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Phylogeography of the flathead mullet *Mugil cephalus* in the north-west Pacific as inferred from the mtDNA control region

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The population genetic structure and historical demography of the flathead mullet *Mugil cephalus* were investigated using the mtDNA control region (CR) sequences (909–1015 bp) of 126 individuals collected from seven locations in the north-west Pacific between 2005 and 2007. Haplotype diversity ($h = 0{\cdot}9333–1{\cdot}000$) and nucleotide diversity ($\pi = 0{\cdot}0046–0{\cdot}1467$) varied greatly among the sampling locations. Phylogenetic analysis of the CR sequences indicated that *M. cephalus* in the north-west Pacific belongs to two highly divergent lineages (lineages 1 and 2), with the inferred population structure being closely associated with the distribution of both lineages. Two populations were identified, one from the East China Sea and the other from the South China Sea. The former samples were obtained from Taiwan and Qingdao of north China and associated with lineage 1 haplotypes. The latter samples were collected from the Philippines, Pearl River of South China and two samples from Japan, all of which were associated with lineage 2. Japanese samples from Okinawa and Yokosuka had different degrees of mixing between lineages 1 and 2. Historical demographic variables in both populations indicated that Pleistocene glaciations had a strong impact on *M. cephalus* in the north-west Pacific, resulting in a recent demographic decline of the East China Sea population but in demographic equilibrium for the South China Sea population. Japan appears to be a contact zone between lineages 1 and 2, but it may also be indicative of coexistence between resident and migratory populations. Further global studies are required to clarify the taxonomic status of this cosmopolitan species.

Key words: geological events; mtDNA control region; *Mugil cephalus*; north-west Pacific; phylogeography; population genetics.

INTRODUCTION

The flathead mullet *Mugil cephalus* L. is a euryhaline marine fish with a global distribution in coastal and estuarine waters of subtropical and tropical regions (42° N–42° S). This species occurs at water temperatures of 12–25° C and tolerates a wide range of salinities from freshwater to hypersaline conditions of 100 (Thomson,

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1966; Kuo *et al*., 1973; Collins, 1985; Vega-Cendejas *et al*., 2004). The *M. cephalus* fishery is one of the most important coastal fisheries in Taiwan, China and Japan. The gonads of ripe *M. cephalus* are considered a delicacy by local residents, with high market prices being attained, particularly in Taiwan and Japan (Liao, 1981).

Mugil cephalus makes an annual spawning migration from the coastal waters of northern China to the offshore waters of south-western Taiwan. This migration occurs around the winter solstice, especially between 10 days before and after 21 or 22 December (Tung, 1981; Huang & Su, 1989). The females become mature at about 3 years of age and spawn once a year for at least 2 or more years thereafter (Tung, 1981). Newly hatched larvae drift with coastal currents towards estuarine nursing areas along the western coast of Taiwan, where they grow for 1–2 months. The juveniles then migrate to the coastal waters of northern China to be recruited into the adult stock (Chang *et al*., 2004).

The life history and population dynamics of *M. cephalus* in Taiwan, such as age, growth, reproduction, recruitment, mortality and fishing conditions have been intensively studied (Liao, 1981; Tung, 1981; Su & Kawasaki, 1995; Chang *et al*., 2000; Chang *et al*., 2004; Hsu *et al*., 2007). However, genetic structuring of the north-west Pacific populations remains unclear (Huang *et al*., 2001). The worldwide genetic structure of *M. cephalus* has been studied using allozyme (Rossi *et al*., 1998) and mtDNA techniques (Crosetti *et al*., 1994). These studies revealed marked genetic divergences among different geographical populations, raisings questions about its true taxonomic status. Analysis of mtDNA control region (CR) sequences also indicated that *M. cephalus* had high genetic divergence between Atlantic and Pacific populations, but there were few samples collected at regional scales, with only a single sample from the Gulf of Mexico in the north-east Atlantic (Rocha-Olivares *et al*., 2000). At present, because the CR sequences show exceptionally high divergences among populations on a geographical scale, Rocha-Olivares *et al*. (2005) have suggested that *M. cephalus* should be considered a species complex. Resolving the taxonomic status of *M. cephalus* requires, however, detailed investigations of genetic structures on regional scales (Crosetti *et al*., 1994; Rossi *et al*., 1998; Rocha-Olivares *et al*., 2000).

Control region sequences have been successfully used by Rocha-Olivares *et al*. (2000, 2005) to reveal extensive polymorphic sequences that were useful in evaluating the population structures and historical demography of *M. cephalus*. The current study used this gene to investigate the genetic structure of *M. cephalus* in the north-west Pacific region, to infer its present-day migration pattern in relation to the past climatic and geological evolution within the region, and to contribute information towards future global assessment of the taxonomic status of this cosmopolitan species.

