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Genetic isolation among populations of the landlocked freshwater shrimp *Macrobrachium* yui in the Mekong River basin

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Abstract. The landlocked freshwater shrimp *Macrobrachium yui* has a high economic value for the rural villages in the northern area of Laos. In order to manage shrimp stock, we investigated genetic stock identification and genetic diversity of this shrimp in the Mekong and Ma River basins using mitochondrial DNA control region sequences. In total, 570 nucleotide sequences were obtained from 355 specimens at seven sampling sites of tributaries of the Xuang River, Ou River, Khan River and Houng River from the Mekong River basin and the Et River from the Ma River basin, in which 58 variable sites and 74 haplotypes were identified. Only Et River has low value in haplotypes and nucleotide diversities. Mismatch distribution analyses was used to evaluate possible historical events of population growth and decline. Minimum spanning tree was separated into the major two clades among localities. Pairwise F_{st} values showed strongly significant genetic differences and these indicate that this species has a restricted migration and gene flow among the rivers. For sustainable shrimp management we propose to ban stocking the shrimp from different river population to avoid genetic contamination and to conduct stock management of the shrimp independently for each river.

Key words: *Macrobrachium yui,* gene flow, conservation, mitochondrial DNA

Introduction

The genus *Macrobrachium* is distributed in tropical and semi-tropical areas, with more than 200 species in the world (Short, 2000). Species of the genus *Macrobrachium* have the highest species diversity in the Indo-West Pacific accounting for 68% of the total number of species. Furthermore, the West Pacific is considered to be biogeographical source of Palaemonidae (Shokita, 1979; 1985). The life history of the genus *Macrobrachium* mainly has two groups. One is amphidromous species, which needs saline (10 ‰~35 ‰) for larval development. The females swim down to the estuary before the hatch of their eggs (Albertoni *et al*., 1999; Short, 2000). The other one is landlocked species, which spend whole life in freshwater (Holthuis, 1950; Shokita, 1979). These species which have an abbreviated larval period have a limited dispersal due to not undergoing larval

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migratory drift (Shokita, 1979; Jalihal *et al*., 1993). Therefore, the landlocked species have the restricted distribution, causing bigger genetic difference among habitat regions in comparison with amphidromous species (Cameron, 1986; Cook *et al*., 2002; Murphy & Austin, 2004).

The target species, *Macrobrachium yui* Holthuis, 1950, is landlocked species distributed from Yunnan province, southern part of China to a northern mountainous area of Indochina countries (Cai & Dai, 1999; Cai *et al*., 2004; Hanamura *et al*., 2011). Adult of this species moves up to a cave stream from a river for spawning. Hatched larvae in the cave stream undergo planktonic larval period and remain there until settling to the bottom (Ito *et al*., 2011). Egg number and size of this shrimp are quite similar to those of other landlocked *Macrobrachium* species (Ito, 2008). In Laos, the shrimp *M. yui* is distributed in tributaries of the upper reaches of the Mekong and Ma River basins. