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Significant population genetic structure of yellowfin seabream *Acanthopagrus latus* in China

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The yellowfin seabream *Acanthopagrus latus* is widely distributed throughout the Indo-West Pacific. The genetic analyses of mtDNA control region sequence variation in samples from Chinese waters revealed a pattern of genetic structure between southern and northern samples ($P < 0{\cdot}05$) and high levels of genetic diversity in this species. Significant isolation by distance between southern and northern locations (Mantel test $r = 0{\cdot}35$, $P = 0{\cdot}04$) and among northern populations ($r = 0{\cdot}55$, $P = 0{\cdot}01$) indicated that ocean straits and other barriers substantially limit gene flow. Phylogeographical analysis also revealed two major divergent mtDNA lineages across Chinese waters. For conservation, *A. latus* populations in Chinese waters should be divided into at least two management units for protection.

Key words: conservation; control region; isolation by distance; marine fish; mtDNA.

INTRODUCTION

The investigation of molecular genetic variation within species has become a common tool for studying patterns of biodiversity and diversification (Roberts, 2006). An understanding of the processes that lead to observed patterns of genetic diversity and genetic structure in a species of interest can provide insights into the mechanisms responsible for divergence and speciation. These insights can guide the management and conservation of at-risk marine populations (Palumbi, 1993; Mathews, 2006).

Yellowfin seabream *Acanthopagrus latus* (Houttuyn) is a commercially and ecologically important species that is widely distributed throughout the Indo-West Pacific, from The Gulf and along the coast of India to the Philippines and from Japan to Australia. Like many other sparids, this fish is a protandrous hermaphrodite and usually inhabits warm shallow and coastal waters, but often entering river mouths and estuaries (Buxton & Garratt, 1990; Li & Ou, 2000). The spawning period of *A. latus* varies markedly between regions (Alex Hesp *et al.*, 2004). For example, *A. latus* spawns in late winter along

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the coast of Guangdong Province, China (Li & Ou, 2000), typically in late winter and early spring in Shark Bay, Western Australia (Alex Hesp *et al.*, 2004), but over a prolonged period from February to April in Kuwait Bay (Abou-Seedo *et al.*, 2003). Gene flow between the geographically distant populations is expected to be limited because fish apparently do not disperse far (Yang *et al.*, 2004*a*). However, no obvious morphological differences arising from this apparent isolation have been reported across its distributional range.

Acanthopagrus latus population sizes have declined in several areas. Over the past 20 years, *A. latus* populations, and those of other sparids in Chinese waters such as *Chrysophrys major* (Temminck & Schlegel) and *Parargyrops edita* Tanaka have been greatly affected by human activities and have rapidly declined from overfishing (Chen & Qin, 2003; Chen *et al.*, 2003; Ye *et al.*, 2004; Xia *et al.*, 2005). *Acanthopagrus latus* populations in Shark Bay, Australia, have also declined, and this decline led the Western Australian Department of Fisheries to reduce the number of commercial licences for this species (Shaw, 2000; Alex Hesp *et al.*, 2004).

Several researchers have used molecular genetic methods to resolve the genetic structure of seabream populations in Chinese waters. Yang *et al.* (2004*a*) suggested that there were distinct genetic differences between populations at Xiamen, Fujian Province ($n = 8$) and Zhujiangkou, Guangdong Province ($n = 8$) with an analysis of random amplified polymorphic DNA (RAPD) data. However, an analysis of mtDNA control region (CR) data indicated that these populations were genetically similar (Liu *et al.*, 2004). A cluster analysis of these data placed Xiamen ($n = 8$) and Zhujiangkou ($n = 8$) in one group and Beibu Bay ($n = 8$) in another (Liu *et al.*, 2004). Recently, an analysis of amplified fragment-length polymorphism (AFLP) variation showed significant frequency differences between pooled samples from Xiamen and Shenzhen of Guangdong Province and a sample from Beibu Bay (Xia *et al.*, 2005). These studies, however, had several shortcomings. For example, sample sizes were small and molecular results were inconsistent. Until the present study, no reliable genetic data for *A. latus* have been available to develop a sound management plan for this sparid.