MATERIALS AND METHODS

FISH COLLECTION

A total of 126 adult *M. cephalus* were collected from seven locations within the East China Sea and South China Sea during the winters (December to February) of 2005 to 2007. The distribution of samples was: Qingdao in northern China (22 individuals), Pearl River in southern China (17), Keelung in northern Taiwan (22), Kaohsiung in southern Taiwan (26),

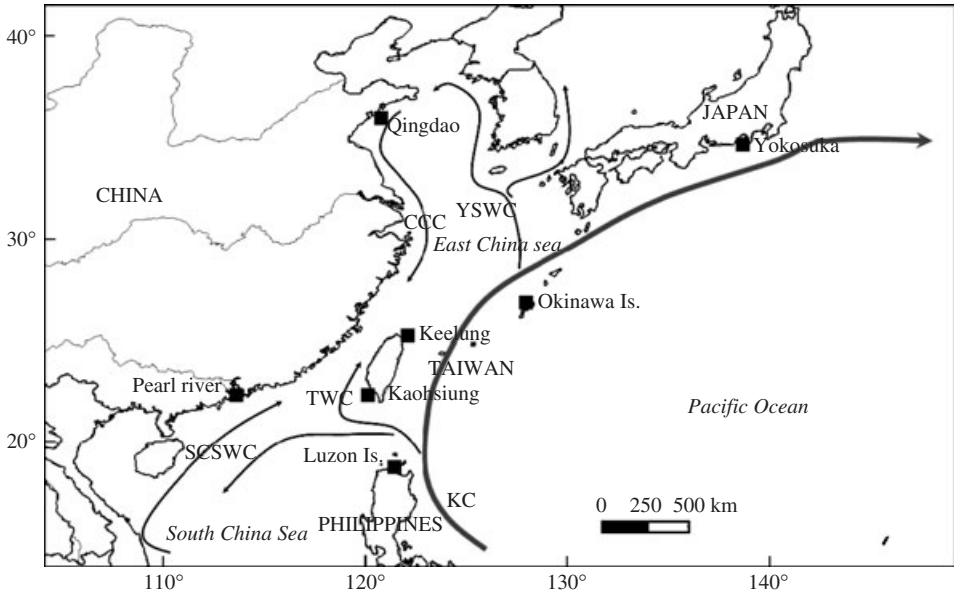


FIG. 1. Sampling locations (■) and ocean currents affecting the distribution and dispersal of *M. cephalus* in the north-west Pacific (KC, Kuroshio Current; SCSWC, South China Sea warm current; TWC, Taiwan warm current; CCC, China coastal current; YSWC, Yellow Sea warm current).

Okinawa in southern Japan (10), Yokosuka in central Japan (13) and Luzon in the Philippines (16) (Fig. 1). The specimens from Taiwan and Okinawa were collected from offshore waters, while those from Pearl River, Yokosuka and Luzon were collected from estuaries.

DNA EXTRACTION AND SEQUENCING

For each specimen, muscle tissues and fin clips were collected and preserved in the 95% ethanol–water solution and returned to the laboratory. Genomic DNA was then extracted from a small piece of either muscle tissue or fin clips, using a commercially available total genomic DNA extraction kit (Bioman Scientific Co. Ltd.; www.bioman.com.tw).

The whole CR of the mitochondrial DNA (CR) was amplified using modified primers MulPro (McepCR-F - CCAAGGCCAAGATTTTTACATT) and MulPhe (TCTTGACATCTTCAGCGTTCGC) of Rocha-Olivares *et al.* (2000) primers. Polymerase chain reaction (PCR) amplification was carried out in a 25 μl total volume containing 0·1 ng DNA, 1·25 pmol reverse primer, 1·25 pmol forward primer, 5 mM deoxyribonucleotide triphosphate (dNTP), 1·5 mM $MgCl_2$ and 0·5 U Taq polymerase (Bioman) at the temperature of 94° C for 2 min. This was followed by 35 temperature cycles, with each cycle comprising denaturation at 94° C for 30 s, annealing at 58° C for 45 s, extension at 70° C for 45 s and then a final elongation at 70° C for 10 min. PCR products were checked on a 1% agarose gel stained with ethidium bromide and then sequenced with forward and reverse primers using dye-terminator cycle sequencing using an ABI 373A instrument (Applied Biosystems; www.appliedbiosystems.com).

PHYLOGENETIC ANALYSIS

The CR sequences determined in this study and those previously obtained from Hawaii and the Atlantic–Gulf of Mexico (Rocha-Olivares *et al.*, 2000) from GenBank (www.ncbi.nlm.nih.gov) were aligned, using the software MAFFT ver. 6 (Katoh *et al.*, 2002) with default parameters and certain manual improvements. This software is a multiple sequence alignment programme based on fast Fourier transformation (FFT), that implements

two different computing methods, the progressive method and the iterative refinement method (Katoh *et al*., 2002).

The phylogenetic relationships among haplotypes were explored using the neighbour-joining (NJ) method (Saitou & Nei, 1987) in conjunction with the Tamura–Nei model. Trees were constructed with MEGA 4.02 (Kumar *et al*., 2004) and their statistical significance was assessed using the bootstrap resampling technique (Felsenstein, 1985).

