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Full Length Research Paper

Population genetic structure and demographic history of small yellow croaker, *Larimichthys polyactis* (Bleeker, 1877), from coastal waters of China

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Small yellow croaker, *Larimichthys polyactis* (Bleeker, 1877), a commercially important benthopelagic fish, is widely distributed in the Bohai, Yellow and East China Seas. To evaluate the population genetic structure and demographic history of *L. polyactis*, we sequenced the complete mitochondrial deoxyribonucleic acid (mtDNA) control region (798 to 801 bp) in 127 individuals sampled from seven localities throughout its distribution region in China. A total of 136 polymorphic sites were detected, which defined 125 haplotypes. High haplotype diversity (1.000 ± 0.013 to 1.000 ± 0.034) and moderate nucleotide diversity (0.0112 ± 0.0061 to 0.0141 ± 0.0075) were detected in the species. The neighbor-joining tree of haplotypes was assigned into two closely related clades, but did not appear to have any geographic genealogic structure. Hierarchical molecular variance analysis (AMOVA), pair wise F_{ST} comparisons and the nearest-neighbor statistic (S_{nn}) showed no significant genetic differences among populations in the Bohai, Yellow and East China Seas. The demographic history of *L. polyactis* was examined by using neutrality tests and mismatch distribution analysis, which revealed that the species had undergone a Pleistocene population expansion. The results based on the complete mtDNA control region sequences analysis indicate that within its distribution range, *L. polyactis* constituted a panmictic mtDNA gene pool. Factors such as dispersal capacity, ocean currents and insufficient evolution time could be responsible for the lack of population genetic differentiation in *L. polyactis*.

Key words: *Larimichthys polyactis*, mitochondrial control region, population genetic structure, demographic history.

INTRODUCTION

The degree and distribution of genetic diversity in marine biota is determined not only by contemporary levels of gene flow, but also by demographic processes, population history, and selection (Hewitt, 2000; D'Amato and Carvalho, 2005). The insight into historical processes can enhance the understanding of population structure underlying evolutionary processes (Grant and Bowen, 1998). The amount and pattern of polymorphism in deoxyribonucleic acid (DNA) sequences are informative for the inference of the history of a population as well as the mechanisms responsible for generating and maintaining the polymorphism (Li, 1997). Such information could provide insight into the spatial components of phylogeographic lineages and explain the evolutionary process of geographically related populations (Avise, 2000).

The late Pleistocene period (the past one million years) was characterized by a series of large glacial-interglacial changes (Imbrie et al., 1992). This climatic oscillation produced great changes in the sea levels. For example, declines in sea levels of 120 to 140 m were noted during

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glacial maxima (Lambeck et al., 2002). Such a sea-level-induced environment signal was amplified in the marginal seas of the Northwestern Pacific, giving rise to drastic changes in areas and configurations of these seas during the late Quaternary glacial cycles (Wang, 1999; Liu et al., 2006a). The range contractions and expansions for marine biota during the periodic climatic oscillations over the Pleistocene were generally much more dramatic in marginal seas (Bohai Sea, Yellow Sea and East China Sea) than in open sea systems (Pacific Ocean), due to the shallow shelf in marginal seas. The consequence was that extinctions and population expansions of marine biota might have occurred within marginal seas during the Pleistocene ice ages. Such changes were considered to have a great influence on the amount and distribution of intraspecific genetic variation in some marine fishes in the Northwestern Pacific (Liu et al., 2006a, b; 2007; Han et al., 2008a, b; Shui et al., 2009).

Small yellow croaker, *Larimichthys polyactis* (Bleeker, 1877), is a benthopelagic fish species of family Sciaenidae, which is widely distributed in the Bohai, Yellow and East China Seas of the Northwestern Pacific. It is an asynchronous and multiple spawner from late spring to early summer (Wu, 1981). *L. polyactis* spawn pelagic eggs and their spawning grounds are always located in estuaries and mixed areas with low and high salinity. They center spawning grounds according to changes in the mixed areas (Seikai National Fisheries Research Institute, 2001). The species is characterized by long-distance seasonal migration; it moves into shallow waters during warmer seasons to breed and spawn, and moves back to deeper waters in the cooler seasons (Seikai National Fisheries Research Institute, 2001).

L. polyactis is one of the most important fishery resources in China, Korea and Japan. However, this resource has been decreasing significantly due to overfishing (Jin et al., 2005). This will decrease its genetic variation and create an increasingly serious population status for the species. Determination of population genetic structure provides essential information to underpin resource recovery and to aid in delineating and monitoring populations for fishery management (Han et al., 2008a). Some studies on genetic diversity, population genetic structure and demographic history of *L. polyactis* have been completed recently by using mitochondrial DNA (mtDNA) [the control region (CR) and cytochrome *b* (Cyt *b*) sequences] (Xiao et al., 2009; Kim et al., 2010; Wu et al., 2009), random amplified polymorphic DNA (RAPD) (Meng et al., 2003) and amplified fragment length polymorphism (AFLP) markers (Han et al., 2009; Lin et al., 2009).

However, small sample sizes (for example, only 48 individuals in the RAPD study by Meng et al., (2003), and 53 individuals in the AFLP study by Han et al., (2009)), limited primer combinations (for example, only 3 and 4 primer combinations in the AFLP studies by Lin et al. (2009) and Han et al., (2009) respectively), and large geographical gaps in sampling design prevented the drawing of more robust conclusions. The aim of this study was to determine the population genetic structure and demographic history of *L. polyactis* sampled throughout its distribution region in China. For this purpose, we have analyzed a more broad-scale sampling locations and a larger sample size by using the highly variable mitochondrial CR as genetic marker. This marker has a high resolving power at the population level in many marine fishes (Liu et al., 2006a; Liu et al., 2007; Han et al., 2008b). We discuss the results with regards to the biological characteristics, geological factors, and severe climatic oscillations in Pleistocene ice ages that influence the population genetic structure and demographic history of *L. polyactis*. Our findings may provide insight into the conservation and sustainable fisheries management of this species.

MATERIALS AND METHODS

A total of 127 individuals of *L. polyactis* were sampled from seven sampling localities (A, B, C, D, E, F, G) in the Bohai, Yellow and East China Seas from 2007 to 2008 (Figure 1, Table 1). Muscle samples were preserved in 90% ethanol for DNA extraction.

Amplifying and sequencing

Genomic DNA was isolated from muscle tissue by proteinase K digestion followed by a standard phenol-chloroform method (Sambrook et al., 1989). The polymerase chain reaction (PCR) amplification of the complete CR sequence was conducted using primers CR-s (5′- CCCACCACTAACTCCCAAAGC-3′) and 12S-CR (5′- GTGCGGATACTTGCATGTGT-3′), which targeted a portion of transfer ribonucleic acid (tRNA-pro) and 12S rRNA, respectively. Polymerase chain reaction (PCR) was carried out in 50 µl volumes containing 20 to 50 ng template DNA, 5 µl of 10× reaction buffer, 5 µl of $MgCl_2$ (25 mM), 1 µl of dNTPs (10 mM), 0.2 uM of each primer, and 2.5 units of *Taq* DNA polymerase (Takara Co.). Amplification of the products was carried out in Biometra thermal cycler (Biorad, USA) under the following conditions: 3 min initial denaturation at 94°C, followed by 35 cycles of 45 s at 94°C for denaturation, 45 s at 57°C for annealing, and 45 s at 72°C for extension; and a final extension at 72°C for 7 min. All sets of PCR included a negative control reaction tube in which all reagents were included, except the template DNA. PCR products were separated on a 1.2% agarose gel and purified with the Gel Extraction Mini Kit (Tiangen BioTech Co., Beijing). PCR products were sequenced with both primers on an ABI Prism 3730 (Applied Biosystems) automatic sequencer.

Data analysis

The mitochondrial CR sequences of both directions in each *L. polyactis* specimen were edited and aligned using Dnastar software (DNASTAR, Inc., Madison, USA). In addition, the complete CR sequences of *L. polyactis* were verified by making a comparison with the known sequences from the large yellow croaker *L. crocea* (Cui et al., 2009). Haplotypes were defined by DnaSP4.0 software. Molecular diversity indices such as number of haplotypes, polymorphic sites, transitions, transversions, and indels, were obtained by applying the program ARLEQUIN (Ver. 3.1). The haplotype diversity (h), nucleotide diversity (π), the mean number of