Genetic variation is one of the three levels of biodiversity that the International Union for the Conservation of Nature (IUCN) has recommended for conservation (WEHAB Working Group, 2002; Reed & Frankham, 2003; Primmer *et al.*, 2006). To overcome the limitations of previous studies and to aid in the development of management strategies aimed at ensuring the sustainable use of this valuable natural resource, the present study included more sample locations and larger sample sizes. Genetic assessment of this species was conducted by surveying the patterns of sequence variation of the mtDNA CR.

MATERIALS AND METHODS

SAMPLING

The study included 169 individuals from eight locations collected from fish markets and included specimens from the northern extreme of the species' range, its southern limit and the middle coast of China (Fig. 1 and Table I). A total of 22 additional sequences used in this study were published in Liu *et al.* (2004) (GenBank accession

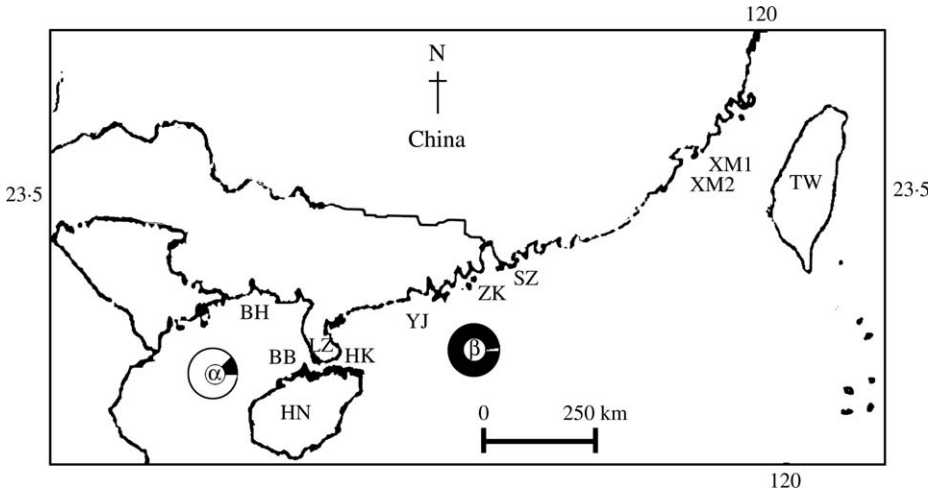

FIG. 1. Geographical locations and phylogeography of the *Acanthopagrus latus* sampled in coastal waters of China. The following abbreviations for sampled locations (Table 1) and additional three locations were used: XM1, Xiamen-1; XM2, Xiamen-2; SZ, Shenzhen; ZK, Zhujiangkou; YJ, Yangjiang; HK, Haikou; BB, Beibu Bay; BH, Beihai; HN, Hainan Island; TW, Taiwan Island; LZ, Leizhou Peninsula. Pie charts depict the relative proportion of southern sample (white semi-circle) and northern sample (black semi-circle) in two mtDNA groups: alpha (α) and beta (β) identified in haplotype phylogeny.

numbers AY549503–AY549524). Specimens were identified according to descriptions in Meng *et al.* (1997).

DNA EXTRACTION, CR AMPLIFICATION AND SEQUENCING

Muscle tissue was preserved in 75% alcohol, stored in 20% dimethyl sulphoxide with saturated salt solution or frozen at −20° C until DNA extraction. Tissue was dissolved in a solution of sodium dodecyl sulphate/proteinase K, and total genomic DNA was isolated with standard phenol–chloroform extraction and ethanol precipitation (Sambrook *et al.*, 1989) or with Qiagen DNeasy tissue kits following the manufacturer's protocol (Qiagen, Valencia, CA, U.S.A).

