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Full Length Research Paper

AFLP analysis on genetic diversity and population structure of small yellow croaker *Larimichthys polyactis*

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The population genetic structure and diversity of small yellow croaker *Larimichthys polyactis* in the Bohai Bay, Yellow Sea and East China Sea were analyzed using amplified fragment length polymorphism (AFLP). Ninety-one individuals were collected from six locations representing three stocks of small yellow croaker. A total of 218 putative loci were detected by 3 primer combinations, 148 of which were polymorphic (67.89%). The proportion of polymorphic loci and Nei's genetic diversity for six populations ranged from 55.34 - 60.09%, and from 0.1244 - 0.1378. AMOVA analysis and pairwise F_{ST} revealed significant genetic differentiation among the three groups based on the breeding migration routes and over-wintering grounds, supporting separate stocks in this species. The result shows the migratory behavior might be an important factor which influences the genetic structure of this species. The UPGMA tree also revealed the significant geographic structure in this species. Pattern of isolation by distance was observed in this species, indicating that significant genetic differentiation among localities of small yellow croaker might be due to the geographic distance.

Key words: Small yellow croaker, *Larimichthys polyactis*, genetic population structure, genetic diversity, AFLP.

INTRODUCTION

The small yellow croaker, *Larimichthys polyactis* (Bleeker) is a benthopelagic fish species of family Sciaenidae which inhabits coastal waters and estuaries (Seikai National Fisheries Research Institute, 2001). Extensively distributed in the Bohai Sea, Yellow Sea and the East China Sea, small yellow croaker has supported a major fishery for decades in China and Korea. Global capture production for small yellow croaker reached 320 thousand metric tones in 2000 (Seikai National Fisheries Research Institute, 2001).

However, the resource of small yellow croaker has faced to a high fishing pressure and has been now considered over-exploited (Jin and Tang, 1996; Jin, 2004; Lin et al., 2008).

The small yellow croaker spawn pelagic eggs and breeding grounds of the species always located in estuaries and mixed areas with low salinity and high salinity. The species spawn eggs in East China Sea from March to April but in May in Yellow Sea and Bohai Bay (Seikai National Fisheries Research Institute, 2001). Based on the studies of spawning migration routes and morphological variation, Liu (1962) and Ikeda (1964) suggested that three wild stocks existed throughout the range of small yellow croaker.

Determination of population genetic structure provides essential information to underpin resource recovery and to aid in delineating and monitoring populations for fishery management. Moreover, understanding fish stock structure is an important component of successful and sustainable long-term management and has attracted considerable interest because of a fundamental interest in biotic evolution (Tudela et al., 1999). Molecular genetic techniques offer the ability to identify and delineate fish stock structure. Nowadays, many molecular genetic techniques have been used successfully to understand the structure of marine fish species (Viñas et al., 2004; Kochzius and Blohm, 2005; Durand et al., 2005; Han et al., 2008a).

Amplified fragment length polymorphism (AFLP) analy-

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sis (Vos et al., 1995), a multilocus marker technique is a PCR-based multilocus fingerprinting technique that combines the strengths and overcomes the weaknesses of PCR-RFLP and RAPD. The major strengths of the AFLP method include simultaneous screening of a large number of polymorphic loci, high reproducibility due to high stringency of PCR, and relative cost effectiveness (Liu and Cordes, 2004). Because of such advantages, AFLP marker has been advocated as a powerful genetic marker system for assessing population structure and individual identity (Gerber et al., 2000; Bensch and Akesson, 2005). And AFLP analysis has also been widely applied to study species, strain and hybrid identification, gene mapping, linkage and genetic diversity of species and populations in a wide variety of organisms including fishes and shrimp(Liu and Cordes, 2004; Wang et al., 2004; Kakehi et al., 2005; Kassam et al., 2005; Gwo et al., 2008). Moreover, AFLP method has already been shown to be a valuable tool for detecting subtle but significant genetic subdivisions in animal studies (Campbell et al., 2003; Whitehead et al., 2003; Wang et al., 2004).