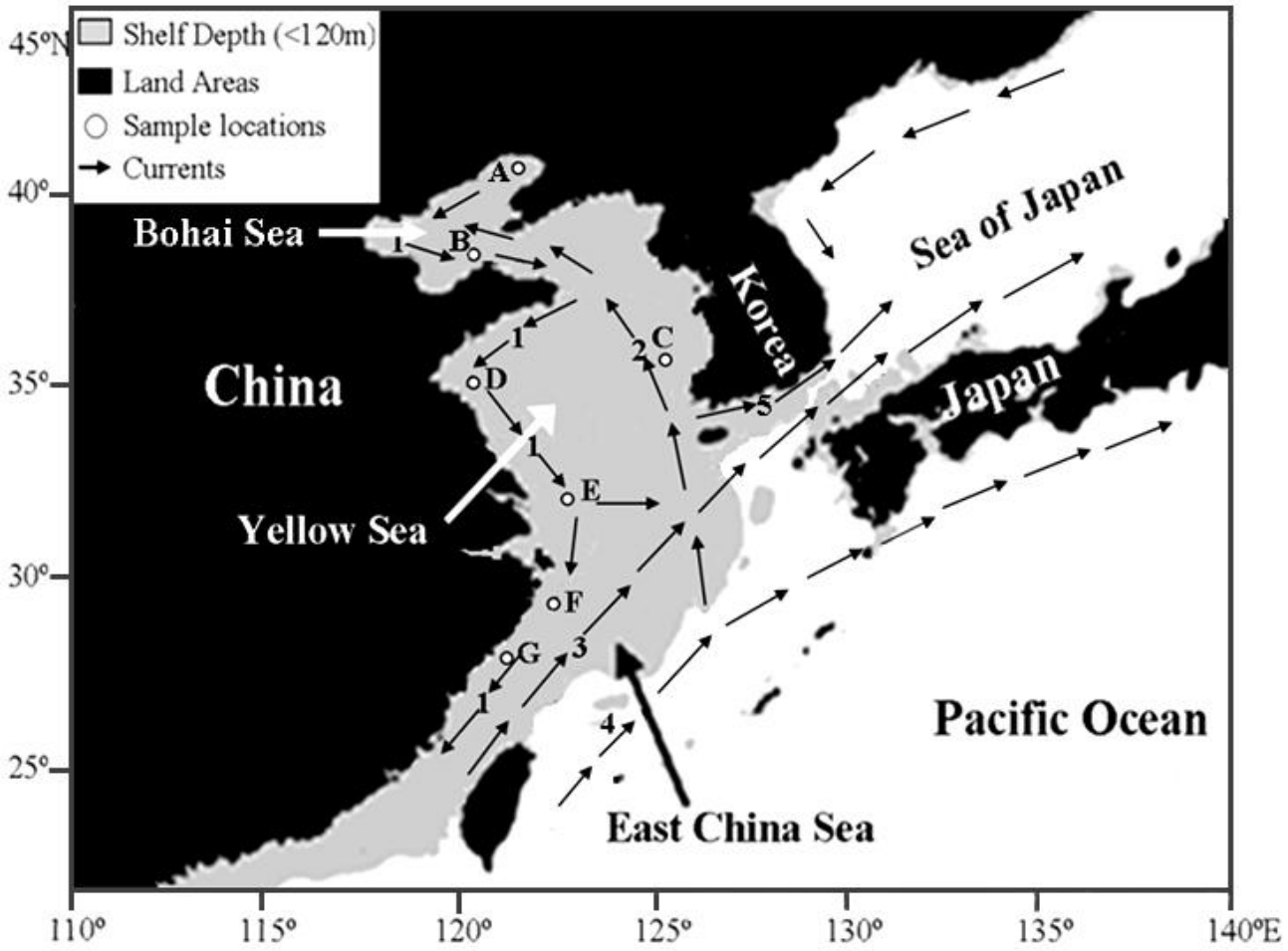

Figure 1. Map showing sample locations (A to G) of *L. polyactis* and currents (1 to 6). Detailed sample information is shown in Table 1. Shaded sea areas are continental shelves that were exposed during periods of low sea-level. 1, China Coastal Current; 2, Yellow Sea Warm Current; 3 Taiwan Warm Current; 4, Kuroshio Current; 5, Tsushima Current.

Table 1. Sampling data of *L. polyactis* including sample name, sample size and date of collection. Several diversity indices for control region were also indicated.

Sample	Sample size	Date of collection	Number of haplotype	*h*	*л*	*K*
A	23	23 Jul.2008	23	1.000 ± 0.013	0.0135 ± 0.0071	10.84 ± 5.12
B	15	19 Oct.2007	15	1.000 ± 0.024	0.0133 ± 0.0072	10.64 ± 5.14
C	22	Jul.2008	22	1.000 ± 0.014	0.0130 ± 0.0069	10.42 ± 4.94
D	17	16 Oct.2007	17	1.000 ± 0.020	0.0141 ± 0.0075	11.31 ± 5.40
E	20	7 May.2007	20	1.000 ± 0.016	0.0131 ± 0.0069	10.48 ± 4.99
F	18	11 May.2007	18	1.000 ± 0.019	0.0112 ± 0.0061	8.98 ± 4.34
G	12	13 May.2007	12	1.000 ± 0.034	0.0128 ± 0.0071	10.25 ± 5.04
Pooled	127	-	125	1.000 ± 0.001	0.0130 ± 0.0067	10.51 ± 4.82

pairwise sequences differences (*k*), and their corresponding variances were calculated after Nei (1987) as implemented in ARLEQUIN. Nucleotide sequence evolution models were evaluated using likelihood-ratio tests implemented by Modeltest Version 3.06 (Posada and Crandall, 1998).

For phylogenetic reconstruction, the genetic distances were generated with MEGA3.1 using the best fit model of Tamura and Nei (TrN) (Tamura and Nei, 1993), corrected with the shape parameter of a gamma distribution (TrN+I+G, I = 0.7137, G = 0.6004). The genetic relationships among haplotypes were reconstructed

using the neighbor-joining method (Saitou and Nei, 1987) implemented in MEGA3.1 and evaluated with 1000 bootstrap replicates.

