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Molecular Phylogenetics and Evolution 39 (2006) 799–811
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Differential population structuring and demographic history of two closely related fish species, Japanese sea bass (*Lateolabrax japonicus*) and spotted sea bass (*Lateolabrax maculatus*) in Northwestern Pacific

Jin-Xian Liu [a,b,c,1], Tian-Xiang Gao [b,1], Koji Yokogawa [d], Ya-Ping Zhang [a,c,*]

[a] *Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China*
[b] *The Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao, Shandong 266003, China*
[c] *Laboratory for Conservation and Utilization of Bio-resource, Yunnan University, Kunming, Yunnan 650091, China*
[d] *13-5 Higashihama, Tadotsu-cho, Nakatado-gun Kagawa Pref., 764-0016, Japan*

Received 28 September 2005; revised 23 December 2005; accepted 6 January 2006
Available online 28 February 2006

Abstract

The Quaternary cold periods in the Northwestern Pacific are thought to have heavily influenced the amount and distribution of intraspecific genetic variation in marine fishes. To estimate the demographic history and genetic structure of *Lateolabrax maculatus* and *L. japonicus* in the Northwestern Pacific, 256 individuals were sampled from 19 localities throughout the distribution range of the two species. Mitochondrial DNA variation was analyzed using DNA sequence data from the cytochrome *b* gene and control region. Nucleotide diversity was much higher in *L. japonicus* (0.030) than in *L. maculatus* (0.012). The demographic history of the two species was examined using neutrality tests and mismatch distribution analyses and results indicated Pleistocene population expansion in both species. Estimates of population expansion time suggested earlier population expansion in *L. japonicus* than in *L. maculatus*. Molecular variance analyses showed differential genetic structuring for these two closely related species. The results indicated that *L. japonicus* is panmictic throughout its range. In contrast, populations of *L. maculatus* showed statistically significant levels of genetic structuring. Pattern of isolation by distance was observed in *L. maculatus*, suggesting that *L. maculatus* is in genetic equilibrium. In contrast, *L. japonicus* did not exhibit isolation by distance.

Keywords: mtDNA; Population expansion; Population genetics; Isolation by distance; Climatic oscillations

1. Introduction

Due to periodic climatic oscillations over the Pleistocene, range contractions and expansions are thought to have greatly influenced the amount and distribution of intraspecific genetic variation in many species (Avise, 2000; Hewitt, 2000). The late Quaternary period (the past one million years) was characterized by a series of large glacial–interglacial changes (Imbrie et al., 1992). The major climatic oscillations occurred during the past ~800 kyr with a ~100 kyr dominant cycle. During glacial maxima, declines in sea levels of 120–140 m have been noted (Lambeck et al., 2002). Severe climatic shifts can produce great changes in species' geographical distribution and abundance, which can be expected to have genetic consequences (Dynesius and Jansson, 2000; Hewitt, 2000).

More than 75% of the marginal basins in the modern global sea are in the Western Pacific continental margin (Tamaki and Honza, 1991). During late Quaternary glacial cycles, the sea-level-induced environmental signal was amplified in the marginal seas of Western Pacific, giving rise to drastic changes in areas and configurations of these seas (Wang, 1999). The consequence is that the Northwestern

* Corresponding author. Fax: +86 871 519 5430.
E-mail addresses: zhangyp1@263.net.cn, zhangyp@mail.kiz.ac.cn (Y. -P. Zhang).
[1] These authors contributed equally to this work.

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doi:10.1016/j.ympev.2006.01.009

Pacific biota have experienced some dramatic climatic changes during the last few million years, with extensive oscillations during the last 800 kyr. For these reasons the Northwestern Pacific appears to provide one of the best natural settings to study how colonization events, population bottlenecks, long term isolation, and subsequent mixing have affected the lineage structure and geographical differentiation of marine species.

Spotted sea bass, *Lateolabrax maculatus* (M′Clelland) is a newly redescribed species (Kim et al., 2001) and recently distinguished from the Japanese sea bass, *L. japonicus*. It is widely distributed along the Chinese coast, reaching south to the borders of Vietnam, and north to Korea (Yokogawa and Seki, 1995). Compared with *L. maculatus*, *L. japonicus* is more geographically restricted, being distributed in coastal Japan from southern Hokkaido to the southeast coast of the Korean Peninsula (Kim and Jun, 1997; Yokogawa and Seki, 1995). *L. maculatus* is characterized by many clear black dots on the lateral body region. While sea bass from the Ariake Sea, Japan, are known to have similar external features (Katayama, 1965), some meristic characters of sea bass were intermediate between *L. maculatus* and *L. japonicus* (Yokogawa et al., 1997). Genetic features, evaluated by isozyme analysis (Yokogawa et al., 1997) and AFLP analysis of nuclear genome (Nakayama, 2002), indicated that populations from Ariake Sea and Yatsushiro Sea are putative hybrids of the two species. The two species are sympatric in some coastal areas of South Korea (Kim and Jun, 1997). Genetic analysis by isozyme and morphological examination of the Mokpo sample revealed that it was a population mixture of *L. japonicus* and *L. maculatus* (Yokogawa, 2004). Both species are reef-associated fishes, mainly found in moving waters of inshore rocky reefs. Spawning occurs on rocky reefs and juveniles may enter rivers (Sadovy and Cornish, 2000).

As one of the most extensive continental shelves in the Western Pacific, the East China Sea Shelf with a total area of 850,000 km^2 was exposed during the Pleistocene ice ages (Fig. 1). We expect that *L. maculatus*, whose present-day distribution range was almost completely eradicated during the last glacial maximum (LGM) (Fig. 1), should have been most severely impacted by the past glaciations. Under the most severe environmental conditions in glacial periods, *L. maculatus* might have become extinct over large parts of its range and survived in glacial refugium, which was likely to have been located in the basin of East China Sea. Population expansions from glacial refugium are expected in *L. maculatus* when more favorable conditions returned during interglacials and lower genetic diversities are expected in the postglacially colonized regions (Hewitt, 1996). However, changes in area and configuration in the Japan Sea and the Pacific side of the Japanese archipelago was mild compared with that in the East China Sea (Fig. 1), which would maintain a larger effective population size and retain much more genetic diversity in *L. japonicus* (Hewitt, 2000).