According to the recommendations of McMillan & Palumbi (1997) and Rocha-Olivares *et al*. (2005) regarding estimations of net average distances between lineages, the gamma corrected version of Tamura & Nei's (1993) model (TrN-G) was used and the gamma distribution (a) was set at 0·604. Population genetic diversity was measured within each sample as the number of distinct haplotypes (n) and mean nucleotidic divergence (π), using the software DnaSP 4.10.9 (Rozas *et al*., 2003) and Arlequin ver. 3.01 (Excoffier *et al*., 2005).

POPULATION STRUCTURE AND HISTORICAL DEMOGRAPHY

To investigate the genetic structure of *M. cephalus* in the north-west Pacific a hierarchical analysis of molecular variance (AMOVA) was performed (Excoffier *et al*., 2005), implemented in Arlequin ver. 3.01. Geographic partitionings, consistent with different scenarios of dispersal (historical *v.* contemporary), were tested. Historical delineation of coastlines were based on results from Liu *et al*. (2007), who showed that in the north-west Pacific region there were three biogeographic areas during the Pleistocene era. Therefore, the geographic partitioning tests were conducted with respect to (1) Pleistocene period separation, (2) north-west Pacific marginal seas separation, (3) Present oceanic currents system separation and (4) population structure separation as inferred from the Φ_{ST} matrix.

The historical demography of *M. cephalus* was inferred from mismatch distribution analysis as performed by Arlequin software. A unimodal pattern of mismatch distribution indicates that the population has experienced a rapid expansion in the recent past, while multimodal distributions indicate populations that are in demographic equilibrium (Rogers & Harpending, 1992). The parameter τ was estimated from the data and calculations were carried out using the method outlined in Rogers (1995). The goodness of fit of the observed data to a simulated model of expansion was tested using the raggedness index (Harpending, 1994) and a corresponding P-value was also obtained.

RESULTS

GENETIC DIVERSITY

The sizes of the 126 CR sequences from the north-west Pacific varied between 909 and 1015 base pairs (bp). The sequences have been deposited at GenBank under accession numbers EU663629–EU663754. Multiple alignments of the sequences made numerous indels (insertion and deletion) at the 3′ end of the CR due to the presence of tandem repeats. To avoid large variance and limit homoplasy, the hyper variable 3′ region was excluded, so that the CR sequence sizes used in the phylogenetic and further analyses were 901 bp.

For the 126 sequences, 105 haplotypes were observed, of which seven haplotypes were shared by the Philippines, Pearl River and Okinawa, and 14 haplotypes by Pearl River, Japan, Taiwan and Qingdao. The haplotype diversity (h) was high and ranged between 0·933 and 1·000 (Table I). Samples from Taiwan (KL, KS) and northern China (QD) presented the lowest genetic diversity ($\pi = 0{\cdot}005–0{\cdot}008$) (Table I), close to that of samples from the Atlantic (0·013). On the other hand, the Philippines (PH) and southern China (PR) samples presented a higher genetic diversity ($\pi = 0{\cdot}032$and 0·066, respectively) comparable to that observed from

TABLE I. Genetic diversity of *Mugil cephalus* and historical demographic parameters at seven of the sampling locations

		Diversity index				Demographic parameter		
Location	code	*N*	*n*	*h*	π	*t*	RI	*P*
Luzon	PH	16	16	1·000	0·032	32·12	0·005	0·870
Pearl River	PR	17	15	0·978	0·066			
Okinawa	OK	10	7	0·933	0·112	—	—	—
Yokosuka	YK	13	12	0·987	0·147			
Qingdao	QD	22	22	1·000	0·008	3·46	0·010	0·999
Keelung	KL	22	20	0·987	0·005			
Kaohsiung	KS	26	22	0·979	0·007			
Hawaii*	HA	19	19	1·000	0·031	28·70	0·011	0·810
Atlantic*	AT	95	94	1·000	0·013	5·40	0·003	0·660

N, sample size; *n*, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity; τ, divergence time; RI, Raggedness index.