For many species, the success of conservation programs and the creation of effective management policies depend on determining the levels of genetic divergence within and between populations and developing strategies to maintain genetic diversity. Because of small yellow croaker is an important economic resource, it is necessary to confirm whether different genetic stocks occur in over its wide distribution would represent a great contribution to conservation policies and population management. The objectives of this study were to investigate the genetic structure among the small yellow croaker populations in the Bohai Bay, Yellow Sea and East China Sea, and to examine the degree of genetic diversity among them, by using AFLP method.

MATERIALS AND METHODS

Sampling

Ninety-one specimens were collected from 6 geographic locations throughout the distribution area of *L. polyactis* from 2005 - 2007 (Figure 1 and Table 1). All individuals were identified based on morphological characteristics and a piece of muscle tissue was obtained from each individual. Muscle samples were preserved in 70 - 90% ethanol before DNA extraction.

Genomic DNA was isolated from muscle tissue by proteinase K digestion followed by a standard phenol-chloroform method. DNA was subsequently resuspended in 100 µl of TE buffer (10 mmol/l Tris-Cl, 1 mmol/L EDTA, PH = 8.0). Procedures of AFLP were essentially based on Vos et al. (1995) and Wang et al. (2000). About 100 ng genomic DNA was digested with 1unit of *Eco*R I and *Mse* I (NEB) at 37˚C for 6 h. Double-stranded adapters were ligated to the restriction fragments at 20℃ overnight after adding 1 µl 10 × ligation buffer, 5 pmol E*co*R I adapter (E*co*R I-1/E*co*R I-2; Table 2), 50 pmol *Mse* I adapter (*Mse*I-1/*Mse*I-2; Table 2), 0.3 unit of T4 DNA ligase (Promega) with a final volume of 10 µl. Preamplification PCR reaction was conducted using an Eppendorf Thermocycler (Mastercycler 5334) with a pair of primers containing a single selective nucleotide. Amplification was performed at an annealing temperature of 53℃ for 30 s. The 20 µl PCR product mixture was diluted 10-fold with distilled water and used as templates for the subsequent selective PCR amplification. The selective amplifications were carried out in 20 µl PCR reaction volume containing 1 µl productions of preamplifications, 1 × PCR reaction buffer, 150 µM of each dNTP, 30 ng of each selective primer and 0.5 unit of Taq DNA polymerase on a gradient thermal cycler (Mastercycler 5334) with a touchdown cycling profile of nine cycles of 30 s at 94℃, 30 s at 65℃ (–1℃ at each cycle), and 30 s at 72℃ followed by the cycling profile of 28 cycles of 30 s at 94 ℃, 30 s at 56℃, and 1 min at 72℃. The final step was a prolonged extension of 7 min at 72℃. PCR products were run on 6.0 % denaturing polyacrylamide gel electrophoresis (PAGE) for 2.5 h at 50℃ on the Sequi-Gen GT Sequencing Cell (Bio-Rad, USA), and finally detected using the silver staining technique modified from Merril et al. (1979). Sequences of AFLP adapters and primers are listed in Table 2. Sequences of AFLP adapters and primers are listed in Table 2. Three primer combinations (E-AAC/M-CAC, E-AAG/M-CAT and E-AAG/M-CTA) were chosen for AFLP analysis (Table 2).

AFLP bands were scored for presence (1) or absent (0), and transformed into 0/1 binary character matrix. Proportion of polymorphic loci, Nei's genetic diversity and Shannon diversity index were calculated by POPGEN. Similarity indices were calculated using the formula $S = 2N_{ab}/ (N_a+N_b)$ (Nei and Li 1979), where N_a and N_b are the number of bands in individuals a and b respectively and N_{ab} is the number of sharing bands. Genetic distances between individuals were computed using the formula $D = -\ln S$ (Nei and Li 1979). Genetic relationships among populations were estimated by constructing UPGMA tree based on Nei's genetic distance (Nei, 1978) in Mega 3.0. Population structure of small yellow croaker was investigated using the molecular variance software package (AMOVA) and *F*-statistics in ARLEQUIN 2.000. In additon to a one gene pool analysis, the AMOVA analysis were also carried out with three groups representing three groups defined by the species breeding migration routes and over-wintering grounds (Liu 1962), the Bohai Bay and North Yellow Sea population (>35℃N; BBA, BBB, YSA, YSB), South Yellow Sea population (32℃N - 34℃N; YSC) and East China Sea population (29℃N - 31℃N; ECS). To test for isolation by distance (Slatkin, 1993), pairwise values of $F_{ST}/ (1-F_{ST})$ were plotted against geographical distance (one-dimensional stepping-stone model) between sample sites of small yellow croaker. The strength and significance of the relationship between genetic distances and geographic distances was assessed using reduced major axis regression and Mantel tests using RMA.