Marine organisms generally show low levels of genetic differentiation over large geographic distances (Avise, 2000; Grant and Bowen, 1998; Palumbi, 1994). Higher dispersal potential during planktonic egg, larval, or adult history stages coupled with the absence of physical barriers to movement seem to greatly facilitate extensive gene flow among populations of marine organisms (Grant and Bowen, 1998; Hewitt, 2000). However, a growing number of genetic surveys have found evidence of restricted dispersal

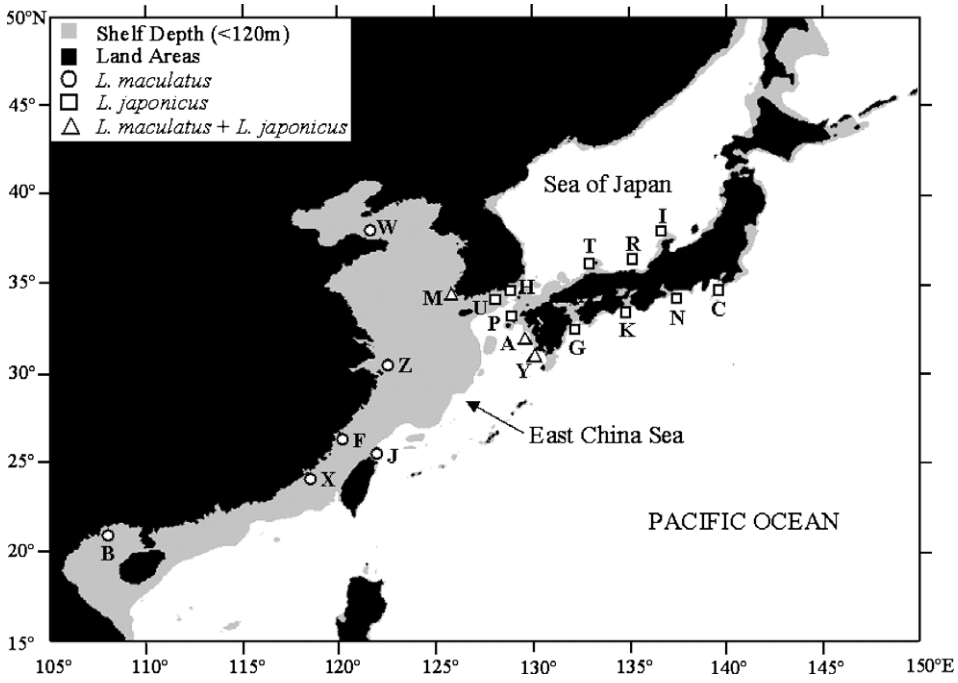

Fig. 1. Map showing sample locations of *L. maculatus* and *L. japonicus*. Details of the sampling locations are described in Tables 1 and 2. Shaded sea areas are continental shelves that would have been exposed to the air during periods of low sea level.

in marine organisms with planktonic larvae (Barber et al., 2000; Miya and Nishida, 1997; Roman and Palumbi, 2004; Wirth and Bernatchez, 2001). An accurate definition of population structure is important for the management of commercial marine fishes (Utter, 1991). Failure to detect population units can lead to local overfishing and ultimately to severe declines (Waples, 1998).

The amount and pattern of polymorphism in DNA sequences are informative to infer the history of a population as well as the mechanism responsible for generating and maintaining the polymorphism (Li, 1997). In the present study, populations of *L. maculatus* and *L. japonicus* were collected throughout their natural range to investigate the evolutionary history of the two species. Haplotype sequences for the mitochondrial (mt) DNA noncoding control region and the cytochrome *b* gene were surveyed. The population history of *L. japonicus* and *L. maculatus* were inferred and results can illuminate how these species have responded to the severe climatic oscillations in Pleistocene ice ages. These data were also used to characterize population structuring of both commercially important species throughout their ranges. Such information may assist the fisheries management of the two species. Mitochondrial DNA polymorphism was also analyzed for samples from Ariake Sea and Yatsushiro Sea to test whether haplotypes of both species existed in these putative hybridized samples or not.

2. Materials and methods

2.1. Sample collection

Two hundred and fifty-six specimens were collected from 19 geographic locations throughout the distribution area of the two species from 1992 to 2003 (Fig. 1). Taxonomic status of the fishes was defined morphologically except those from Ariake Sea and Yatsushiro Sea. Both morphological species were found in Mokpo. Muscle or fin clips samples were obtained and preserved in 95% ethanol or frozen for DNA extraction. Two individuals of the congener *Lateolabrax latus* were also collected to serve as outgroup for phylogenetic analyses.

2.2. DNA extraction, amplification, and sequencing

Genomic DNA was isolated from muscle tissue and fin clips by proteinase K digestion followed by a standard phenol–chloroform method. Fish primers L16528 (5′-TCA CCC CTG GCT CCC AAA GCC AG-3′) and H427 (5′-TGC ATA TAA AAG AAT GCY CGG CAT G-3′), which target a portion of transfer RNA (tRNA)-pro and the central conserved region of the control region (Kong et al., 2003) were used in a polymerase chain reaction (PCR) to amplify the first hypervariable region. The 5′-end of the mitochondrial cytochrome *b* gene was amplified for a subset of the samples with the primers L15312 (5′-ATG GCA ARC CTA CGA AAA AC-3′) and H15682 (5′-TGT CCT CAT GGA AGG ACR TA-3′) (Gao et al., 2001). All individuals analyzed for cytochrome *b* were selected based on the topology of the control region sequences to detect whether it was suitable for population level analysis or not.

PCR were carried out with 1.25 U *Taq* DNA polymerase (TaKaRa, Dalian) in 50 μl volumes. The final concentrations were 200 nM forward and reverse primers, 200 μM of each dNTP, 10 mM Tris, pH 8.3, 50 mM KCl, and 1.5 mM $MgCl_2$. All sets of PCR included one negative control reaction to check for contamination. PCR products were purified with Gel Extraction Mini Kit (Watson BioTechnologies, Shanghai). Both strands were sequenced using the BigDye Terminator Cycle Sequencing Kit (ver.2.0, PE Biosystems, Foster City, California) and run on an ABI Prism 377 (Applied Biosystems) automatic sequencer according to the manufacturer's recommendations. All sequences were deposited in GenBank under Accession Nos. AY820976–AY821307.

2.3. Sequence alignment and data analyses

Sequences were aligned and edited using Dnastar software (DNASTAR, Madison, USA). The net average distance between two species given by $dA = dXY - (dX + dY)/2$ where dXY is the average distance between species X and Y, and dX and dY are the mean within-species distances, was calculated with MEGA2.0 (Kumar et al., 2001). Population genetic statistics were estimated using the program ARLEQUIN (ver. 2.000; Schneider et al., 2000). The level of polymorphism for each population was estimated as the number of polymorphic sites (S), haplotype diversity (h; Nei, 1987), nucleotide diversity (π; Nei, 1987), and the average number of pairwise nucleotide differences (k; Tajima, 1983). Nucleotide sequence evolution models were evaluated using likelihood-ratio tests implemented by Modeltest v.3.06 (Posada and Crandall, 1998).