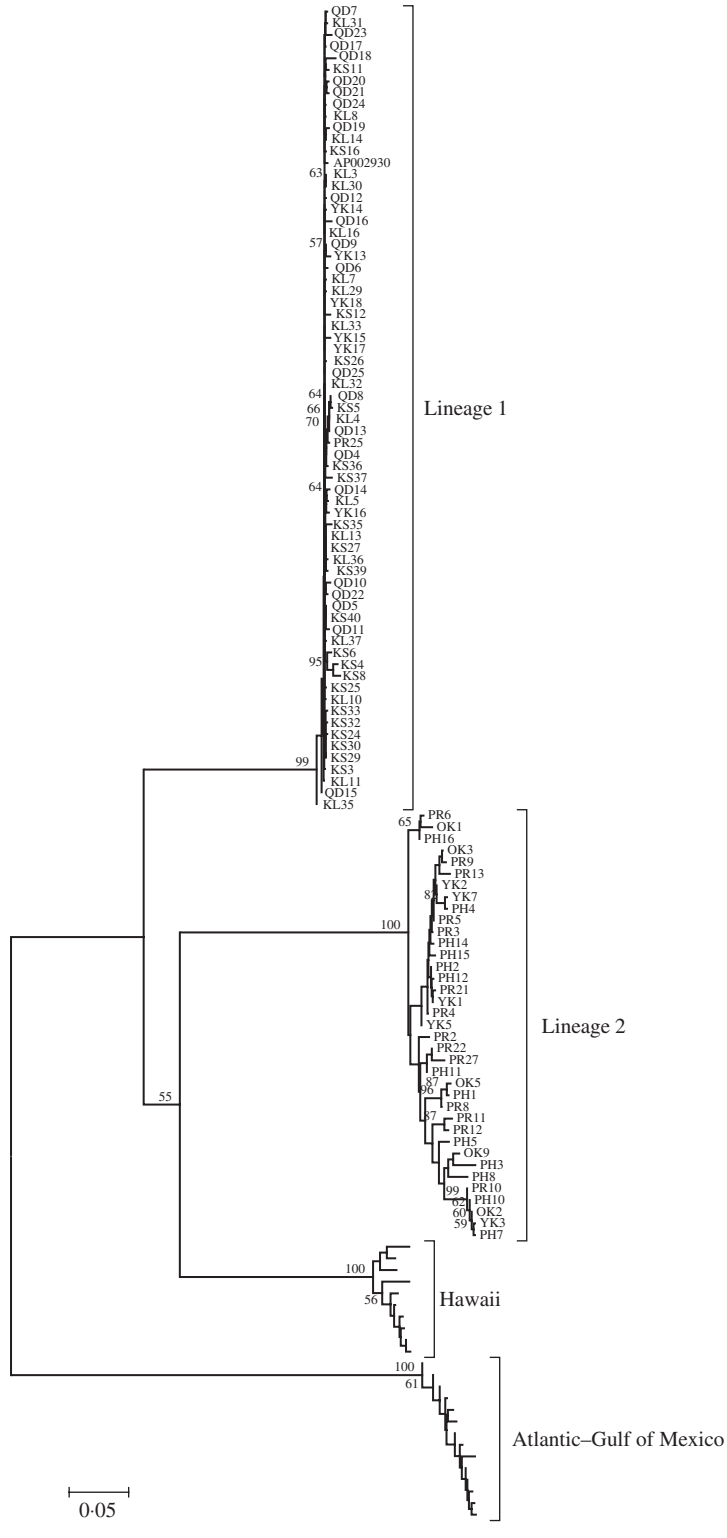
*Data from Rocha-Olivares *et al*. (2000).

Hawaii ($\pi = 0{\cdot}031$). The Japanese samples (OK, YK) exhibited the highest genetic diversity observed ($\pi = 0{\cdot}112–0{\cdot}147$) as both lineages occurred sympatrically.

The NJ tree reconstructed from the CR sequences illustrated the divergences among populations of *M. cephalus* on a global and regional scale (Fig. 2). There were four lineages observed in the tree: lineages 1 and 2 for the specimens from the north-west Pacific, and lineages 3 and 4 from Hawaii and Atlantic–Gulf of Mexico. All individuals from Qingdao, Kaohsiung and Keelung belonged to lineage 1, whereas all individuals from Luzon and Pearl River (with exception of one haplotype) belonged to lineage 2. Yokosuka and Okinawa samples consisted of a mixture of haplotypes belonging to both lineages 1 and 2 (Figs 2 and 3). Within each individual lineage there was no significant phylogenetic relationship observed. The NJ tree suggested a rapid ancestral radiation except for the Atlantic lineage. The genetic distances among the Hawaii lineage and lineages 1 and 2 were similar, ranging between 45·6 and 48·0% for corrected sequence divergences (TrN-G). Net average genetic distances (Tamura & Nei, 1993), with gamma correction between lineages 1 and 2, was 48·0% (Table II).

GENETIC STRUCTURE AND HISTORICAL DEMOGRAPHY

Significant differences in genetic structure were detected in the CR haplotypes ($\Phi_{ST} = 0{\cdot}7280$; $P < 0{\cdot}05$) among the samples from different geographical areas. Within the north-west Pacific area, two geographic populations were identified from the pair-wise Φ_{ST} matrix (Table III): the East China Sea population (ECS, Qingdao, Keelung, Kaohsiung) and the South China Sea population including Japan (SCS, Philippines, Okinawa, Yokosuka). Between these two populations, there was little or no genetic heterogeneity ($\Phi_{ST} = 0{\cdot}153$, $P = 0{\cdot}009$, $\Phi_{ST} = 0{\cdot}079$ NS, respectively) but with extreme interpopulation differentiations ($\Phi_{CT} = 0{\cdot}77$, $P = 0{\cdot}000$). Differentiation of these two populations was mainly due to the distribution of lineages 1 and 2, with more genetic heterogeneity present in the SCS population when compared to the ECS population where only lineage 1 occurred (Fig. 3).