RESULTS

A total of 218 putative loci were detected by the three primer combinations, 148 of which were polymorphic (67.89%, Table 3). The average number of bands scored per primer pair was 73, ranging from 51 - 85. The number of polymorphic loci amplified by each primer combination over all populations ranged from 35 to 57, with the average of 49 polymorphic loci per prime combination (Table 3).

The population with the highest proportion of polymorphic loci (60.09%) was population BBB, whereas that with the lowest value was population YSA, in which the proportion of polymorphic loci and number of polymorphic loci was 54.34% and 100, respectively. The population with the highest Nei's genetic diversity was YSC population, with a value of 0.1378, the lowest Nei's genetic diversity was found in YSA population, only with a value of 0.1244 (Table 1).

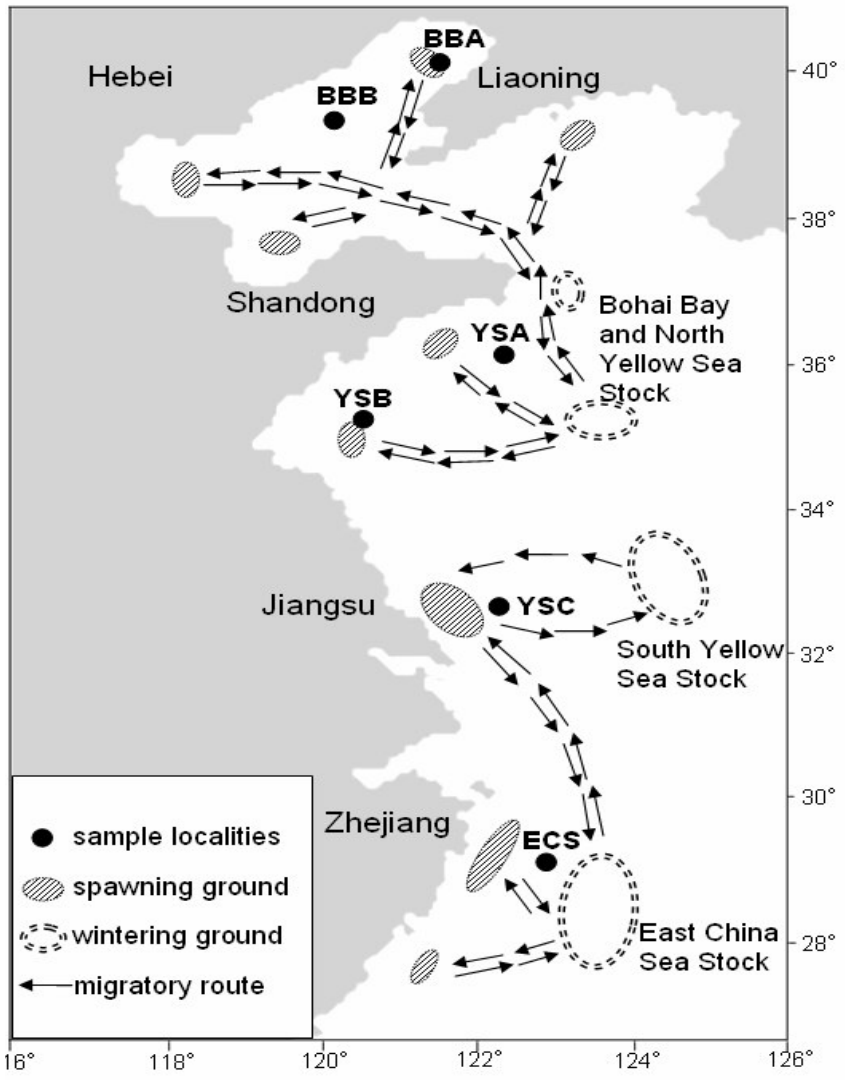

Figure 1. Sample sites and migration routes for small yellow croaker. Three fishery stocks were also shown in the map.