An analysis of molecular variation (AMOVA; Excoffier et al., 1992) was used to test for significant population structure within species. Both AMOVAs were performed in ARLEQUIN and the haplotypes were permuted 1000 times. Genetic distances between haplotypes were corrected for multiple hits by using Tamura and Nei model of nucleotide substitution (Tamura and Nei, 1993) with a gamma correction for heterogeneity of mutation rates (models given by Modeltest for the two species (the Tamura and Nei model with gamma shape parameter and invariant sites, TrN + I + Γ) are not available in ARLEQUIN). A one-factor AMOVA was employed to assess the degree of population structure over all populations. F_{ST} genetic distances between populations were calculated considering the Tamura and Nei model assuming a gamma shape parameter equal to the ones given by Modeltest. To test for isolation by distance (Slatkin, 1993; Wright, 1943), pairwise values of $F_{ST}/(1 - F_{ST})$ (Rousset, 1997) were plotted against geographical distance (one-dimensional stepping-stone model) between sample sites of *L. maculatus*. Considering the two-dimensional habitats of *L. japonicus* (along the

coast of Japan as well across the coast of Korea), pairwise values of $F_{ST}/(1-F_{ST})$ were plotted against logarithm of distance (two-dimensional stepping-stone model) (Rousset, 1997). Under the stepping-stone model, genetic differentiation can build up between distant populations even when adjacent populations remain indistinguishable due to high pairwise gene flow. In such cases, there can be a positive relationship between geographic and genetic distance (Palumbi, 2003). The strength and significance of the relationship between genetic distances and geographic distances was assessed using reduced major axis (RMA) regression and Mantel tests using IBDWS (Isolation by distance web service at http://phage.sdsu.edu/~jensen/) (Bohonak, 2002; Jensen et al., 2005). Estimations of F_{ST} genetic distances were calculated using the software ARLEQUIN.

Genetic relationships among haplotypes of the two species for both cytochrome *b* and control region data were reconstructed using the neighbour-joining method (Saitou and Nei, 1987) implemented in PAUP* (Swofford, 2002). The congener, *L. latus* was used as outgroup for both datasets. Genetic distances were generated for phylogenetic reconstruction using models of substitution suggested by Modeltest. The Modeltest revealed that the model that best fit the control region data was Tamura and Nei model with gamma shape parameter (TrN + Γ, Γ = 0.24). For cytochrome *b* data, the best model suggested was HKY85 model (Hasegawa et al., 1985) with gamma shape parameter (HKY + Γ, Γ = 0.08). We used bootstrap analysis with 1000 replicates to evaluate support for phylogenetic relationships (Felsenstein, 1985). Relationships of intraspecific haplotypes within each species were assessed by minimum spanning trees created via the MINSPNET algorithm as employed in ARLEQUIN and drawn by hand. Minimum spanning trees of haplotypes were constructed under haplotype pairwise differences, showing the number of mutation steps between haplotypes.

The historical demographic expansions were examined by two different approaches. First the *D* test of Tajima (1989a) and F_S test of Fu (1997) were used to test if the neutrality holds. Significant negative *D* and F_S statistics can be interpreted as signatures of population expansion. Historic demographic expansions were also investigated by examination of frequency distributions of pairwise differences between sequences (mismatch distribution), which is based on three parameters: θ_0, θ_1 (θ before and after the population growth) and τ (time since expansion expressed in units of mutational time (Rogers and Harpending, 1992)). The distribution is usually multimodal in samples drawn from populations at demographic equilibrium, but it is usually unimodal in populations following a recent population demographic expansion and population range expansion (Excoffier, 2004; Ray et al., 2003; Rogers and Harpending, 1992; Slatkin and Hudson, 1991). The concordance of the observed with the expected distribution under the sudden expansion model of Rogers (1995) was tested by means of a least squares approach (Schneider and Excoffier, 1999). Both mismatch analysis and neutrality tests were performed in ARLEQUIN. For distribution that did not differ significantly ($P > 0.05$) from the expectation of the sudden expansion model, the parameter of the demographic expansion τ was estimated by a generalized nonlinear least-square approach, and confidence intervals was computed using a parametric bootstrap approach (Schneider and Excoffier, 1999). The values of τ were transformed to estimates of real time since expansion with the equation $\tau = 2ut$, where u is the mutation rate for the whole sequence under study and t is the time measured in years since expansion.

An appropriate nucleotide substitution rate had not been calibrated for this lineage. Generally, a 2%/MY divergence rate has been calibrated for the cytochrome *b* locus in multiple bony fishes (Bermingham et al., 1997; Bowen et al., 2001). Taking for valid the divergence time estimated with the cytochrome *b* clock (3.9 MY, see Section 3), an approximation of 6%/MY divergence for control region was retro-calculated based on the genetic distance between the two species (0.226, see Section 3).

3. Results

3.1. Divergence between the two species

Evidence from both cytochrome *b* gene and the control region indicated the existence of two clearly distinguishable clades that were sympatric within some coastal areas of South Korea and Kyushu of Japan (Figs. 2 and 3). Of the 256 individuals, 105 belonged to *L. maculatus* and 151 belonged to *L. japonicus*. Average mean nucleotide distances between the two species were 0.226 for the control region and 0.078 for cytochrome *b* gene. No haplotypes were shared between morphological species. Haplotypes of both species were detected in Ariake Sea and Yatsushiro Sea, consistent with the results of previous studies based on nuclear loci (Nakayama, 2002; Yokogawa et al., 1997). Haplotypes from these two samples were analyzed together with those of the two species. The divergence time between the two species based on the cytochrome *b* and control region mutation rate was about 3.9 MY, indicating middle Pliocene divergence between the two species.

3.2. Intraspecific sequence variation

The analyzed section of the control region comprised 457 nucleotides in *L. maculatus* and 455 nucleotides in *L. japonicus*, with the first nucleotide in the sea bass transfer RNA (tRNA)-pro corresponding to nucleotide position 16551 in the *Cyprinus carpio* mitochondrial genome (Chang et al., 1994). In *L. maculatus*, there were 60 polymorphic sites (44 parsimony informative) within the fragment, which defined 63 substitutions: 56 transitions and 7 transversions. No indels were found. Sixty-eight haplotypes were identified among 105 individuals belonging to *L. maculatus*, 12 of which were shared among populations. In *L. japonicus*,

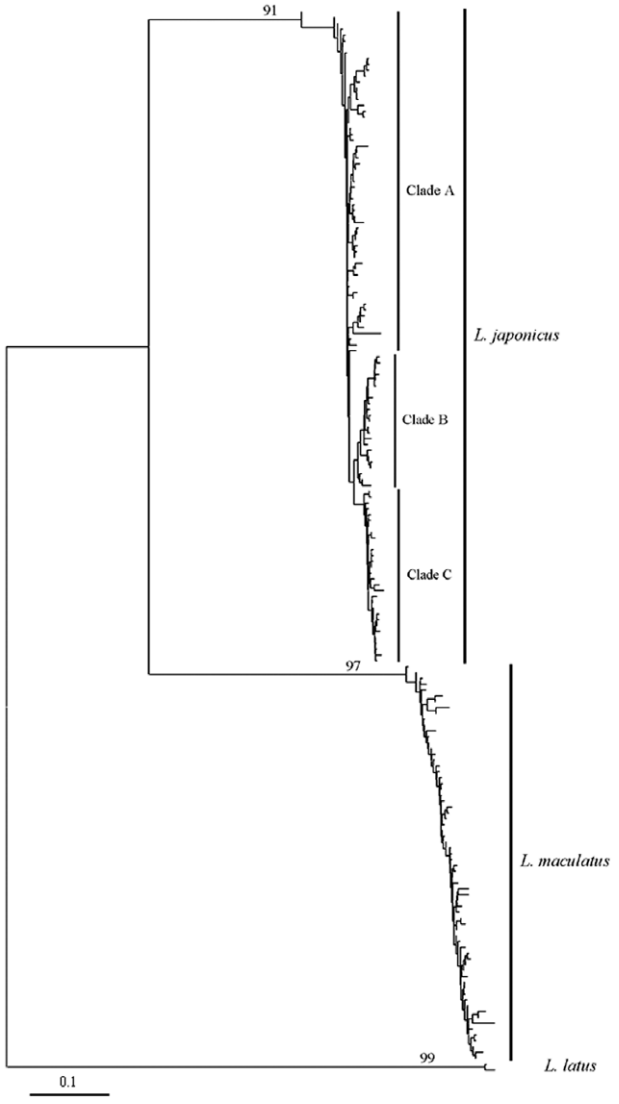

Fig. 2. Neighbor-joining tree constructed using Tamura and Nei distances with a gamma shape parameter of 0.24 for 180 control region haplotypes of *L. maculatus* and *L. japonicus*. The congener *L. latus* was chosen as outgroup. Bootstrap supports of >90% in 1000 replicates are shown. Bootstrap values were 54 and 76% for clade B and clade C, respectively.