Population structure was measured with an analysis of molecular variance (AMOVA) (Excoffier et al., 1992) incorporating both sequence divergences and haplotype frequencies among populations. The significance of the covariance components was tested using 1000 permutations. Pairwise genetic divergences between populations were estimated using the fixation index, F_{ST}, which included information on mitochondrial haplotype frequency and genetic distances. F_{ST} statistics were also calculated between pairs of populations. The significance of the F_{ST} was tested by 1000 permutations for each pairwise comparison. In addition to a single gene pool analysis, AMOVA analyses of two formats of three gene pools were used to examine the population structure of *L. polyactis*. Firstly, we conducted AMOVA analysis with three groups representing three conventional spawning stocks (Seikai National Fisheries Research Institute, 2001): samples from A, B, C and D were grouped as the Bohai Sea and northern Yellow Sea stock, samples from E was grouped as the Southern Yellow Sea stock, samples from F and G were grouped as the East China Sea stock. Secondly, we conducted AMOVA analysis with three groups representing three sampled seas: samples from A and B were grouped as the Bohai Sea group, samples from C, D and E were grouped as the Yellow Sea group, and samples from F and G were grouped as the East China Sea group.

AMOVA analysis was performed using ARLEQUIN 3.1, and genetic distances between haplotypes determined using the TrN model of nucleotide substitution with a gamma correction for heterogeneity of mutation rates. The nearest-neighbor statistic (S_{nn}) (Hudson, 2000) was estimated using DnaSP4.0 software. This statistic measures population differentiation by testing whether low divergent sequences are from the same location, and it is particularly useful when populations show high levels of haplotype diversity (Hudson, 2000).

The historical demographic expansions were examined by the neutrality tests and mismatch distribution analysis. We used Tajima *D* test (Tajima, 1989) and Fu F_S test (Fu, 1997) to test whether neutrality holds. The concordance of the observed mismatch distribution between control region haplotypes with the expected distribution under the sudden expansion model was tested by means of a least squares approach (Rogers and Harpending, 1992). The parameter of the demographic expansion, τ, was estimated by a generalized nonlinear least square approach, and confidence intervals were computed using a parametric bootstrap approach (Schneider and Excoffier, 1999). The values of τ were transformed to estimates of real time since expansion using the equation $\tau = 2ut$, where t is the expansion time measured in generations and u is the mutation rate for the whole sequence under study ($u = m_T \mu$, m_T is the number of nucleotide and μ is the mutation rate per nucleotide) (Rogers and Harpending, 1992).

An appropriate nucleotide substitution rate had not been calibrated for this lineage. Assuming the validity of the divergence time estimated with the Cyt *b* clock of *L. polyactis* in the studied area (120,000 years, Wu et al., 2009), an approximation of 4%/million year (μ=4×10^{-8}) divergence for CR was applied to provide a time frame for *L. polyactis* population expansion. We assumed an average generation time of 1 year because this is the time that most individuals reach sexual maturity (Jin et al., 2005). Both neutrality tests and mismatch distribution analysis were performed in ARLEQUIN3.1.

RESULTS

The complete CR was sequenced in 127 individuals of *L. polyactis* from seven populations (GenBank accession numbers: HM234851–HM234975), and the length of these sequences ranged from 798 to 801 bp (excluding partial segment of the tRNA-Pro and 12S rRNA genes), with a consensus length of 806 bp. The average cleotide composition of these sequences were T = 30.4%, C = 23.0%, A = 32.3%, G = 14.3%. A total of 136 polymorphic sites were found, containing 77 parsimony informative sites and 59 singleton polymorphic sites, which defined 147 substitutions including 106 transitions, 41 transversions and 11 indels. A total of 125 haplotypes were identified among 127 individuals. Only 2 haplotypes (1.6%) were shared between two individuals, the other 123 haplotypes (98.4%) were singletons.

Haplotype diversity (*h*) was high for all populations, with all the observed values being 1.000 (Table 1). In contrast, the values of nucleotide diversity (π) within each population were moderate, ranging from 0.0112 to 0.0141 (Table 1). Among all individuals of *L. polyactis*, h = 1.000 and π = 0.0130. Both the haplotype diversity and nucleotide diversity estimates varied little among populations, which implies that there was no clear geographical trend in terms of the control region diversity values for *L. polyactis*. The haplotypes of *L. polyactis* were assigned to two closely related clades (A and B) in the neighbor-joining tree, but with low support values (<50) (Figure 2). Clade A and clade B included 103 and 22 haplotypes comprising 104 and 23 individuals, respectively. However, these clades did not appear to have any geographic structure, and there were no significant genealogical branches or cluster of samples corresponding to sampling locations.

All the pairwise comparisons of F_{ST} were low, ranging from -0.0181 to 0.0259, without statistical significance (Table 2), suggesting a pattern of the overall control region homogeneity of *L. polyactis* in the seven populations. Two patterns of gene pools with four formats of AMOVA analysis were performed on the genetic structure of *L. polyactis* populations (Table 3). AMOVA analysis showed that most of the variances were found within populations: 99.70 and 99.92% in the single gene pool pattern, and 99.74 and 99.76% in the three gene pools pattern. Overall φ_{st} did not differ from zero (φ_{st} = 0.001, P = 0.376) among Bohai Sea and northern Yellow Sea stock, Southern Yellow Sea stock and East China Sea stock. The values of φ_{ct} in the two patterns of three gene pools were low: group subdivisions were not significant using either the spawning stocks (φ_{ct}=-0.001, P=0.596) or the sampled seas (φ_{ct} = -0.002, P = 0.658) as the grouping criteria. Genetic differentiation among seven populations was further tested by the nearest-neighbor statistic (S_{nn}). The test was not significant (S_{nn}=0.138, P=0.504) for all sequences. Thus, both the hierarchical AMOVA analysis and S_{nn} showed that no significant population genetic differentiation existed throughout the examined range of *L. polyactis*.