Table 1. Parameters of genetic diversity for populations of small yellow croaker.

Populations	n	Date of collection	Coordinates	Number of loci	Number of polymorphic loci	Proportion of polymorphic loci	Nei's genetic diversity
BBA	15	Oct.r2007	120°30'E 39°30'N	202	121	59.90%	0.1298
BBB	16	Oct. 2007	121°45'E 40°15'N	203	122	60.09%	0.1273
YSA	11	Apr. 2005	122°30'E 36°30'N	184	100	54.34%	0.1244
YSB	15	Apr. 2005	122°00'E 35°00'N	196	116	59.18%	0.1304
YSC	13	Apr. 2005	123°15'E 32°45'N	196	115	58.67%	0.1378
ECS	21	Sep. 2007	123°00'E 28°30'N	198	117	59.09%	0.1275

Table 2. Adaptor and primer sequences used in AFLP analysis.

Primer	Sequence
Adapters	
*Eco*RI-adapter	5'-CTCGTAGACTGCGTACC-3' 5'-AATTGGTACGCAGTCTAC-3'
*Mse*I-adapter	5'-GACGTGAGTCCTGAG-3' 5'-TACTCAGGACTCAT-3'
Pre-amplification primer	
*Eco*RI	5'-GACTGCGTACCAATTC-3'
*Mse*I	5'-GATGAGTCCTGAGTAA-3'
Selective amplification primer	
E-AAC/M-CAC	5'-GACTGCGTACCAATTCAAC-3' 5'-GATGAGTCCTGAGTAACAC-3'
E-AAG/M-CAT	5'-GACTGCGTACCAATTCAAG-3' 5'-GATGAGTCCTGAGTAACAT-3'
E-AAG/M-CTA	5'-GACTGCGTACCAATTCAAG-3' 5'-GATGAGTCCTGAGTAACTA-3'

Table 3. Number of bands generated by primer combinations.

Loci	E-AAC/M-CAC	E-ACG/M-CAC	E-ACT/ M-CTT	Total
Number of loci	85	82	51	218
Number of polymorphic loci	56	57	35	148
Proportion of polymorphic loci	65.88%	69.51%	68.62%	67.89%

Table 4. Nei's genetic distance (above) and pairwise F_{ST} (below) between populations.

	BBA	BBB	YSA	YSB	YSC	ECS
BBA		0.0091	0.0107	0.0105	0.0160	0.0141
BBB	-0.0207		0.0086	0.0108	0.0139	0.0149
YSA	0.0054	-0.0073		0.0078	0.0150	0.0149
YSB	0.0088	0.0108	-0.02628		0.0141	0.0151
YSC	0.0396*	0.0456*	0.0433*	0.0291*		0.0130
ECS	0.0482*	0.0589*	0.0580*	0.0568*	0.0297*	

According to AMOVA analysis, overall genetic differentiation among small yellow croaker from the six populations was small but significant (F_{ST} = 0.0305 P < 0.001), suggesting significant genetic differentiation among localities, and a significant genetic structure (F_{ST} = 0.0583 P < 0.05) was detected among the three groups based on the breeding migration routes and over-wintering grounds. Moreover, significant genetic structure (P < 0.05) was also detected from the pairwise F_{ST} values between populations, though there was lack of genetic structure among populations in the group of Bohai Bay and North Yellow Sea (Table 4). These analyses indicated that several distinct populations of small yellow croaker existed in Bohai Bay, the East China Sea and Yellow Sea. Nei's genetic distance analysis shows lower genetic distances among the populations of the Bohai Bay and North Yellow Sea group, but higher among each of them and other two populations (Table 4). Further, cluster analysis placed populations BBA, BBB, YSA and YSB in a group, while populations YSC and ECS as another group (Fig.2). Among sample sites for small yellow croaker, a Mantel test indicated a significant relationship (P=0.02, r=0.76) between $F_{ST}/(1-F_{ST})$ and geographic distance indicating isolation by distance (Figure 3), with geographic distance explaining 76% of the variation in genetic differentiation for species.