there were 107 polymorphic sites (73 parsimony informative) defined by 119 substitutions: 100 transitions and 19 transversions, and 4 indels were found. One hundred and twelve haplotypes were identified among 151 individuals of *L. japonicus*, 14 of which were shared among populations. There were more haplotypes than variable sites in both species, which indicated homoplasy, a common outcome in control region surveys. The intraspecific divergence was 0.012 and 0.030 for *L. maculatus* and *L. japonicus*, respectively. The gamma distribution shape parameter was 1.08 for *L. maculatus* and 0.74 for *L. japonicus*.

Partial sequence of cytochrome *b* gene was determined for 21 individuals of *L. maculatus* and 51 of *L. japonicus* with the first base corresponding to position 15313 in the (*Cyprinus carpio*) mitochondrial genome. Only three polymorphic sites were observed which defined four haplotypes in *L. maculatus*. All the substitutions were synonymous substitutions and were in third codon positions. In *L. japonicus*, nine polymorphic sites were found, which result in 10 haplotypes. All the substitutions were synonymous substitutions. Most of the substitutions (eight) were in third codon positions and one in the first codon positions. The intraspecific polymorphism was low for cytochrome *b* and was only used in the phylogenetic analysis of the two species. Nucleotide diversities (π) for both the control region and cytochrome *b* gene were much higher in *L. japonicus* than in *L. maculatus*.

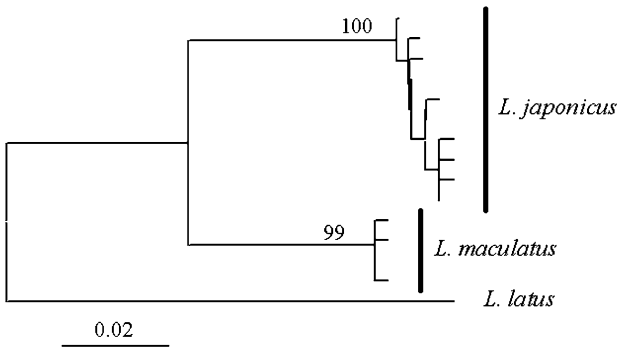

Fig. 3. Neighbor-joining tree for cytochrome *b* haplotypes of *L. maculatus* and *L. japonicus*. The tree was constructed under HKY + Γ model (Γ = 0.08) using *L. latus* as outgroup. Bootstrap supports of >90% in 1000 replicates are shown.

3.3. Genetic relationships

The NJ tree constructed with 180 control region haplotypes of both species showed that the 112 haplotypes of *L. japonicus* were assigned into three closely related groups (clades A, B, and C) (Fig. 2). The clades A, B, and C included 59, 23, and 30 haplotypes comprising 77, 26, and 48 individuals, respectively. However, the genealogical structure among the 68 haplotypes in *L. maculatus* was shallow. As expected from sequence variability and the NJ tree, minimum spanning tree appeared much more complex in *L. japonicus* than in *L. maculatus* (Figs. 4A and B). In *L. japonicus*, the minimum spanning tree also identified three clades, corresponding to those defined in the NJ tree. However, these clades did not appear to have geographic structure (Fig. 4B). Two subclades separated by three mutation steps were revealed in clade C. The structures of clade A and clade B were much more variable compared to clade C. The structure of minimum spanning tree for *L. maculatus* was simple and no clustering that corresponded to sampling localities was detected (Fig. 4A).

3.4. Inter- and intrapopulation variation

All the samples of the two species showed high haplotype diversity and the minimal haplotype diversity was 0.96 (Mokpo). Nucleotide diversities varied in different