TABLE I. Sample locality, sample size (n) and mtDNA nucleotide (π) and haplotype (h) diversities in *Acanthopagrus latus*

Locality	n	h	π	Reference	Grouping
Xiamen-1	29	0·995	0·025	This study	Northern sample
Xiamen-2	8	0·952	0·015	Liu *et al.* (2004)	
Shenzhen	40	0·951	0·023	This study	
Zhujiangkou	8	0·964	0·013	Liu *et al.* (2004)	
Yangjiang	20	0·989	0·026	This study	
Haikou	34	0·884	0·024	This study	
Beibu Bay	8	0·972	0·017	Liu *et al.* (2004)	Southern sample
Beihai	22	0·974	0·023	This study	

A portion of the CR was amplified with the polymerase chain reaction (PCR) using primers 16086F: 5′ TTA GTA TGG TGA CAA TGC AT 3′ and 16621R: 5′GAC ACC ATT AAC TTA TGC AA 3′ (Liu *et al.*, 2004). PCR amplifications were carried out on a gradient thermal cycler (Biometra, Göttingen, Germany) in 50 μl aliquots of a mixture containing 100 ng genomic DNA, 1× Pfu PCR buffer, 240 μmol dNTPs, 0·2 μmol of each primer and 2 U Pfu DNA polymerase according to the manufacturer's instructions (Tianwei, Beijing, China) with the following cycling profile: one denaturation step for 3 min at 94° C was followed by 35 cycles of 30 s at 94° C, 30 s at 52° C and 1 min at 72° C. The final extension lasted 5 min at 72° C. A 5 μl sample of each PCR product was electrophoresed in 1% agarose gel and stained with ethidium bromide for visualization. PCR products were sequenced directly using an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.) after purification using V-gene PCR clean up kit (V-Gene, Hangzhou, China). Sequences were aligned in ClustalX (Thompson *et al.*, 1997) and Seaview (Galtier *et al.*, 1996). Sequences were deposited in GenBank under accession numbers EF506765–EF506875.

PHYLOGENETIC AND SEQUENCE DIVERSITY ANALYSIS

Phylogenetic analyses included 111 unique haplotypes (289 bp) defined with the ARLEQUIN 3.1 (Schneider *et al.*, 2000; http://cmpg.unibe.ch/software/arlequin3) in 169 individuals and one outgroup sequence (Gong *et al.*, 2006) from the close relative *Acanthopagrus schlegelii* (Bleeker). A neighbour-joining (NJ) tree (Saitou & Nei, 1987) of haplotypes was constructed with MEGA 3.1 (Kumar *et al.*, 2004) with Kimura's two-parameter genetic distance. One thousand bootstrap replicates were used to assess the statistical support for nodes in the tree. Estimates of nucleotide diversity, haplotype diversity, D_{xy} (average number of nucleotide substitution per site between populations) and D_a (Number of net nucleotide substitution per site between populations) were made with DnaSP 4.0 (Rozas *et al.*, 2003).

NETWORK ANALYSIS

As gene flow can lead to reticulated genealogies, haplotype networks are generally better suited than phylogenetic trees to depict lineages within species (Verovnik *et al.*, 2005). NETWORK 4.1.0.8 (www.fluxus-engeering.com) was used to infer the most parsimonious solution of the median-joining (MJ) network (Bandelt *et al.*, 1999), as described by Verovnik *et al.* (2005). To enhance the transparency of connections in the network, CR sequences differing by a single substitution were pooled. The network was further simplified by excluding singleton haplotypes. All positions were assigned equal masses, and the homoplasy level parameter (ε) was set to zero. Networks produced with original CR sequences had the same rooted topologies as the displayed network.

POPULATION PAIR-WISE F_{ST} AND AMOVA ANALYSIS

Linearized pair-wise F_{ST} were used as temporally short-term genetic distances between populations (Reynolds *et al.*, 1983; Slatkin, 1995). Differentiation between populations was assessed with population pair-wise F_{ST} values (10 100 permutations) using ARLEQUIN (http://www.cmpg.unibe.ch/software/arlequin3/). ARLEQUIN was also used to estimate the number of migrants per generation (*Nm*). Genetic structuring among groups was examined with an analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992), as implemented in ARLEQUIN (10 100 permutations), with distances measured as the number of pair-wise nucleotide differences. In this AMOVA, samples from eight localities were divided into two geographical groups: one group consisted of individuals from Beibu Bay ($n = 30$, southern sample) and the other of the remaining samples ($n = 138$, northern sample; Table I).