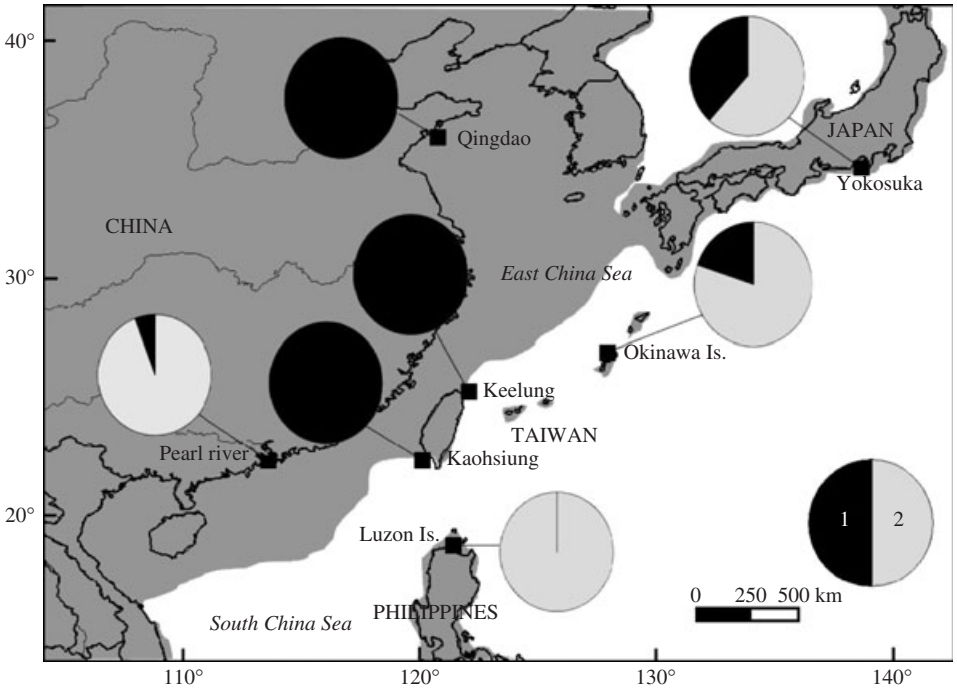


FIG. 3. Geographical distribution of lineages among seven sampling locations of *Mugil cephalus* in the north-west Pacific (■, land areas at the lowest sea level during the last glacial maximum of the Pleistocene period).

TABLE II. Mean TrN-G uncorrected (below diagonal) and TrN-G corrected (above diagonal) sequence divergences (%) among *Mugil cephalus* CR haplotypes

	Lineage 1	Lineage 2	Hawaii	Atlantic
Lineage 1	*0·5/0·5*	48	45·9	74·7
Lineage 2	29·5	*2·6/2·8*	45·6	77·8
Hawaii	28·3	27·4	*2·5/2·7*	71·1
Atlantic	38·7	38·9	35·8	*1·1/1·1*

Diagonal: within-region mean uncorrected/corrected per cent sequence divergences.

The genetic structure of the *M. cephalus* in the north-west Pacific was further investigated with hierarchical AMOVA that took into account different environmental scenarios (Historical–Pleistocene *v.* Contemporary–Sea currents) (Table II). The best subdivision that would limit the intrapopulation variation and maximize the interpopulation differentiation of the samples was found to accord with the present

FIG. 2. Neighbour-joining tree inferred from the mtDNA CR sequences of *Mugil cephalus* in the north-west Pacific, Hawaii and Atlantic–Gulf of Mexico (QD, Qingdao of China; PR, Pearl River of China; YK, Yokosuka of Japan; OK, Okinawa of Japan; KL, Keelung of Taiwan; KS, Kaohsiung of Taiwan; PH, Luzon of the Philippines).

TABLE III. Pair-wise Φ_{ST} of *Mugil cephalus* collected from seven locations

Φ_{ST}		South China Sea				East China Sea			
		PH	OK	YK	PR	QD	KL	KS	HA
North-west Pacific	OK	0·0851							
	YK	**0·374*****	0·057						
	PR	0·0153	−0·029	0·220*					
	QD	**0·941*****	**0·809*****	**0·556*****	**0·869*****				
	KL	**0·948*****	**0·819*****	**0·567*****	**0·876*****	0·002			
	KS	**0·945*****	**0·826*****	**0·584*****	**0·879*****	0·021*	0·016		
	HA	**0·891*****	**0·730*****	**0·643*****	**0·808*****	**0·951*****	**0·960*****	**0·955*****	
	AT	**0·945*****	**0·861*****	**0·802*****	**0·896*****	**0·978*****	**0·983*****	**0·979*****	**0·951*****

PH, the Philippines; OK, Okinawa; YK, Yokosuka; PR, Pearl River; QD, Qingdao; KL, Keelung; KS, Kaohsiung; HA, Hawaii; AT, Atlantic.

*$P < 0·05$;

**$P < 0·01$;

***$P < 0·001$;

Bold values denote significant value after Bonferroni correction.