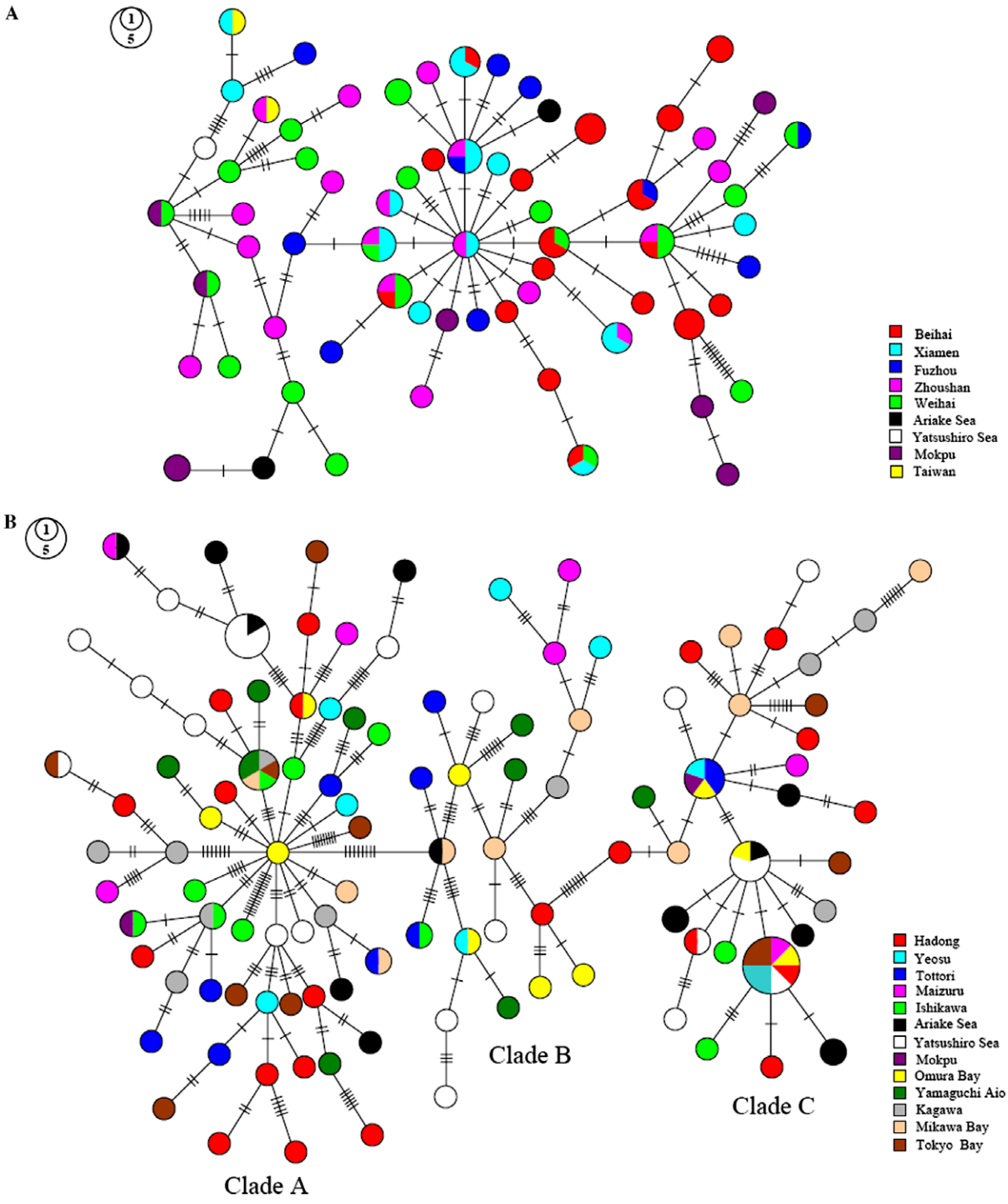

Fig. 4. Unrooted minimum spanning trees showing genetic relationship among control region haplotypes for *L. maculatus* (A) and *L. japonicus* (B). The sizes of circles are proportional to haplotype frequency. Perpendicular tick marks on the lines joining haplotypes represent the number of nucleotide substitutions.

populations of *L. maculatus*, ranging from 0.008 (Beihai) to 0.015 (Weihai). In general, the northern populations of *L. maculatus* showed higher nucleotide diversity than southern populations. Nucleotide diversities in populations of *L. japonicus* were higher than those of *L. maculatus*, ranging from 0.026 (Omura Bay) to 0.035 (Maizuru) (Tables 1 and 2).

The genetic structures of the populations of the two species were investigated by AMOVA. In *L. maculatus*, 93.72% of the genetic variation was found within populations, whereas 6.28% of the variation ($P=0.00$) was among populations. Most of the variance (99.12%) was found within population and a small amount (0.88%, $P=0.23$) among populations in *L. japonicus*, indicating that no population structure existed in *L. japonicus*. Among sites for *L. maculatus*, a Mantel test indicated a significant relationship ($P=0.007$) between $F_{ST}/(1-F_{ST})$ and geographic distance among six samples

Table 1
Sampling data of *Lateolabrax maculatus* including sample abbreviation (ID), sample name, sample size, site (country), and date of collection

ID	Sample name	Country	Date of collection	Sample size		No. of haplotypes	*h*	π	*S*	*k*
				Cytb	CR					
B	Beihai	China	Nov. 1999	4	25	17	0.97 ± 0.05	0.008 ± 0.005	18	3.63 ± 1.90
X	Xiamen	China	Nov. 1999	2	16	12	0.97 ± 0.03	0.009 ± 0.006	20	4.31 ± 2.25
F	Fuzhou	China	Nov. 1999		10	10	1.00 ± 0.04	0.012 ± 0.007	21	5.80 ± 3.03
Z	Zhoushan	China	Nov. 2001	5	19	19	1.00 ± 0.02	0.012 ± 0.007	32	5.70 ± 2.86
W	Weihai	China	Nov. 2001	5	22	19	0.99 ± 0.02	0.015 ± 0.008	36	6.64 ± 3.26
M	Mokpo	Korea	Sep. 2002	1	8	7	0.96 ± 0.08	0.012 ± 0.007	14	5.43 ± 2.93
J	Taiwan	China	Nov. 2003	1	2	2				
A	Ariake Sea	Japan	18–26 May 1993	2	2	2				
Y	Yatsushiro Sea	Japan	14 Sep. 1995	1	1	1				

Several diversity indices for control gene were also indicated.

Table 2
Sampling data of *Lateolabrax japonicus* including sample abbreviation (ID), sample name, sample size, site (country), and date of collection

ID	Sample name	Country	Date of collection	Sample size		No. of haplotypes	*h*	π	*S*	*k*
				Cytb	CR					
I	Ishikawa	Japan	26 Aug.–2 Sep. 1995	3	10	10	1.00 ± 0.04	0.030 ± 0.017	48	13.84 ± 6.80
R	Maizuru	Japan	14 May 2000	5	10	10	1.00 ± 0.04	0.035 ± 0.019	46	15.94 ± 7.78
T	Tottori	Japan	17–25 Sep. 1994	4	10	9	0.98 ± 0.05	0.027 ± 0.015	41	12.37 ± 6.11
U	Yeosu	Korea	Sep. 2002		9	8	0.97 ± 0.06	0.034 ± 0.019	42	13.37 ± 6.26
H	Hadong	Korea	Sep.1994	6	21	21	1.00 ± 0.01	0.029 ± 0.015	51	15.26 ± 7.54
P	Omura Bay	Japan	13 Oct. 1999	2	10	10	1.00 ± 0.04	0.026 ± 0.015	35	11.78 ± 5.83
A	Ariake Sea	Japan	18–26 May 1993	3	14	12	0.98 ± 0.04	0.033 ± 0.017	42	15.76 ± 7.49
Y	Yatsushiro Sea	Japan	14 Sep. 1995	6	25	19	0.96 ± 0.03	0.033 ± 0.017	55	14.85 ± 6.88
G	Yamaguchi Aio	Japan	6–19 Dec. 1993	6	10	9	0.98 ± 0.05	0.028 ± 0.016	41	12.88 ± 6.35
K	Kagawa	Japan	14 Dec. 1996	4	10	10	1.00 ± 0.04	0.030 ± 0.017	38	13.56 ± 6.66
N	Mikawa Bay	Japan	25 Aug. 1995	7	10	10	1.00 ± 0.04	0.026 ± 0.015	35	11.80 ± 5.84
C	Tokyo Bay	Japan	Apr. 1995	4	10	9	0.98 ± 0.05	0.030 ± 0.017	41	14.14 ± 6.94
M	Mokpo	Korea	Sep. 2002	1	2	2				

Several diversity indices for control gene were also indicated.

(Fig. 5A) indicating isolation by distance, with geographic distance explaining 70% of the variation in genetic differentiation for *L. maculatus* ($r = 0.84$). However, there was no evidence of isolation by distance in *L. japonicus* (Fig. 5B). The matrix correlation explained very little of the variation ($r = 0.14$) and was not statistically significant ($P = 0.16$).