ISOLATION BY DISTANCE ANALYSIS

When migration rates are small, populations at migration–drift equilibrium are expected to exhibit isolation by distance (Slatkin, 1993). Pair-wise F_{ST} was used as genetic distance between localities and the shortest sea distance between localities as geographical distance to test for isolation by distance. However, as the fish were captured by fisherman over a range of several kilometres in one location, the shortest sea distance between localities could not be measured accurately. Isolation by distance was tested with a Mantel non-parametric permutation test (Mantel, 1967), as implemented in ARLEQUIN with 100 000 permutations in two groups of samples. One group included all eight localities, and another group consisted of six localities, excluding the southern sample from Beibu Bay. This analysis can provide insights into the genetic structure of *A. latus* as numerous samples were collected over a range of 1000 km.

DEMOGRAPHIC ANALYSIS

The distribution of pair-wise differences between haplotypes, or mismatch distribution, is potentially influenced by rapid population growth (Slatkin & Hudson, 1991; Rogers & Harpending, 1992; Roberts, 2006). Maximum likelihood estimates of $\Theta = 2N_f\mu$ (where N_f is the effective number of females in the population and μ is the mutation rate per site per generation) from the mismatch distribution were estimated with DnaSP (http://www.ub.es/dnasp/). The immigration parameter M, expressed as m/μ, where m is the immigration rate per generation. The exponential growth rate, g, at time t before was estimated with $\Theta_t = \Theta_{present}\exp(-gt)$, was estimated with likelihood analysis with metropolis algorithm using random coalescence (LAMARC; Kuhner *et al.*, 2004; http://evolution.gs.washington.edu/lamarc/). Gene flow into a population was estimated by $2Nm = M\Theta$ under the assumption of migration-drift equilibrium. Positive values of g indicate population growth, and negative values indicate a declining population.

RESULTS

SEQUENCE VARIATION

A total of 111 mtDNA haplotypes were detected in the CR sequences (289 bp) among 169 *A. latus* from coastal waters of China (excluding the outgroup; Table I). Of these, 96 haplotypes were limited to one locality, and 15 were shared among localities. One haplotype was found in 11·8% of the fish, and an additional five haplotypes had frequencies >2%. Large nucleotide diversities ranged from 0·013 (Zhujiangkou) to 0·026 (Yangjiang), and haplotype diversities ranged from 0·884 (Haikou) to 0·995 (Xiamen-1) (Table I).

GENETIC DIFFERENCE AND ISOLATION BY DISTANCE

F_{ST} between samples ranged between −0·046 and 0·544 (Table II). Pair-wise comparisons indicated significant differentiation between the southern sample and the pooled northern samples, and between the samples from Haikou–Xiamen-1 and Shenzhen. An AMOVA indicated that 46·1% ($P < 0·05$) of the total variation was because of differences between the southern sample and the pooled northern sample, but only 1·1% ($P < 0·05$) was because of variation among samples from the northern area (Table III). The average sequence divergence between the southern sample and the pooled northern sample was 4%. Estimates of migration indicated little restriction between nearby localities,

TABLE II. Pair-wise F_{ST} (below diagonal) and $2Nm$ (above diagonal) between samples of *Acanthopagrus latus*

	Xiamen-1	Xiamen-2	Shenzhen	Zhujiangkou	Yangjiang	Haikou	Beibu Bay	Beihai
Xiamen-1		inf	inf	inf	28·183	6·020	0·687	0·566
Xiamen-2	−0·017		inf	inf	25·001	6·803	0·489	0·481
Shenzhen	−0·003	−0·017		inf	inf	10·365	0·656	0·541
Zhujiangkou	−0·033	−0·046	−0·042		inf	19·428	0·419	0·437
Yangjiang	0·017	0·020	−0·003	−0·012		14·974	0·853	0·679
Haikou	0·077**	0·069	0·046*	0·025	0·032		0·581	0·485
Beibu Bay	0·421***	0·506***	0·433***	0·544***	0·370***	0·462***		109·789
Beihai	0·469***	0·510***	0·480***	0·534***	0·424***	0·507***	0·005	

Inf, infinite; significance level: $*P < 0·05$, $**P < 0·01$, $***P < 0·001$.

but only limited migration between the southern and the northern populations with $Nm < 1$.