The mismatch distributions of *L. polyactis* haplotypes were unimodal for the two clades and the pooled sample

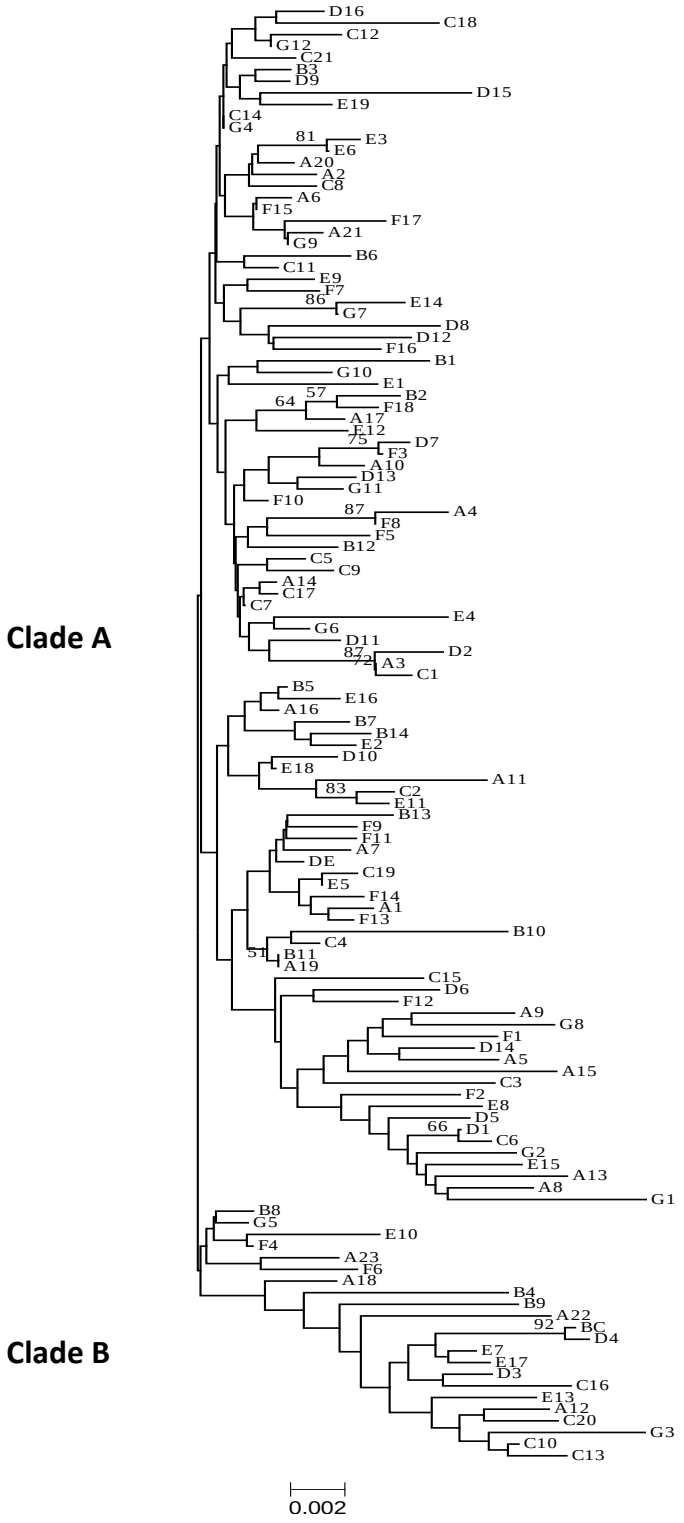

Figure 2. Neighbor-joining tree of haplotypes constructed using TrN distances for *L. polyactis*. Bootstrap support of > 50% in 1000 replicates is shown above branches.

and closely matched the expected distributions under the sudden expansion modal (Figure 3). Small values for the SSD and Raggedness index associated with a lack of significance were of further support for unimodal interpretation of the mismatch distribution for the two clades (Table 4). Tajima's *D* test was negative for the two clades of *L. polyactis*, and highly significant for clade A (P=0.00), but not significant for clade B (P=0.19) (Table 4). The Fu's tests of the two clades of *L. polyactis* were negative and highly significant (P=0.00), which indicated population expansion. For the clade A, the observed value of τ was 8.59 units of mutational time (Table 4), it was estimated that population expansion occurred approximately at 133,000 years ago; while for the clade B τ was 9.46 (Table 4), approximately at 147,000 years ago. Estimated effective female population size after expansion (θ_1) was 178 times higher than before expansion (θ_0) for the pooled sample. For clade A and clade B, these estimates (θ_1/θ_0) were 124 and 36, respectively.