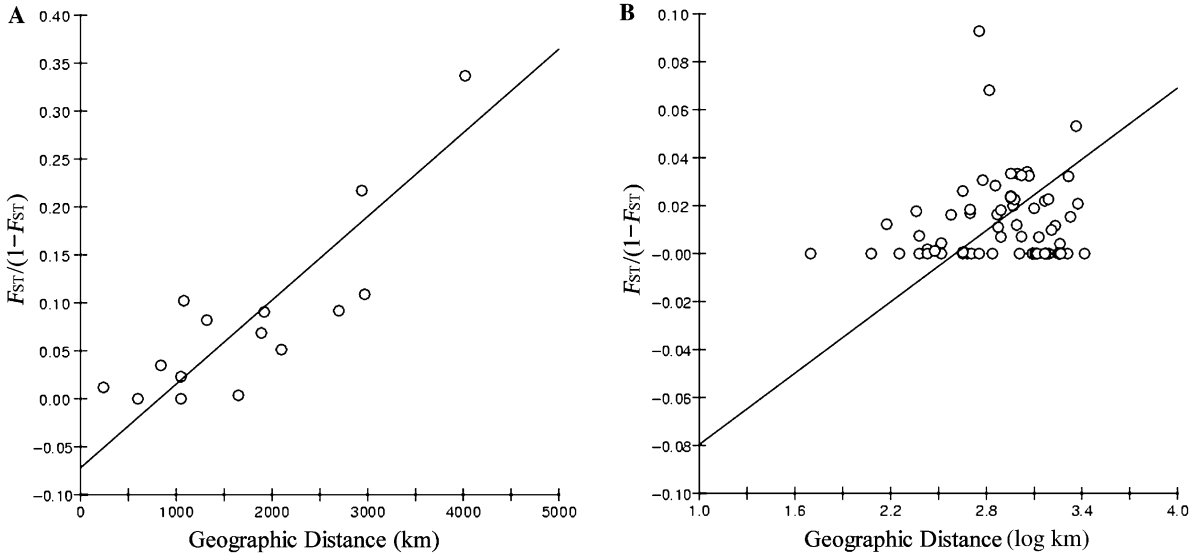

Fig. 5. Plot of pairwise estimates of $F_{ST}/(1 - F_{ST})$ vs. geographic distance between samples of (A) *L. maculatus* and log geographic distance between samples of (B) *L. japonicus*. The RMA regression line overlays the scatter plots. The regressions are $y = 8.77\ 10^{-5} \times -0.072$ for *L. maculatus* and $y = 0.049 \times -0.012$ for *L. japonicus*.

3.5. Historic demography

The mismatch distribution was unimodal for *L. maculatus* (Fig. 6) and was characteristic of populations that have undergone large-scale expansion. The mismatch distribution of all *L. japonicus* sequences was bimodal (Fig. 6), one small mode corresponded to within clade comparisons and the other to differences between individuals among clades. Three clades were detected in *L. japonicus* and these clades did not appear to have geographic structure, which might be the signature of admixture following past divisions. This genealogical structure of *L. japonicus* drives much older τ in mismatch distribution including all samples (Table 3). To obtain more precise estimates, the neutrality tests and mismatch distribution analysis were performed for each clade in *L. japonicus*. Although the mismatch distributions for the three clades in *L. japonicus* did not fit the expected distributions under sudden expansion model nicely (for example, it was bimodal for clade C) (Fig. 6), they did not differ significantly ($P > 0.05$) from the sudden expansion model and therefore were suitable for analyses of demographic patterns. The F_S test and D test agreed well with the mismatch analyses. The F_S tests of *L. maculatus* and the three clades of *L. japonicus* were negative and highly significant ($P = 0.00$) (Table 3), which indicated population demographic expansion for the two species. Tajima's D test was negative (–1.66) and statistically significant for *L. maculatus* ($P = 0.02$). For the three clades of *L. japonicus*, the D statistics were negative and significant for clade C ($P = 0.04$), nearly significant for clade A ($P = 0.07$), but insignificant

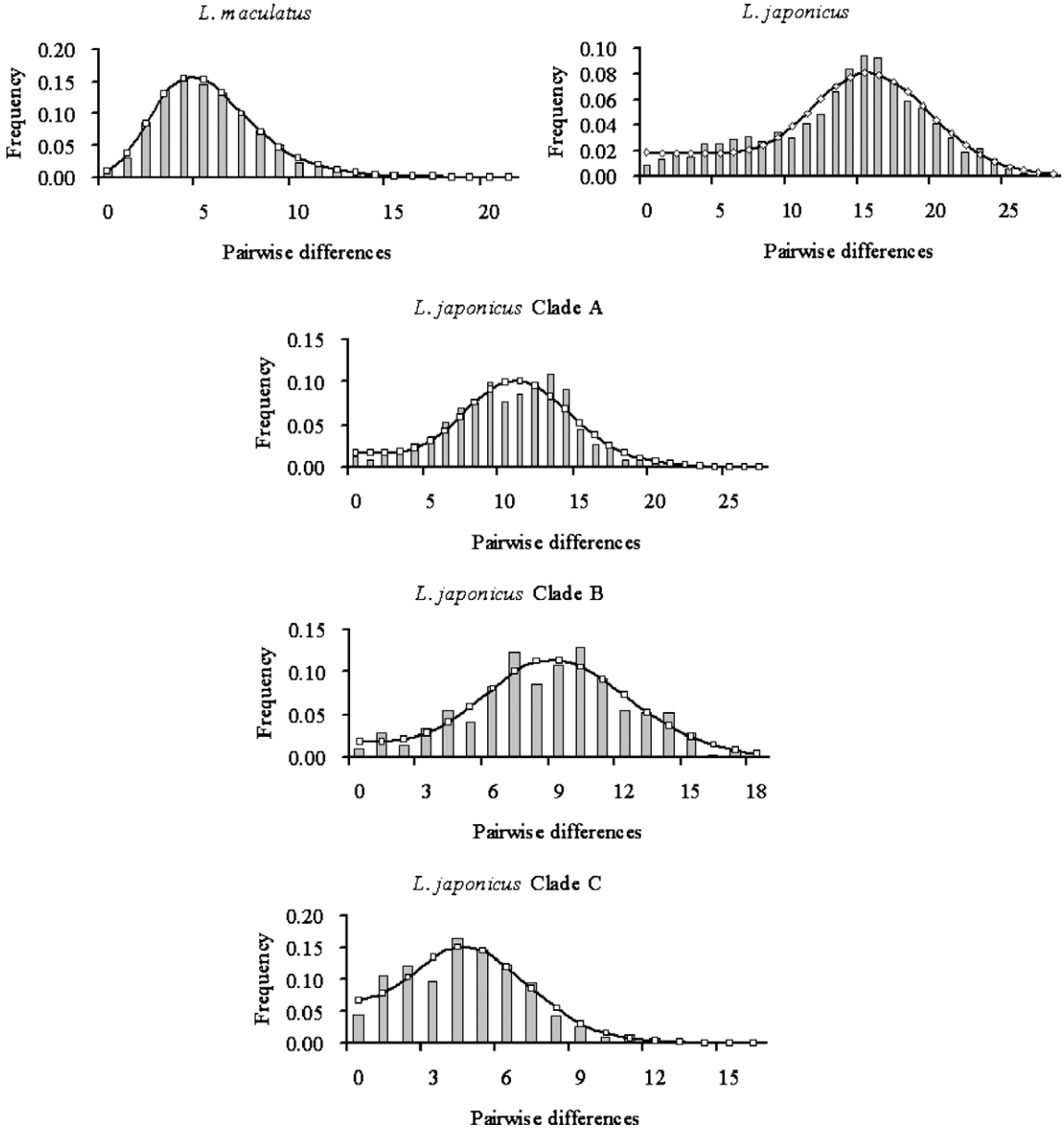

Fig. 6. The observed pairwise difference (bars), and the expected mismatch distributions under the sudden expansion model (solid line) of control region haplotypes in *L. maculatus* and *L. japonicus*.

