus*

Table 1. Sampling sites, date of collection, sample size, and genetic diversity indices for each sample population of yellowfin seabream.

ID	Sampling sites	Date	Number	No. of haplotype	*h*	π	*k*	GeneBank No.
XM	Xiamen	2019.10	23	20	0.9881±0.0163	0.0155±0.0083	8.5138±4.0859	MT312259-MT312328, MT312330-MT312358
DS I	Dongshan I	2019.10	22	21	0.9957±0.0153	0.0183±0.0097	10.0519±4.7771	
DS II	Dongshan II	2019.11	17	16	0.9926±0.0230	0.0133±0.0073	7.3382±3.6127	
YJ	Yangjiang	2019.11	10	9	0.9778±0.0540	0.0130±0.0075	7.1333±3.6568	
FC	Fangchenggang	2019.10	20	18	0.9875±0.0193	0.0161±0.0087	8.8894±4.2774	
BB	Beibu Gulf	2019.11	31	28	0.9935±0.0100	0.0209±0.0108	11.4860±5.3517	
	Total		123	100	0.9959±0.0018	0.0207±0.0105	11.4237±5.2159	

Note: *h,* haplotype diversity; *π*, nucleotide diversity; *k,* mean number of pairwise differences.

Table 6. Results of neutral tests, *τ* value, and test of goodness of fit for yellowfin seabream.

Group	Number	Number of haplotype	Tajima's *D*	*P*	Fu's *Fs*	*P*	*τ*	SSD	*P*	Raggedness	*P*
Lineage 1	92	73	-1.841	0.011	-24.722	0.000	6.227	0.005	0.254	0.007	0.458
Lineage 2	31	27	-2.189	0.002	-25.854	0.000	3.748	0.007	0.241	0.038	0.261
All	123	100	-1.533	0.030	-24.271	0.000	3.760	0.008	0.363	0.004	0.552

Table 2 Distribution of haplotypes in yellowfin seabream sample populations: XM (Xiamen), DS I (Dongshan I), DS II (Dongshan II), YJ (Yangjiang), FC (Fangchenggang), BB (Beibu Gulf).

	XM	DS I	DS II	YJ	FC	BB	Total
Hap_1	1						1
Hap_2	1						1
Hap_3	1						1
Hap_4				1			1
Hap_5			1	1			2
Hap_6				1			1
Hap_7				1			1
Hap_8				1			1
Hap_9		1		2			3
Hap_10				1			1
Hap_11				1			1
Hap_12			1	1			2
Hap_13						1	1
Hap_14						1	1
Hap_15						1	1
Hap_16						1	1
Hap_17						1	1
Hap_18						1	1
Hap_19						1	1
Hap_20					1	1	2
Hap_21					1	2	3
Hap_22						1	1
Hap_23						1	1
Hap_24						1	1
Hap_25						2	2
Hap_26						1	1
Hap_27						1	1
Hap_28						1	1
Hap_29					2	1	3
Hap_30						2	2
Hap_31						1	1
Hap_32						1	1
Hap_33						1	1
Hap_34						1	1
Hap_35						1	1
Hap_36						1	1
Hap_37						1	1
Hap_38						1	1
Hap_39						1	1
Hap_40			2			1	3
Hap_41			1				1

Genetic characteristics of *Acanthopagrus latus*

Hap_42			1			1
Hap_43			1			1
Hap_44			1			1
Hap_45	2	1	1			4
Hap_46			1			1
Hap_47			1			1
Hap_48		1	1			2
Hap_49			1			1
Hap_50			1			1
Hap_51			1			1
Hap_52	2		1			3
Hap_53			1			1
Hap_54				1		1
Hap_55				1		1
Hap_56				2		2
Hap_57				1		1
Hap_58				1		1
Hap_59				1		1
Hap_60				1		1
Hap_61				1		1
Hap_62				1		1
Hap_63				1		1
Hap_64				1		1
Hap_65				1		1
Hap_66				1		1
Hap_67				1		1
Hap_68				1		1
Hap_69		1				1
Hap_70		1				1
Hap_71		1				1
Hap_72		1				1
Hap_73		1				1
Hap_74		1				1
Hap_75		1				1
Hap_76		2				2
Hap_77		1				1
Hap_78		1				1
Hap_79		1				1
Hap_80		1				1
Hap_81	1	1				2
Hap_82		1				1
Hap_83		1				1
Hap_84		1				1
Hap_85		1				1

Hap_86		1	1
Hap_87	2		2
Hap_88	1		1
Hap_89	1		1
Hap_90	1		1
Hap_91	1		1
Hap_92	1		1
Hap_93	1		1
Hap_94	1		1
Hap_95	1		1
Hap_96	1		1
Hap_97	1		1
Hap_98	1		1
Hap_99	1		1
Hap_100	1		1