DISCUSSION

The genetic signal of population differentiation observed in the reproductive areas of highly migratory species is

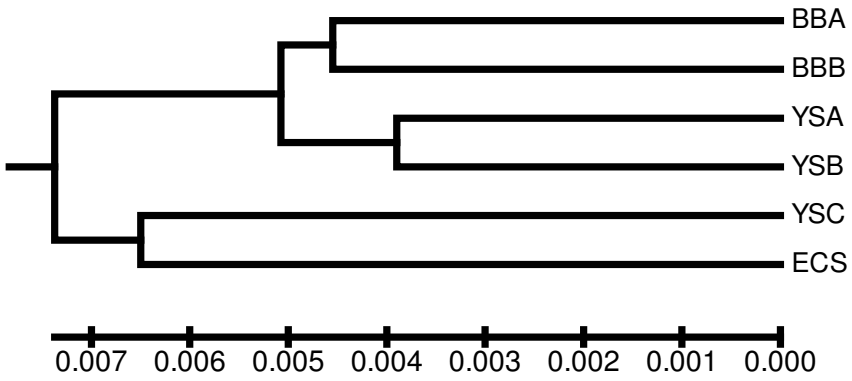

Figure 2. UPGMA cluster analysis based on Nei's genetic distances among four populations. See Table 1 for the different populations.

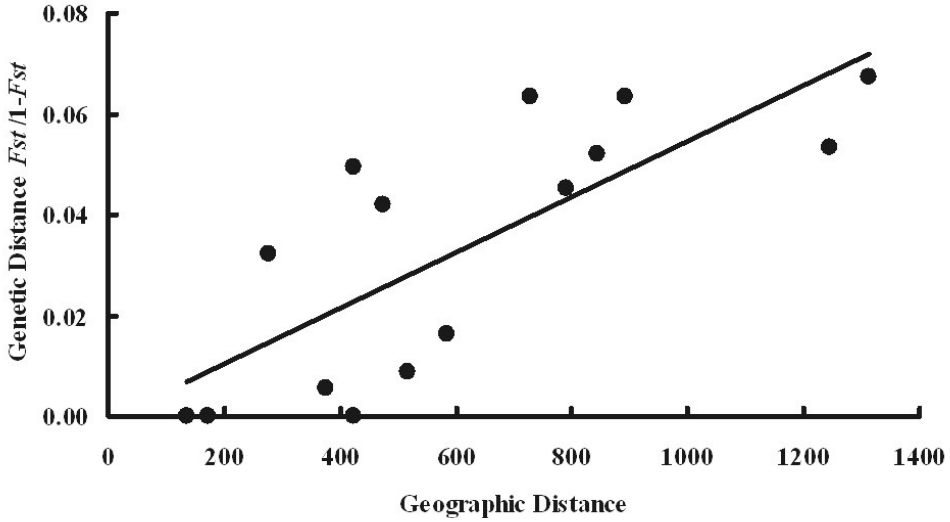

Figure 3. Plot of pairwise estimates of F_{ST}/ (1- F_{ST}) and geographic distance between samples of small yellow croaker.

often obscured by population admixture in wintering or feeding areas (Van Wagner and Baker, 1990; Bowen et al., 1992; Wenink et al., 1994). In previous study, three stocks (North Yellow Sea stock, South Yellow Sea stock and East China Sea stock) were identified throughout the range of small yellow croaker based on the species breeding migration routes and over-wintering grounds (Liu, 1962). The southern coastal waters of Zhejiang Province is the over-wintering ground for the East China Sea stock, the Zhoushan archipelago is the main spawning areas for this stock. For South Yellow Sea stock, the over-wintering ground is located in 32°00' - 34°00'N, 123°00' - 126°00'E; the adults migrate from this over-wintering ground west to Yangze Rivers estuary for spawning in March, the migration distance is no longer 300 sea miles. The over-wintering ground for Bohai Bay and North Yellow Sea stock is located in 33°30' - 35°30'N, 127°00' - 125°00'E in March, the adults migrate from the over-wintering ground north to the Haizhou Bay, Laizhou Bay, Bohai Bay for spawning (Table 1; Seikai National Fisheries Research Institute, 2001). The migratory behavior including the different migration routes and different over-wintering grounds, and different mating period in the Bohai Bay and Yellow and East China Sea might predispose small yellow croaker to genetic structuring along its geographical distribution, although the species showed strong larval dispersal ability. According to the AMOVA analysis a significant genetic structure (F_{ST} = 0.0583 P < 0.05) was detected among the three groups based on the breeding migration routes and over-wintering grounds. Significant genetic structures (P < 0.05) were detected from the pairwise F_{ST} values among populations, except these values among populations in the group of Bohai Bay and North Yellow Sea. The results were consistent with the previous study, supporting at least three stocks in small Yellow croaker (Liu et al.,