Table 3
Tajima's D and Fu's F_S, corresponding P-value, and mismatch distribution parameter estimates

Species	Tajima's D		Fu's F_S		Mismatch distribution		
	D	P	F_S	P	τ (95% CI)	θ_0	θ_1
L. maculatus	−1.66	0.02	−25.32	0.00	3.87 (2.53, 6.11)	1.52	3121.25
L. japonicus	−0.90	0.19	−24.03	0.00	16.08 (10.58, 19.86)	0.00	52.74
L. japonicus Clade A	−1.30	0.07	−24.54	0.00	10.64 (7.06, 14.51)	0.85	54.79
L. japonicus Clade B	−0.72	0.26	−13.14	0.00	8.68 (5.41, 12.57)	0.80	53.07
L. japonicus Clade C	−1.50	0.04	−21.91	0.00	5.10 (2.37, 7.83)	0.08	14.64

for clade B ($P = 0.26$) (Table 3). Tajima's D test compares two estimators of the mutation parameter θ, Watterson's estimator θ_S and Tajima's estimator $\theta\pi$ (Tajima, 1989a). As S is more dependent on the present-day population size and π is more strongly influenced by the size of the original population, a history of population growth inflates S relative to π and would generate a negative value of Tajima's D (Fay and Wu, 1999; Tajima, 1989b).

The tau value (τ), which reflects the location of the mismatch distribution crest, provides a rough estimate of the time when rapid population expansion started. The observed value of the age expansion parameter (τ) was 3.87 (95% CI: 2.52–6.07) units of mutational time in *L. maculatus*. The τ values of clade A and clade B in *L. japonicus* were much larger than those of clade C and *L. maculatus* (Table 3). The estimate of time of expansion for *L. maculatus*, based on the rates mentioned above for control region, was ~141 kyr ago (95% CI between 92 and 221 kyr). For clade A of *L. japonicus*, this estimate was ~390 kyr ago (95% CI between 259 and 532 kyr), and for clade B, it was ~318 kyr ago (95% CI between 198 and 460 kyr). In contrast, the estimate for clade C was ~187 kyr ago (95% CI between 87 and 287 kyr). Estimated effective female population size after expansion (θ_1) was ~2050 times higher than before expansion (θ_0) for *L. maculatus*. This estimate (θ_1/θ_0) was 64, 66, and 183 for clades A, B, and C of *L. japonicus*, respectively.

4. Discussion

The mtDNA survey indicates middle Pliocene divergence between the two sea bass species and Pleistocene population expansion for both species. Before dissecting these results, it is appropriate to address the caveats of molecular clock. A molecular clock for the control region has not been determined with precision for marine fishes and seems to vary among major taxonomic groups. In some bony fishes, the control region seems to mutate at about the same rate as protein-coding mtDNA regions. This has been demonstrated for some groups of fishes including salmonids (Shedlock et al., 1992), East African cichlids (Sato et al., 2003; 2.2–4.5% divergence per million years), Australian rainbow fishes (Zhu et al., 1994; 3%/MY), and snooks (Donaldson and Wilson, 1999; 3.6%/MY). However, evolution of the control region seems to be much faster in other bony fishes including Lake Malawi cichlids (Sturmbauer et al., 2001; 6.5–8.8%/MY), Arctic charr (Brunner et al., 2001; 5–10%/MY), and butterfly fishes (McMillan and Palumbi, 1997) with as much as 33–100%/MY at selected hotspots. Occasionally, pronounced rate heterogeneity is reported even among closely related lineages. Large variance might exist in the divergence rate used in the present study (6%/MY for control region and 2%/MY for cytochrome *b*). However, a molecular clock-based time estimate provides an approximate time frame for evaluating phylogeographic hypotheses. Overall, we regard time estimates as approximations on the scale of geological eras.

4.1. Comparative phylogeography in the Northwestern Pacific

The cytochrome *b* and control region data suggests a divergence time of 3.9 MY between the two species, indicating isolation in the middle Pliocene. The dating of divergence coincides with geological events that may have created a vicariant barrier between populations of the Japan Sea and the East China Sea. During middle Pliocene, the Japan Sea was a semi-closed area, being strongly influenced by a cold northern surface water until 3.5 MY ago when warm surface water first reappeared corresponding to the reopening of the southern channel (Kitamura et al., 2001; Tada, 1994; Yamamoto, 1993), with concurrently a huge enclosed sea existing in the region of the present East China Sea (Kimura, 1996; Kizaki and Oshiro, 1977). These processes may separate populations and create conditions favorable for allopatric speciation. Around the target area of the present study, two species of mitten crab *Eriocheir* show similar phylogeographic pattern to that of the two *Lateolabrax* species. *Eriocheir japonica* is distributed around the Japanese archipelago, eastern and southern coasts of the Korean Peninsula, while *Eriocheir chinensis* is distributed in western coast of the Korean Peninsula and throughout the Chinese coasts (Gao and Watanabe, 1998). Similarly, two forms of red ark shell *Scapharca (Andara) broughtonii* show quite similar phylogeographic pattern. Yokogawa (1997) examined morphological and genetic differences between Japanese and Chinese forms of the red ark shell, concluding that they should be taxonomically separated as subspecies or species. Yamashita et al. (2002) investigated shell fauna around South Korean coast, and reported that the two forms of the red ark shell show distributional pattern similar to that of the freshwater crabs and sea basses. Comparative phylogeography describes the

evolution of landscapes and permits analyses of the effects of history and geography on organismal community structure at both local and regional levels (Bermingham and Moritz, 1998). In comparative phylogeography, the most parsimonious explanation for multiple taxonomic groups that exhibit patterns of phylogeographic congruence is that they have a shared biogeographic history (Avise, 2000). Divergence times between the two species (forms) of mitten crab and red ark shell were not estimated in the previous studies (Gao and Watanabe, 1998; Yokogawa, 1997). Levels of isozyme divergence (Nei's *D* distance) between the two species (forms) of the three taxonomic groups vary (0.174 for sea bass, 0.108 for red ark shell, and 0.052 for mitten crab), which suggests that the three taxonomic groups may have diversified at different times in the past. However, among lineage isozyme rate heterogeneity (Nei, 1975; Vawter et al., 1980) could also contribute to the differing amount of isozyme divergence detected in the three taxonomic groups. Therefore, it is difficult to reject a hypothesis of contemporaneous divergence for each of the three taxonomic groups based on the available data. Further studies using data from multiple independent loci in each taxonomic group would be helpful to test whether the divergence for each group were contemporaneous or not.