DISCUSSION

Genetic diversity and historical demography

Our results show high levels of *h* (1.000) and moderate π (0.0112-0.0141) within each population of *L. polyactis*. This pattern of genetic diversity is typical for large populations which have recently expanded rapidly from a small population caused by bottlenecks or founder events (Grant and Bowen, 1998). Both the neutrality tests and mismatch distribution analysis indicated a late Pleistocene expansion (133,000 to 147,000 years ago) for *L. polyactis*. The effective female population size of *L. polyactis* was considerably increased after expansion. Additionally, population range expansion may lead to a basically similar molecular signature to that of a pure demographic expansion (Ray et al., 2003).

During the Pleistocene ice periods, all the Bohai Sea Shelf and the Yellow Sea Shelf, and a total area of 850,000 km^2 of the East China Sea Shelf were exposed when the sea level declined about 130 m (Figure 1) (Li et al., 2000). Under this condition, *L. polyactis* might have become extinct above the continental shelf, and survived in a glacial refugium which was located in the basin of East China Sea (Xiao et al., 2009). During the postglacial periods, population expansion of *L. polyactis* from the glacial refugium may have gone back to the shelf region of the Bohai, Yellow and East China Seas.

In such cases, the sudden expansion enhances the retention of new mutations without sufficient time for the accumulation of large differentiation among haplotypes (Grant and Bowen, 1998). So, both the Pleistocene population demographic expansion and the geographical range expansion could be responsible for the observed genetic diversity with high *h* and moderate π in *L. polyactis*. Similar patterns of genetic diversity were also found in other marine fishes in the studied area, such as sea bass *Lateolabrax maculatus* (Liu et al., 2006a), and

Table 2. Pairwise F_{ST} values (below diagonal) and significant *P* values (above diagonal) among populations of *L. polyactis.*

Sample	A	B	C	D	E	F	G
A		0.26	0.62	0.23	0.32	0.45	0.79
B	0.0092		0.21	0.46	0.64	0.28	0.07
C	-0.0077	0.0124	-	0.30	0.46	0.16	0.51
D	0.0095	-0.0009	0.0054	-	0.22	0.14	0.32
E	0.0027	-0.0084	-0.0043	0.0128	-	0.33	0.51
F	-0.0017	0.0065	0.0168	0.0193	0.0024	-	0.36
G	-0.0181	0.0259	-0.0117	-0.0018	-0.0022	0.0012	-

Table 3. Analysis of molecular variance (AMOVA) of *L. polyactis.*

Source of variation	Variance component	Percentage of variation (%)	φ-statistics	*P*-value
One gene pool (A, B, C, D, E, F, G)				
Among populations	0.016	0.30	Φ_{st}=0.003	0.336
Within populations	5.243	99.70		
One gene pool (Bohai Sea and northern Yellow Sea stock, Southern Yellow Sea stock, East China Sea stock)				
Among populations	0.004	0.08	Φ_{st} = 0.001	0.376
Within populations	5.255	99.92	-	-
Three gene pools (A, B, C, D) (E) (F, G)				
Among stocks	-0.006	-0.11	Φ_{ct} = -0.001	0.596
Within stocks	0.020	0.38	Φ_{sc} = 0.004	0.313
Within populations	5.243	99.74	Φ_{st} = 0.003	0.334
Three gene pools (A, B) (C, D, E) (F, G)				
Among groups	-0.012	-0.23	Φ_{ct} = -0.002	0.658
Within groups	0.025	0.48	Φ_{sc} = 0.005	0.254
Within populations	5.243	99.76	Φ_{st} = 0.002	0.302

Table 4. Tajima's *D* and Fu's *Fs*, corresponding P-values, and mismatch distribution parameter estimates.

Sample	Tajima's *D*		Fu's *Fs*		Mismatch distribution			Goodness of fit test			
	D	P	*Fs*	P	τ	θ_0	θ_1	SSD	P	Raggedness index	P
Clade A	-2.04	0.00	-24.44	0.00	8.59	2.04	253.12	0.001	0.33	0.004	0.60
Clade B	-0.89	0.19	-10.94	0.00	9.46	3.82	135.78	0.006	0.51	0.009	0.75
Pooled	-1.97	0.00	-24.24	0.00	8.44	3.85	342.50	0.001	0.33	0.003	0.76

white croaker *Pennahia argentata* (Han et al., 2008b).