Genetic distances between pairs of samples from all eight localities were positively correlated with geographical distance (Mantel's test: $r = 0·35$, $P = 0·04$; Fig. 2). However, F_{ST} values including the southern sample were generally much larger, relative to geographical distance, than those between other population pairs and may have distorted the Mantel analysis. After removing this sample, a highly significant isolation by distance ($r = 0·55$, $P = 0·01$; Fig. 2) appeared among the northern samples.

PHYLOGENETIC ANALYSIS

The MJ network displayed a star-like genealogy. Most haplotypes were connected by one mutation and most were linked to common haplotypes (Fig. 3). Nevertheless, a few divergent haplotypes also appeared in the network. Two major clusters appeared in the network. One, designated α, included 28 (93·3%) of 30 fish from Beibu Bay, and the other group, β, included 135 (97·1%) of the remaining 139 fish in northern samples (Figs 1 and 3). The separation of haplotypes in Beibu Bay from the remaining haplotypes by at least one mutation step indicates strong phylogeographic structure among *A. latus* populations.

TABLE III. AMOVA of mtDNA control region haplotypes in *Acanthopagrus latus* sample from eight localities

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Between groups	1	169·450	3·241	46·10*
Among localities within groups	6	31·998	0·080	1·14*
Within localities	161	597·303	3·710	52·76***
Total	168	798·751	7·032	

Significance level: $*P < 0·05$, $***P < 0·001$.

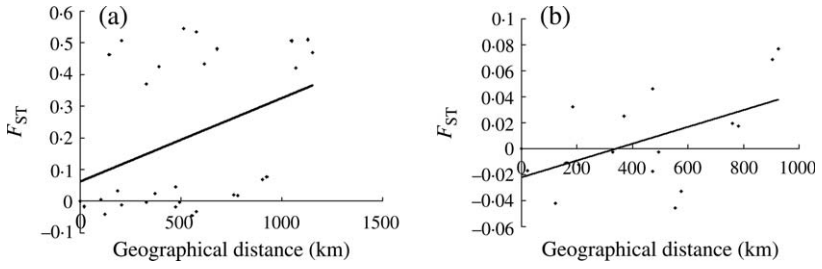

FIG. 2. Isolation by distance. (a) Among all localities (equation of best-fit line: $y = 0{\cdot}0003x + 0{\cdot}0619$); (b) among northern samples ($y = 0{\cdot}00006x - 0{\cdot}0219$).

This phylogenetic pattern was supported by the unweighted pair-group method with arithmetic mean (UPGMA) and the NJ trees of sequence divergences between haplotypes (trees not shown). These trees placed individuals into three major haplotype clades. Two of these clades fell in the α lineage and the third consisted of haplotypes in β lineage. However, most subclades in the tree were located on short branches with low bootstrap support (<50%), and only few nodes had support >80%. There was little obvious geographical pattern in the distribution of lineages in the northern samples.