4.2. Pleistocene ice ages and patterns of genetic diversity

As expected, range contractions and expansions played a central role in shaping the genetic diversity of the two species. Much higher nucleotide diversity was found in *L. japonicus* than in *L. maculatus* (Tables 1 and 2). Changes in area and configuration were much more drastic in the East China Sea than in the Japan Sea and the Pacific side of the Japanese archipelago. During the LGM when the sea level was at its lowest, about 130 m below the present sea level, an extensive area of the continental shelf of the East China Sea was exposed (Fig. 1) (Xu and Oda, 1999). During glacial periods, the displaced populations had to survive along the glacial refugium in a compressed biome, inevitably leading to reduced genetic diversity (Hewitt, 1996, 2000). Similar result was found in coral reef fish species with different habitat preferences (lagoon or outer slope) in French Polynesia, species inhabiting lagoons demonstrated reduced mtDNA diversity compared to species inhabiting stable environments (the outer slope) because lagoons dried out during Holocene sea-level regression (Fauvelot et al., 2003).

Postglacial expansion into new territory was suggested to be important in the geographic distribution of population and species genomes (Hewitt, 2000). If an ancestral population and a derived population are compared, the genetic diversity is expected to be higher in the ancestral population (Savolainen et al., 2002). When haplotype diversity is close to 1, it could not discriminate among different populations and is therefore no longer an informative measure of polymorphism (Li, 1997). This is the case in the present study, so we consider the more appropriate measure of genetic diversity, nucleotide diversity (π). For *L. maculatus*, nucleotide diversity was highest in the northern population of Weihai (0.015) and lowest in the southern peripheral population of Beihai (0.008). In general, northern populations of *L. maculatus* showed higher nucleotide diversities than southern ones (Table 1), which was consistent with the hypotheses that the glacial refugium of *L. maculatus* was located in the basin of East China Sea.

4.3. Population genetic structure and isolation by distance

Marine fishes 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 (Grant and Bowen, 1998; Hewitt, 2000; Palumbi, 1994). According to our results, *L. japonicus* conforms to this pattern whereas *L. maculatus* shows differentiation throughout its range. The differential structuring of the populations of the two closely related species is supported both by the AMOVA test and analyses of isolation by distance. The AMOVA tests showed significant levels of genetic structuring among *L. maculatus* but not in *L. japonicus* populations. Our results on the two species agree with previous findings of genetic structuring based on isozyme data. Xu et al. (2001) examined two samples collected from southern and northern coasts of China by isozyme analyses and concluded that these two samples might represent different populations. Yokogawa et al. (1997) summarized that no genetic structure existed among *L. japonicus* populations based on isozyme analyses. The results have important implications for fisheries management of both species. The lack of structure in *L. japonicus* is consistent with a 'one stock' management policy. In contrast, the finding that *L. maculatus* populations are structured throughout its range points out the need to take into account self-recruitment to avoid local overexploitation and decline.

Marine environments are often seen as open habitats in which isolation by distance is the main mechanism that may promote genetic differentiation (Palumbi, 1994). The plot of $F_{ST}/(1-F_{ST})$ and geographic distance revealed a strong pattern of isolation by distance in *L. maculatus*, indicating that this species is at genetic equilibrium under dispersal and genetic drift (Slatkin, 1993). Simulations of one-dimensional stepping-stone populations with particular larval dispersal regimes shows that isolation by distance is most obvious when comparing populations separated by 2–5 times the mean larval dispersal distance (Palumbi, 2003). Patterns of isolation by distance were also detected in Atlantic cod (*Gadus morhua*) despite weak population structuring (Pogson et al., 2001). Spatial autocorrelation analysis in red drum (*Sciaenops ocellatus*) and black drum (*Pogonias cromis*) from the Gulf of Mexico also suggested a strong isolation-by-distance effect despite very low overall genetic differentiation among localities (Gold et al., 1994). In contrast, *L. japonicus* did not exhibit a pattern of isolation by distance. Absence of isolation by distance results

either when migration is so high that it overcomes the effects of genetic drift, or there has been insufficient time following a recent range expansion and contraction for a balance between migration and drift to be achieved (Slatkin, 1993). Although the potential larval movement of the two species is high (at least 3 weeks of larval duration) (Nakayama et al., 1996; Zhu et al., 2002), the apparent pattern of isolation by distance in *L. maculatus* indicated low levels of migration. So we can be reasonably certain that *L. japonicus* colonized its current habitat recently.

4.4. Population history—crashes and expansions

Both the neutrality tests and the mismatch distribution analysis indicated population expansion in the two species. Estimate of population expansion time indicated an extensive population expansion ~141 kyr ago in *L. maculatus*. Additionally, simulations demonstrated that range expansions may lead to a molecular signature quite similar to that observed after a pure demographic expansion (Ray et al., 2003). Population range expansion must have occurred after the LGM for *L. maculatus*. So both past population demographic expansion and geographical range expansion could explain the observed mitochondrial diversity in *L. maculatus*. Estimate of population expansion time for clade A and clade B in *L. japonicus* was much older than that of *L. maculatus*. However, the estimate for clade C (~187 kyr ago) was similar to that of *L. maculatus*. Pleistocene climatic oscillations produced changes in temperatures, current patterns, upwelling intensity, and the displacement, or eradication of coastal habitats (Bond et al., 1997; Kennett and Ingram, 1995; Kotilainen and Shackleton, 1995; Lambeck et al., 2002; Petit et al., 1999). Deterioration of rocky reef habitats for *L. japonicus* must have occurred when sea levels lowered (120–140 m below present sea level) during glacial maxima, which may be the reason for the population expansion.

The major climatic oscillations occurred during the past ~800 kyr with a dominant ~100 kyr cycle, and associated with glacial maxima were declines in sea levels of 120–140 m (Lambeck et al., 2002). For the uncertainty on the estimates of population expansion time, it is difficult to link population expansion of the two species to any particular Pleistocene paleo-climatic event in the present study. However, it is evident that Pleistocene ice ages had great effect on the demographic history of both species. Similar conclusions have been reached on other marine fishes. Historical population expansions were detected in mackerel *Scomber scombrus* and chub mackerel *Scomber japonicus* (Zardoya et al., 2004), and Pleistocene population expansions were also detected in northern anchovy *Engraulis mordax* and Pacific sardine *Sardinops sagax* (Lecomte et al., 2004).

Acknowledgments

We are grateful to Dr. Bowen B.W. for his insightful comments on the manuscript. The present study could not have been carried out without the willing help of those listed below in collecting sea bass specimens: Prof. Watanabe Seiichi, Mr. Masumi Ozaki, Mr. Minoru Tomiyama, Mr. Hiroshi Kimura, Dr. Tsutomu Tanabe, Mr. Akira Okamoto, Mr. Seiji Matsumura, Mr. Nobuyuki Miyazaki, and Mrs. Sachiko Miyazaki, Mr. Akira Kajikawa, Mr. Kiyomi Mizushima, Mr. Youichi Ohashi, Mr. Takeshi Tajima, Mr. Jeong Haw Bae, Mr. Takashi Nishikawa, Dr. Won-Kyo Lee, Dr. Chung-Bae Kang, and Dr. Yukio Ueta. We are also grateful to Gou S.-K., Zhu C.-L., and Wu S.-F. for technical assistance. Thanks Mr. Nwafili Sylvanus for his checking of writing English. This work was supported by the Bureau of Science and Technology of Yunnan Province, National Natural Science Foundation of China (Nos. 30021004, 39970578), and the Doctoral Foundation of Shandong Province.

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