Phylogeographic patterns

Unlike the shallow haplotypes phylogenetic tree of both the first hypervariable CR (Xiao et al., 2009) and the complete Cyt *b* (Wu et al., 2009), two defined haplotype clades of the complete CR were detected in this study, although these clades did not appear to have any geographic structure. The higher mutation rate in CR than that in Cyt *b* was suggested to explain their different phylogenetic relationships (Theisen et al., 2008) and the same discrepancy between the CR and Cyt *b* was also reported in wahoo *Acanthocybium solandri* (Theisen et al., 2008) and three *Trachurus* species (Karaiskou et al., 2004). Compared to Xiao et al. (2009) study's, the longer segment sequenced (798 to 801 bp vs 411 bp) in this study indicate that more variable sites were detected (136 vs 84); thus, more haplotypes were defined (125 vs 87), since the samples of the two studies were close to each other (127 individuals vs 114 individuals). Moreover,

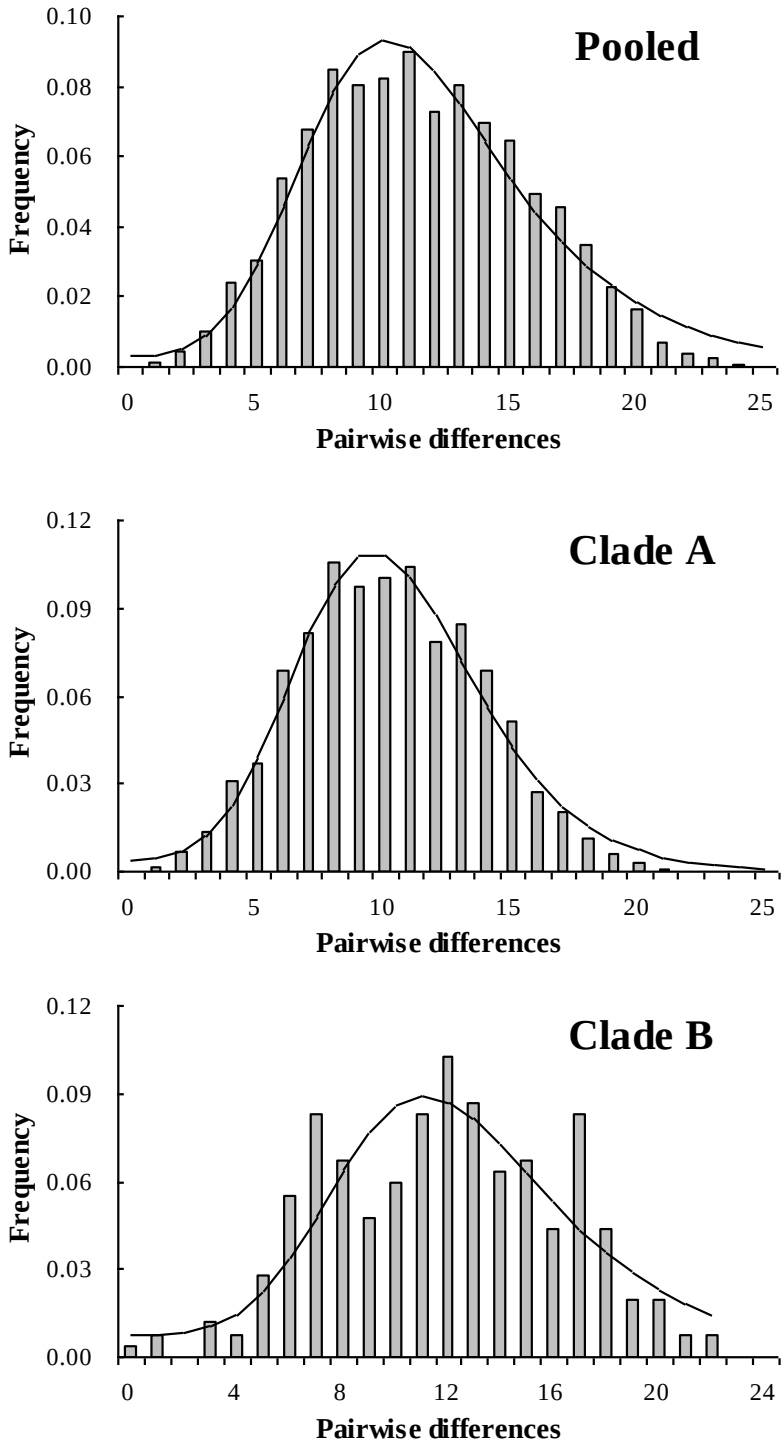

Figure 3. The observed pairwise differences (bars) and the expected mismatch distributions under the sudden expansion model (solid line) for the control region for *L. polyactis*.

the broader sampling range (whole distribution range vs Yellow Sea and north East China Sea) were designed in our sampling strategy. Accordingly, we suggest that the higher resolving power of the complete CR sequence and more larger sample source used in the present study may be responsible for the two clades phylogenetic tree.

It has been suggested that Pleistocene glaciations might play an important role in shaping the

phylogeographic mtDNA patterns and population genetic structure in marine fish species (Planes et al., 2001). Strong geographical differences in haplotype frequencies of the three mtDNA CR lineages were observed in the Sea of Japan, East China Sea and South China Sea in redlip mullet *Chenlon haematocheilus*, which reflected the isolation of the above three marginal seas of the Northwestern Pacific during Pleistocene low sea-level stands (Liu et al., 2007). On the other hand, two haplotype clades of mtDNA CR were detected in Japanese Spanish mackerel *Scomberomorus niphonius* in the Yellow and East China Seas, but these clades did not appear to have any geographic structure (Shui et al., 2009). This phylogeographic mtDNA pattern was also found in *L. polyactis* in this study. The phylogeographic patterns in both *S. niphonius* and *L. polyactis* might be signatures of admixture following past divisions between marginal seas of the Northwestern Pacific.