TABLE IV. Hierarchical AMOVAs

Source of variation	% of variation	Phi(Φ)-stat	*P*
Pleistocene period separation [PH,PR, KS][KL, QD][OK, YK]			
Between groups	11·81	$\Phi_{CT} = 0{\cdot}118$	0·463
Among pops. or groups	64·06	$\Phi_{SC} = 0{\cdot}726$	0
Within populations	24·13	$\Phi_{ST} = 0{\cdot}759$	0
North-west Pacific marginal–seas separation [PH,PR,KS][KL,OK,QD,YK]			
Among groups	−8·17	$\Phi_{CT} = -0{\cdot}082$	0·39
Among pops. or groups	81·02	$\Phi_{SC} = 0{\cdot}749$	0
Within populations	27·14	$\Phi_{ST} = 0{\cdot}729$	0
Oceanic currents system separation: Kuroshio Current [PH,OK,YK][PR,KS,KL,QD]			
Among groups	38·86	$\Phi_{CT} = 0{\cdot}389$	0·106
Among pops. or groups	39·92	$\Phi_{SC} = 0{\cdot}653$	0
Within populations	21·22	$\Phi_{ST} = 0{\cdot}788$	0
Population structure inferred from the Φ st matrix [PH,PR,OK,YK][KS,KL,QD]			
Among groups	79·4	$\Phi_{CT} = 0{\cdot}794$	0·026
Among pops. or groups	3·92	$\Phi_{SC} = 0{\cdot}190$	0·002
Within populations	16·68	$\Phi_{ST} = 0{\cdot}833$	0

PH, Luzon, Philippines; PR, Pearl River; KS, Kaohsiung; KL, Keelung; QD, Qingdao; OK, Okinawa; YK, Yokosuka.

oceanic currents in the area where Philippines, Okinawa and Yokosuka would be preferentially connected through the Kuroshio Current (Fig. 1 and Table IV). The grouping that took into account a potential Pleistocene separation of South China Sea locations (Luzon, Kaohsiung and Pearl River) from more northern locations (Fig. 1), as suggested by the *Chelon haematocheilus* (Temminck & Schlegel) phylogeographic structure (Liu *et al*., 2007), created a poor fit in relation to the observed geographic diversity in *M. cephalus*.

The mismatched distribution pattern provides an insight into the past population demography. Because Japanese samples belonged to a contact zone where the genetic diversity was strongly influenced by recent dispersion of both SCS and ECS populations, Japanese samples were excluded from the mismatch analyses. The mismatch distribution pattern of the SCS population was polymodal, with the main mode at a pair-wise difference of 34 nucleotides [Fig. 4(a)]. The result of the raggedness test rejected a model of sudden expansion (Harpending's raggedness index = 0·0045, $P = 0{\cdot}87$). In contrast, the ECS population was unimodal with the mode at a pair-wise difference of four nucleotides [Fig. 4(b)]. The raggedness test indicated a significant fit of the observed mismatch distribution to a model of sudden expansion (Harpending's raggedness index = 0·01, $P = 0{\cdot}99$).