1962). And these migration behaviors might play an important role on influencing the genetic diversity and population structure of small yellow croaker.

It is essential to analyze the genetic diversity and population differentiation for genetic research of various organisms. Marine fish generally show low levels of genetic differentiation among geographic regions due to higher dispersal potential during planktonic egg, larval or adult history stages coupled with an absence of physical barriers to movement between ocean basins or adjacent continental margins (Hewitt, 2000). However, the present study provided the molecular evidence for the existence of separate small yellow croaker stocks in Bohai Bay, the East China Sea and Yellow Sea. A significant differentiation ($P < 0.05$) was supported by both AMOVA analyses among six populations of small yellow croaker and among the three groups defined by the migration behavior.

The causes of differentiation in marine organisms are not well understood. In marine environments, the geographic structure of populations may be affected by local conditions and species life history. A plausible explanation might be that the gene exchange among populations was limited by some factors such as oceanographic characteristics, geographic distance, and species life history.

Many marine organisms have pelagic larvae that can potentially interconnect distant populations through dispersal on ocean currents (Liu et al., 2007). The ocean current circulation among the Yellow and the East China seas consists of outflow of water from the Yellow Sea to the East China Sea along the China coast (China Coastal Current), and inflow from the East China Sea to the Yellow Sea along the west coast of Korea (Yellow Sea Warm Current) (Li et al., 2000). Previous studies have revealed that the ocean currents in the East China Sea and Yellow Sea facilitate the dispersal of marine larvae among distant populations (Han et al., 2008b). So the ocean currents might be not responsible for the significant genetic differentiation in small yellow croaker.

Besides the behavior of migration, geography distance might also be responsible for the significant genetic differentiation among populations of small yellow croaker. Generally, in our study more geographically related populations were more genetically similar due to gene flow among populations. The pairwise F_{ST} analysis indicated that a significant difference existed in populations YSC and ECS and other four subpopulations ($P < 0.01$).The UPGMA cluster analysis based on the Nei's genetic distance also indicated the samples from the YSC and ECS were obvious separated from the other sites, while populations BBA, BBB, YSA and YSB were clustered in a group. Therefore, the population YSC and ECS were more different genetically from other populations. To further investigate the correlation between the geographic distance and genetic distance, the isolation by distance test (Slatikin, 1993) was conducted in this study. The plot of $F_{ST}/(1-F_{ST})$ and geographic distance revealed a strong pattern of isolation by distance in this species, indicating that population subdivision among localities of small yellow croaker was mainly due to the geographic distance (76%). Marine environments are often seen as open habitats in which isolation by distance is the main mechanism that may promote genetic differentiation among populations. Patterns of isolation by distance are often established over long periods through equilibrium between gene flow and drift (Slatkin 1993). Isolation by distance in small yellow croaker indicated this species is at genetic equilibrium under dispersal and genetic drift.