Comparative phylogeography of the above mentioned three fishes revealed that life histories of the species could play an important role in the degree of the secondary admixture for their mtDNA phylogroups. *C. haematocheilus* inhabits shallow coastal and fresh waters of no more than ten meters depth, and the larvae are typically found in near-shore waters and shallow estuaries (Li, 1992). This suggests limited genetic exchange between populations of this species, which could be responsible for the strong gene frequency changes of the three lineages among the Sea of Japan, East China Sea and South China Sea for *C. haematocheilus* (Liu et al., 2007).

On the other hand, both *S. niphonius* and *L. polyactis* have pelagic larva and a strong capacity for long distance migration (Seikai National Fisheries Research Institute, 2001), which may facilitate the gene flow among populations. This results shows no geographical differences in haplotype frequencies of the two clades in the two species. In addition, the shallow and non-significant genealogical branches of mtDNA haplotypes of both *Engraulis japonicus* (Liu et al., 2006a) and *Nibea albiflora* (Han et al., 2008a) were suggested to relate to their high dispersal capabilities.

Population genetic structure

The results of pairwise F_{ST} comparisons, AMOVA tests and S_{nn} statistic showed no significant population genetic subdivision throughout the Bohai, Yellow and East China Seas of *L. polyactis*. Two studies on *C. haematocheilus* (Liu et al., 2007) and *P. argentata* (Han et al., 2008b) in the Northwestern Pacific, found no significant genetic differences between the Yellow and East China Seas, but significant genetic differences between the Japanese populations and Chinese populations. Lack of genetic break between populations in the China coastal waters have also been reported for *E. japonicus* (Liu et al., 2006a), *N. albiflora* (Han et al., 2008a) and *S. niphonius* (Shui et al., 2009). Compared to the Bohai, Yellow and East China seas, changes in area and configuration in the Japan Sea and the Pacific side of the Japanese archipelago caused by climatic changes were mild (Figure 1). This would have allowed sufficient time for the development of significant genetic differentiation among Japanese populations (Han et al., 2008b).The lack of significant genetic differentiation in the studied area was due to the recent range expansion and insufficient time to attain migration-drift equilibrium (Liu et al., 2007). Given that the time since population expansion in *L. polyactis* (133,000 to 147,000 years ago) is close to that of the fishes cited above, this could explain the lack of significant population genetic differentiation in *L. polyactis*.

Marine fish species with high dispersal often have shallow or no genetic differentiation across large geographic scales, because of a lack of obvious geographical barriers to migration in the oceanic environment (Palumbi, 1994). The pelagic eggs and larvae and strong capability for adult migration suggest the high dispersal of *L. polyactis*. Lacks of obvious barriers to larval dispersal were reported in the studied area (Liu et al., 2007; Han et al., 2008a). The ocean current circulation between the Yellow and East China Seas consists of inflow from the East China Sea to the Yellow Sea along the western coast of Korea (Yellow Sea Warm Current), and outflow of water from the Yellow Sea to the East China Sea along the China coast (China Coastal Current) (Figure 1) (Li et al., 2000). In this case, the East China Sea population could get direct recruitment of *L. polyactis* eggs and larvae from the western coast of the Yellow Sea by the transportation of the southward China Coastal Current, and the connectivity should be high among the six China coastal populations. On the other hand, the Yellow Sea Warm Current could facilitate the dispersal of *L. polyactis* eggs and larvae from the western coast of Korea to the China coast.The movement of eggs and larvae of *L. polyactis* on the above mentioned currents was consistent with a previous fishery investigation which indicated that recruitment phenomena existed from north to south among the Northern Yellow Sea population, the Southern Yellow Sea population and the East China Sea population (Ikeda, 1964). Thus, high dispersal capacity and the ocean circulation among the Bohai, Yellow and East China Seas could be responsible for the genetic homogeneity in *L. polyactis*. Consistent with the previous two mtDNA studies in the Yellow and East China Seas (Xiao et al., 2009; Wu et al., 2009; Kim et al., 2010); our results did support existence of a single panmictic stock of *L. polyactis* throughout its distribution region. However, a significant geographic structure in *L. polyactis* was found in the studied area by using RAPD (Meng et al., 2003) and AFLP (Han et al., 2009; Lin et al., 2009), supporting the concept of separate stocks of this species.

The power to detect genetic structure is influenced by several factors including: the magnitude of genetic

differentiation, intrinsic variability of the population, sample size, spatial replication, and the number and characteristics of the marker loci used (for example, polymorphism, homoplasy, and mutation rates) (Curley and Gillings, 2009). Therefore, it should be acceptable that a different study resulted in a different population genetic structure for *L. polyactis.* Despite this, the complete mtDNA CR analysis of the present study can be particularly informative for inferring historical changes, by reconstructing intraspecific genealogies, and for estimating the population demographic history of *L. polyactis*.

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