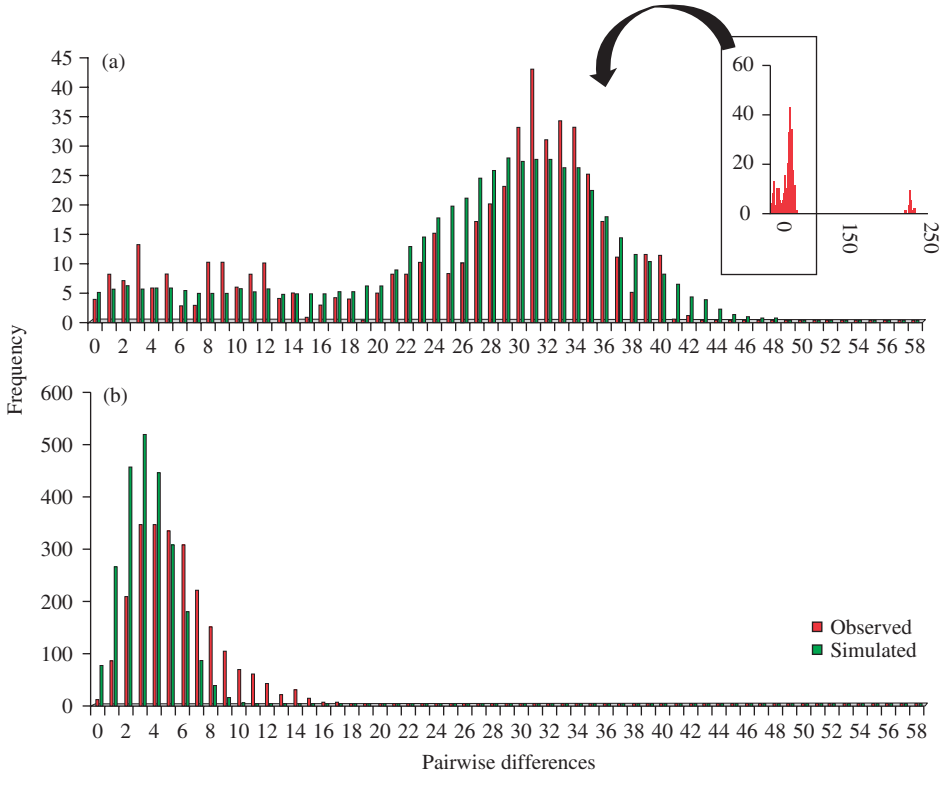


FIG. 4. Mismatch distributions from the mtDNA CR sequences of *Mugil cephalus* from the seven sampling locations: (a) lineage 2; (b) lineage 1; ■, observed distributions; ■, expected distributions from the sudden expansion model.

DISCUSSION

USEFULNESS OF THE CR SEQUENCES IN PHYLOGENETIC ANALYSIS

High genetic divergences were found in the CR sequences among geographical populations of *M. cephalus* in this study. Rocha-Olivares *et al*. (2000, 2005) reported an unexpectedly high level of CR sequence divergence between populations separated by a wide geographic range, as is the case between the Atlantic and the Pacific populations where there is up to 70% corrected sequence divergence (TrN-G). This study has revealed two highly divergent lineages at a regional scale in the north-west Pacific (48·0% TrN-G). Lineage 1 has already been described by Rocha-Olivares *et al*. (2005) using a Japanese *M. cephalus* sequenced by Miya *et al*. (2001), whereas lineage 2 was identified during this study. Atlantic lineages appear to be the most divergent (71·1–77·8% TrN-G), with Pacific lineages less so (45·6–48·0% TrN-G). The above studies have demonstrated that intra and interoceanic divergences in *M. cephalus* greatly exceed the expected level of divergence within a species, as already emphasized by Rocha-Olivares *et al*. (2000). More recently, an investigation into the genetic structure of another mugilid in the north-west Pacific, *C. haematocheilus*,

showed that its CR sequence divergent levels ranged from 0·53 to 0·63% TrN-G (Liu *et al*., 2007) and were considerably lower than those observed for *M. cephalus*.

The high level of genetic divergence in *M. cephalus* led Rocha-Olivares *et al*. (2005) to question whether the CR sequences were based on NUMT (nuclear mitochondrial DNA) pseudogene material, since it had been reported that the NUMT pseudogene evolves faster than their mitochondrial copies. However, upon analysing the CR sequences and using nested PCR, the results obtained in this study demonstrated that the *M. cephalus* material was mitochondrial in origin and not pseudogenetic. Therefore, rapid evolution of the CR gene could be responsible for the high levels of divergence in *M. cephalus*. This is in agreement with Caldara *et al*. (1996) who suggested that, among the species of Mugilidae, the *Mugil* lineage might exhibit a higher rate of evolution. However, it remains unclear as to why levels of divergence in *M. cephalus* exceed the usual intraspecific range, even when compared to species with rapid evolution such as within the Chaetodontidae (McMillan & Palumbi, 1997). Nevertheless, this cannot be used to determine the taxonomic rank, because considerable heterogeneity for evolutionary rate of the mitochondrial CR has been described among species (Bowen *et al*., 2006). Using less variable markers than the CR gene, Fraga *et al*. (2007) and then Heras *et al*. (2008) found much more limited inter *M. cephalus* lineage divergences [1–2% for 16S ribosomal RNA (rRNA) and 4–5% for cytochrome *b*]. These results lead to conflicting interpretations, with Heras *et al*. (2008) concluding there was a species complex due to the allopatry of *M. cephalus* lineages but Fraga *et al*. (2007) proposed that *M. cephalus* is a single species which includes *Mugil liza* Valenciennes and *Mugil platanus* Günther as separate *M. cephalus* lineages.

Because of its high evolutionary rate, the *M. cephalus* CR gene is not reliable as a molecular clock to date lineage divergence. Indeed, parallel mutations quickly blur the relationship between the genetic distance and divergence time. As mentioned by McMillan & Palumbi (1997), the utility of CR sequences to derive phylogenetic information on divergences is restricted to very short periods of time. Therefore, the divergence estimates among the lineages within the geographical populations in the *M. cephalus* phylogenetic tree reconstructed from the CR sequences (Fig. 2) should be considered with caution. Furthermore, this study could not provide a divergence date for *M. cephalus* due to ambiguities in the evolution rate of the species.