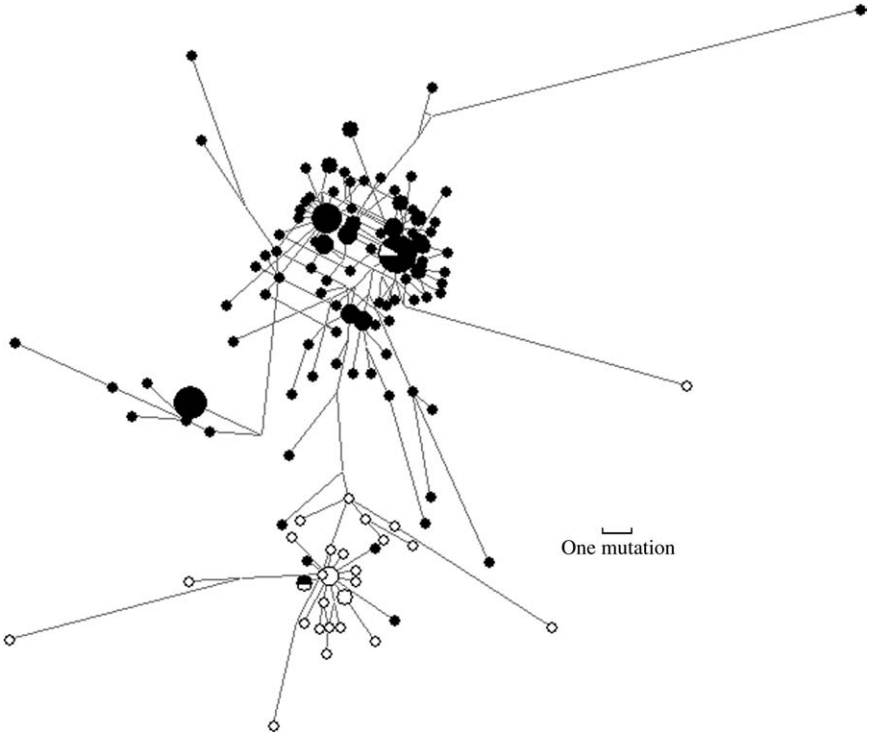

FIG. 3. Haplotype median-joining network of the yellowfin *Acanthopagrus latus*. Open circles represent haplotypes from the southern locality. Closed circles represent samples from northern localities. Circle sizes are proportional to haplotype frequency. Haplotype codes and median vectors representing hypothetical missing or unsampled ancestral haplotypes were omitted for clarity.

However, small clades of a few haplotypes were more often drawn from the same location or from nearby locations.

MISMATCH DISTRIBUTION AND DEMOGRAPHIC ANALYSIS

The mismatch distribution of haplotypes in a pooled sample exhibited two modes at four and 13 mismatches (Fig. 4). The mismatch distributions in the southern and northern samples had major peaks at four mismatches and a small peak at 13 mismatches. The southern sample additionally had a small peak at 25 mismatches. Coalescence analysis indicated moderate to high positive growth rates for the pooled ($g = 211{\cdot}7$) and individual southern ($g = 383{\cdot}7$) and northern ($g = 176{\cdot}4$) samples (Table IV). Estimates of Θ were similar in the southern (0·62) and northern (0·57) samples but larger in the pooled sample (0·90), as expected. Apparent immigration rates were larger in the southern population ($2Nm = 18{\cdot}41$) than in the northern populations (2·67).

DISCUSSION

GENETIC STRUCTURE AND MANAGEMENT UNITS

Pair-wise F_{ST} values, as well as the AMOVA, indicated that samples of *A. latus* along the Chinese coast belong to two genetically differentiated geographic units, a northern population, including populations from North of Qiongzhou Strait to Taiwan Strait and a southern population in Baibu Bay. Significant differentiation among northern populations indicated that population structure generally exists at a relatively small spatial scale in this species. The results presented here are consistent with the results of previous studies of AFLP variation (Xia *et al.*, 2005) and of mtDNA CR sequences (Liu *et al.* (2004), which detected a genetically distinct population in Beibu Bay. The results of both these studies revealed significant genetic structure between populations. Similar patterns of genetic subdivision have also appeared in studies of other sparid species along the coast of China, such as yellowback seabream *Dentex tumifrons* (Temminck & Schlegel) (Xia & Jiang, 2006).

An important step for designing a conservation programme to preserve genetic diversity is to determine the scale of genetic structuring (Primmer *et al.*, 2006). Moritz (1994) proposed the concept of a management unit (MU), defined as a population unit that has statistically significant divergence in allele frequencies (nuclear or mitochondrial). Therefore, the significant haplotype frequency differences among northern and southern populations of *A. latus* in Chinese waters provide a basis for designating these two population groups as separate MUs.

ISOLATION BY DISTANCE AND RESTRICTED GENE FLOW

Isolation by distance has been reported in a wide variety of fish, including Atlantic salmon *Salmo salar* L. (Primmer *et al.*, 2006). Wright (1943) suggested that simple models of restricted migration could result in genetic isolation by