Genetic analysis of fish species in Bohai Bay, Yellow Sea and the East China Sea is still few in number, which can be used for comparison to our present study. Similar to our study, AFLP analysis of Japanese Spanish mackerel *Scomberomorus niphonius* in China coastal waters also revealed significant genetic differentiation among populations in the East China Sea and Yellow Sea (Shui et al., 2008). The explanation for significant genetic differentiation in this species might result from migration behavior. MtDNA analysis of white croaker *Pennahia argentata* in the East China Sea and Yellow Sea revealed two geographic groups, with the similar geographic distribution of small yellow croaker (Han et al., 2008c). This geographic structure of white croaker was partly due to the biological characteristics of this species. However, there are some study show differences from this study. A study on the redlip mullet, *Chelon haematocheilus* in the Northwestern Pacific, found no significant genetic structure between the Yellow and the East China seas, although there were significant genetic differences between the three marginal seas of Northwestern Pacific (the Sea of Japan, East China Sea, and South China Sea) (Liu et al., 2007). Recent range expansion and insufficient time to attain migration-drift equilibrium were reasons for the lack of phylogeographical structure in redlip mullet. Genetic study on the *Nibea albiflora* revealed no significant genetic structure in China coastal waters by mtDNA and AFLP markers, suggesting high gene flow among populations (Han et al., 2006; Han et al., 2008b). The Ocean currents in the East China Sea and Yellow Sea were responsible for the lack of genetic structure in *N. albilfora*.

Our result should be sufficient to generate the preliminary data on genetic structure and population diversity of small yellow croaker in the Bohai Bay and Yellow Sea and East China Sea. The concept of a management unit (MU) proposed by Moritz (1994) was defined as a conservation unit that had statistically significant divergence in allele frequencies (nuclear or mitochondrial). Therefore, the small yellow croaker in the Yellow Sea and East China Sea should be considered to be at least three management units, which provides a guideline for further effective conservation and management of the species, and would help greatly in understand.

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REFERENCES

Bensch S, Akesson M (2005). Ten years of AFLP in ecology and evolution: why so few animals? Mol. Ecol. 14: 2899-2914.

Bowen BW, Meylan AB, Ross JP, Limpus CJ, Balazs GH, Avise JC (1992). Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evol. 46: 865-881.

Campbell D, Duchesne P, Bernatchez L (2003). AFLP utility for population assignment studies: analytical investigation and empirical comparison with microsatellites. Mol. Ecol. 12: 1979-1991.

Durand JD, Tine M, Panfili J, Thiaw OT, Laë R (2005). Impact of glaciations and geographic distance on the genetic structure of a tropical estuarine fish, *Ethmalosa fimbriata* (Clupeidae, S. Bowdich, 1825). Mol. Phylogenet. Evol. 36: 277-287.

Gerber S, Mariette S, Streiff R, Bodénés C, Kremer A (2000). Comparison of microsatellites and amplified fragment length polymorphism markers for parentage analysis. Mol. Ecol. 9: 1037-1048.

Gwo JC, Hsu TH, Lin KH, Chou YC (2008). Genetic relationship among four subspecies of cherry salmon (Oncorhynchus masou) inferred using AFLP. Mol. Phylogenet. Evol. 48: 776-781

Han ZQ, Li YZ, Chen GB, Gao TX (2008a). Population genetic structure of coral reef species *Plectorhinchus flavomaculatus* in South China Sea. Afr. J. Biotechnol. 7: 1774-1781.

Han ZQ, Gao TX, Yanagimoto T, Sakurai Y (2008b). Genetic population structure of *Nibea albiflora* in Yellow Sea and East China Sea. Fish. Sci. 74: 544-552.

Han ZQ, Gao TX, Yanagimoto T, Sakurai Y (2008c). Deep phylogeographic break among white croaker *Pennahia argentata* (Sciaenidae, Perciformes) populations in North-western Pacific. Fish. Sci. 74: 770-780.

Han ZQ, Gao TX, Wang ZY, Zhuang ZM, Su TF (2006). Analysis of genetic diversity of *Nibea albiflora* by AFLP markers. J. Fish. China 30: 640-646.

Hewitt GM (2000). The genetic legacy of the Quaternary ice ages. Nat. 405: 907-913.

Ikeda I (1964). Studies on the Fisheries Biology of the Yellow Croaker in the East China and the Yellow Seas. Seikai. Reg. Fish. Res. Lab. 31: 1-81.

Jin XS (2004). Long-term changes in fish community structure in the Bohai Sea, China. Estuar. Coast. Shelf S. 59: 163-171.

Jin XS, Tang QS (1996). Changes in fish species diversity and dominant species composition in the Yellow Sea. Fish. Res. 26: 337-352

Kakehi Y, Nakayama K, Watanabe K, Nishida M (2005). Inheritance of amplified fragment length polymorphism markers and their utility in population genetic analysis of *Plecoglossus altivelis*. J. Fish. Biol. 66: 1529-1544.