HISTORICAL DEMOGRAPHY OF *MUGIL CEPHALUS* IN THE NORTH-WEST PACIFIC

The two north-west Pacific lineages exhibited contrasting historical demographics, suggesting different evolutionary histories. Lineage 2 has a mismatch distribution that was significantly different from that expected from a model of sudden expansion. The broad, polymodal distribution is typical for a population under long-term equilibrium. A similar distribution was observed for the Hawaiian population of *M. cephalus* and, to a lesser extent, the Gulf of Mexico and Atlantic populations (Rocha-Olivares *et al*., 2000). By contrast, lineage 1 has a unimodal mismatch distribution typical of an expanding population. The raggedness index reflected the degree of departure from a smooth distribution typical of an expanding population. The index was significant for lineage 2 but not for lineage 1 suggesting that, in contrast to lineage 2, lineage 1 experienced a recent bottleneck (demographic crash) and is now in a phase of

population expansion. Since the distribution range of lineage 1 was in the temperate zone, further north than lineage 2 in the tropical zone, it can be assumed that Pleistocene glaciations had a greater effect on lineage 1 than on lineage 2. This result concurs with findings of Liu *et al.* (2007) for *C. haematocheilus*, which found a similar trend in historical demography, *i.e.* population expansion in the northern region (East China Sea) and equilibrium in the southern region (South China Sea). Pleistocene glaciations are known to have had a major effect on the genetic diversity and structure of temperate species (Hewitt, 1996, 2004).

Coastal habitats in the tropical region were also affected by the glaciations. Lowering of the sea level had a profound effect on these ecosystems during Pleistocene glaciations, either promoting differentiation or creating bottlenecks for populations (Chenoweth *et al.*, 1998; Fauvelot *et al.*, 2003; Durand *et al.,* 2005). These geological events limited estuarine connectivity, sometimes drying out coastal lagoons, which increased genetic structuring of fish populations (Chenoweth *et al.*, 1998) and decreased their genetic diversity (Fauvelot *et al.*, 2003). Our results indicate that such effects were more drastic in high than low latitudes, and suggest that lineages 1 and 2 of *M. cephalus* in the north-west Pacific were isolated during the Pleistocene era.

EVOLUTIONARY SIGNIFICANCE OF THE *MUGIL CEPHALUS* LINEAGES

In this study, a high genetic heterogeneity was found among samples of *M. cephalus* in the north-west Pacific, as expressed by the presence of two lineages (Fig. 2). Lineage 1 dominates samples located within the continental shelf of the East China Sea (Qingdao, Keelung and Kaohsiung), whereas lineage 2 (Philippines) was found outside continental shelf waters (with the exception of Pearl River). Both lineages (1 and 2) were found in samples from Japan.

Questions regarding the taxonomic status of *M. cephalus* have been raised in many genetic studies (Crosetti *et al.*, 1994; Rossi *et al.*, 1998; Rocha-Olivares *et al.*, 2000, 2005; Fraga *et al.*, 2007; Heras *et al.*, 2008). These studies have revealed the presence of highly isolated populations but were unable to determine whether the absence of gene flow was due to genetic incompatibility or geographic discontinuities, primarily because the sampling locations covered widely separated areas. In this study, the sampling locations were concentrated within the distributional range of *M. cephalus* in the north-west Pacific. A mixing of the two lineages (1 and 2) in Japan was considered to be the result of a secondary contact of previously geographically isolated lineages. The northward dispersion of both lineages with the Kuroshio Current from the Philippines, Taiwan and Okinawa to Japan may have facilitated the mixing (Fig. 1). Finally, the absence of allopatric distribution of north-west Pacific *M. cephalus* lineages promotes the concept of a single *M. cephalus* species in the region.

Despite the above genetic evidence, the question of the taxonomic status of *M. cephalus* remains largely unresolved. Two forms of *M. cephalus*, with different reproductive behaviours, have been described in the north-west Pacific (Liu, 1986; Su & Kawasaki, 1995). In the coastal waters of Taiwan, both resident and migratory forms of *M. cephalus* have been reported (Huang *et al.*, 2001). The resident form lives in estuarine environments all year and reproduces at sea during late winter

(late December to late January). The migratory form reproduces in mid-winter (October to December) and then migrates from Taiwanese to Chinese coastal waters (Liu, 1986). If reproductive periods of the two forms overlapped, then gene flow between the resident and migratory forms would be possible (Huang *et al*., 2001). However, Huang *et al*. (2001) described extensive genetic differentiation at the glycosylphosphatidylinositol (GPI) enzyme, suggesting an absence of gene flow or strong selective pressure towards GPI polymorphism. Samples used in the present study could not be used to compare resident and migratory forms based on the ECS or SCS population. Samples from Taiwan and China, except those from the Pearl River, were collected from offshore waters during their spawning migration, while the other samples were collected from estuarine waters. Therefore, the resident and migratory forms of *M. cephalus* might be sympatric in the Pearl River, Okinawa and Yokosuka, as indicated by the mixing of the two lineages. Past geological events and contemporary environmental conditions might have favoured the divergence of resident and migratory forms of *M. cephalus*.

Further studies of life history, reproductive behaviour, recruitment and genetic architecture are required to determine whether the inferred secondary contact of previously isolated lineages of *M. cephalus* has resulted in either the formation of introgressed populations (*e.g.* two lineages within one population) or the coexistence in sympatry of genetically isolated races (*e.g.* each population/race/species is composed of only one lineage). A combined analysis of mtDNA and biparentally inherited markers could determine the level of genetic isolation of mtDNA lineages.

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