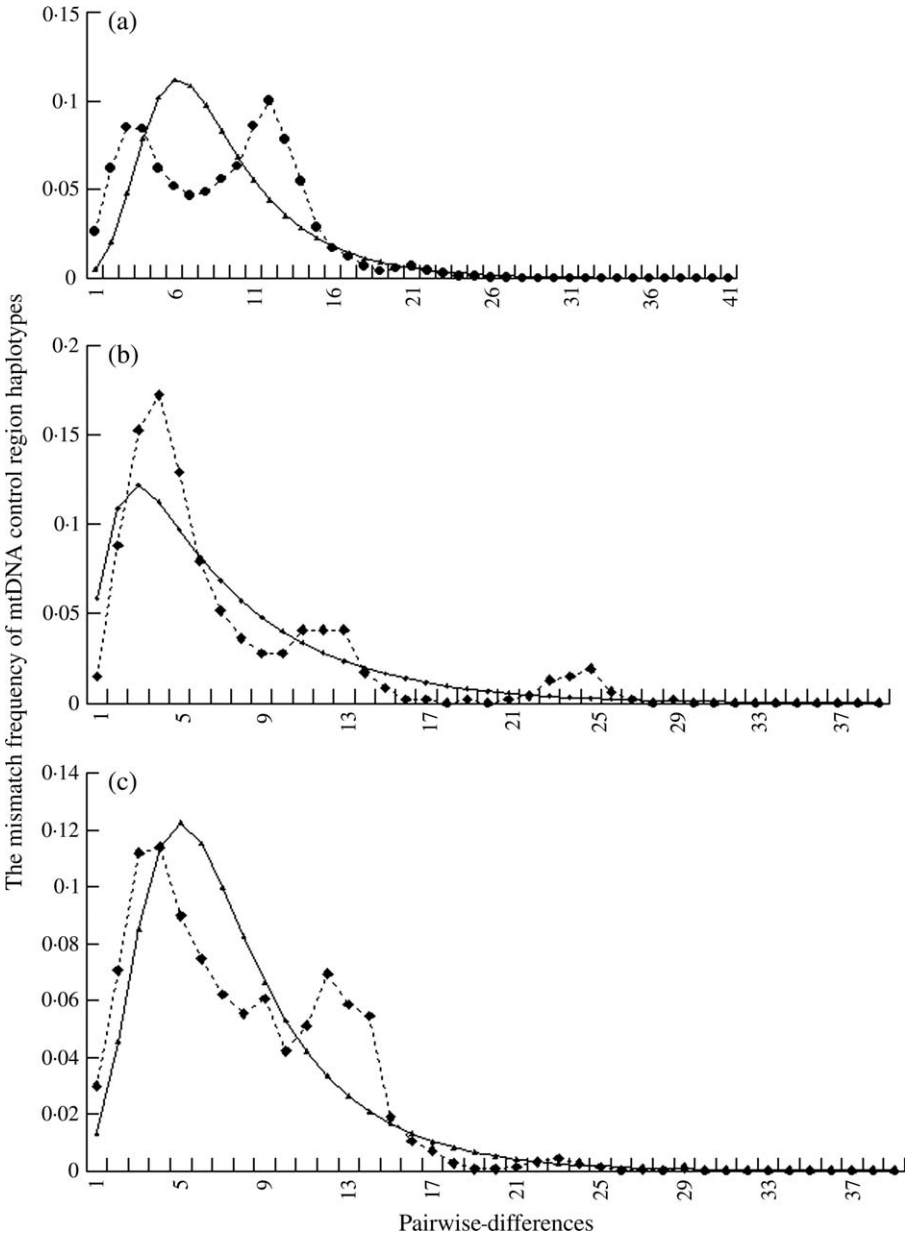

FIG. 4. Mismatch frequency distributions of *Acanthopagrus latus* mtDNA control region haplotypes. Obs, observed distribution; Exp-G, expected distribution under a model of population expansion. (a) both samples; (b) southern sample; (c) northern sample. - - -●- - -; Obs; ——♦——; Exp-G.

distance without physical barriers. The sampling sites ranged across nearly 3° latitude and 9° longitude, with the two most distant sites separated by >1000 km. As the migration distances for *A. latus* are usually short, populations separated by these large distances are likely isolated from one another, even without geographic barriers. The positive correlation between genetic

distance and geographical distance detected for *A. latus* supports a model of isolation by distance. Under these circumstances, isolation is sufficient to lead to population-specific haplotypes but is not so complete that very deep allopatric lineages among populations do not appear (Barton & Wilson, 1995; Roberts, 2006).