Kassam D, Seki S, Rusuwa B, Ambali A, Yamaoka K (2005). Genetic diversity within the genus *Cynotilapia* and its phylogenetic position among Lake Malawis mbuna cichlids. Afr. J. Biotechnol 4:1195-1202.

Kochzius M, Blohm D (2005). Genetic population structure of the lionfish *Pterois miles* (Scorpaenidae, Pteroinae) in the Gulf of Aqaba and northern Red Sea. Genet. 347: 295-301.

Li NS, Zhao SL, Wasiliev B (2000). Geology of Marginal Sea in the Northwest Pacific. Heilongjiang Education Press, Harbin.

Liu JX, Gao TX, Wu SF, Zhang YP (2007). Pleistocene isolation in the Northwestern Pacific marginal seas and limited dispersal in a marine fish, *Chelon haematocheilus* (Temminck & Schlegel, 1845). Mol. Ecol. 16: 275-288.

Liu JZ, Cordes JF (2004). DNA marker technologies and their applications in aquaculture genetic. Aquaculture, 238: 1 -37.

Liu XS (1962). The research of small yellow croaker (*Larimichthys polyactis*) geographic race and gonad. Science Press, Beijing.

Lin LS, Chen JH, Li HY (2008). The fishery biology of *Trichiurus japonicus* and *Larimichthys polyactis* in the East China Sea region. Mar. Fish. 30: 126-134.

Merril CR, Switzer RC, Van Keuren ML (1979). Trace polypeptides in cellular extracts and human body fluid detected by two-dimensional electrophoresis and a highly sensitive silver stain. Proc. Natl. Acad. Sci. USA, 76: 4335-4339.

Moritz C (1994). Defining evolutionary significant units for conservation. Trends Ecol. Evol. 9: 373-375.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genet. 89: 583-590.

Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA, 76: 5269-5273.

Seikai National Fisheries Research Institute (2001). Biological and ecological characteristics of valuable fisheries resources from the East China Sea and the Yellow sea, comparison between the Chinese and Japanese knowledges. Seikai National Fisheries Research Institute, Nagasaki.

Slatkin M (1993). Isolation by distance in equilibrium and non-equilibrium populations. Evol. 47: 264-279.

Shui BN, Han ZQ, Gao TX, Miao ZQ (2008). Genetic structure of Japanese Spanish mackerel *Scomberomorus niphonius* in the East China Sea and Yellow Sea inferred from AFLP data. Afr. J. Biotechnol. 21: 3860-3865.

Tudela S, Garca-Marn JL, Pla C (1999). Genetic structure of the European anchovy, Engraulis encrasicolus, in the north-west Mediterranean. J. Exp. Mar. Biol. Ecol. 234: 95-109.

Van Wagner C, Baker AJ (1990). Association between mitochondrial DNA and morphological evolution in Canada Geese. J. Mol. Evol. 31: 373-382.

Viñas J, Bremer JA, Pla C (2004). Phylogeography of the Atlantic bonito (Sarda sarda) in the northern Mediterranean: the combined effects of historical vicariance, population expansion, secondary invasion, and isolation by distance. Mol. Phylogenet. Evol. 33: 32-42.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. Nucleos. Acids. Res. 23: 4407-4414.

Wang Z, Jayasankar P, Khoo SK (2000). AFLP fingerprinting reveals genetic variability in common carp stocks from Indonesia. Asian Fish. Sci. 13: 139-147.

Wang ZY, Tsoi KH, Chu KH (2004). Applications of AFLP technology in genetic and phylogenetic analysis of penaeid shrimp. Biochem. Syst. Ecol. 399-407.

Wenink PW, Baker AJ, Tilanus MGJ (1994). Hypervariablecontrol region sequences reveal global population structuring in a long-distance migrant shorebird, the Dunlin (*Calidris alpina*). Proc. Natl. Acad. Sci. USA, 90: 94-98.

Whitehead A, Anderson SL, Kuivila KM, Roach JL, May B (2003). Genetic variation among interconnected populations of Catostomus occidentalis: implications for distinguishing impacts of contaminants from biogeographical structuring. Mol. Ecol. 12: 2817-2833.