The results of AMOVA and tests of genetic differentiation among locations showed a strong genetic difference between southern and northern populations. The AMOVA indicated a greater degree of variation between these groups than between localities within the northern group of populations. These might provide evidence of barriers between the two groups. The amount of gene flow between marine populations is generally correlated with individual dispersal potential and is limited by geographic barriers (Scheltema, 1975; Hedgecock, 1986; Taylor & Hellberg, 2006). Although barriers in the ocean are generally less obvious, ocean currents, variation in habitat and topography have proved to be substantial barriers to gene flow in some species (Goldstien *et al.*, 2006; Mathews, 2006; Roberts, 2006; Taylor & Hellberg, 2006). There are many bays and islands in coastal waters of China, and the oceanographic dynamics of coastal waters is complex, especially near Beibu Bay. Seasonal variation in wind, temperature and salinity, among other variables, influence water transport through Qiongzhou Strait and undoubtedly influence the potential for gene flow (Yang *et al.*, 2003; Chen *et al.*, 2006; Yang *et al.*, 2006). Some evidence suggest that Taiwan and Qiongzhou Straits, the Leizhou Peninsula and Hainan Island may represent major barriers to the movement of *A. latus* and other sparid fishes. For example, the northern extreme of *A. latus* off the coast of China is at Taiwan Strait. Some studies have suggested that the red seabream *Pagrosomus major* (Temminck & Schlegel) in Chinese waters consists of two subpopulations, one distributed from the middle of Taiwan Strait to the Yellow Sea and the other from the middle of Taiwan Strait to the South China Sea (Taniguchi & Sugama 1990; Wang *et al.*, 2003). The latter group is further subdivided into two stocks, south and north of Qiongzhou Strait. Significant RAPD frequency differences for the black seabream *A. schlegelii* were detected among locations in Qingdao, Shandong Province, Xiamen and Beihu Bay (Yang *et al.*, 2004*b*), and only a few mtDNA haplotypes were shared among samples from Beihai, Shenzhen and Qingdao (Gong *et al.*, 2006). In addition, genetic differentiation among populations of the New Zealand snapper *Chrysophrys auratus* (Forster) appears to be more influenced by ocean currents than by geographic distance (Bernal-Ramírez *et al.*, 2003). These results indicate that hydrographic conditions in straits might also influence the population genetic structure of *A. latus* and other sparid fishes. Nevertheless, the samples from Beibu Bay and Xiamen, spanning a distance of >1000 km shared several haplotypes, suggesting that historical, long-distance dispersals between *A. latus* populations have occurred sporadically.

PHYLOGENETIC PATTERNS AND DEMOGRAPHIC HISTORY

The results of the present study revealed two divergent mtDNA lineages in *A. latus* across Chinese waters, and CRs haplotype frequency differences indicate deep phylogeographic structure. Whether these two lineages represent

sub-species remains to be evaluated with nuclear DNA markers or with less rapidly evolving mtDNA markers such as cytochrome oxidase I, together with morphological information.

In a recently expanded population, the majority of lineage coalescences are expected to post-date the expansion, producing a smooth unimodal Poisson distribution and a star-like haplotype genealogy because of the accumulation of low frequency mutations (Slatkin & Hudson, 1991; Rogers & Harpending, 1992; Campbell *et al.*, 2006). The sharp peaks in the mismatch distributions in *A. latus* are consistent with a past sudden expansion in each of the two major populations. The LAMARC analysis also indicated moderate to high positive growth rates for both groups. Population growth and the accumulation of numerous recent mutations typically produce numerous short internal branch lengths and low bootstrap support in the phylogenetic trees (Whitfield & Lockhart, 2007). This phylogenetic signature in *A. latus* indicates a recent origin and rapid expansion of the two divergent population groups of *A. latus* in Chinese waters.

In conclusion, the results of this genetic study show that there are at least two population groups of *A. latus* in Chinese coastal waters that should be considered as separate MUs for conservation. However, as these conclusions are based on a single gene, the present results should be complemented with additional sampling and additional analysis with other molecular markers (Audzijonyte & Väinölä, 2006). A multidisciplinary approach integrating genetics with morphology, climatic models and biogeography should provide further valuable insights into the biological diversity and phylogeography of *A. latus*.

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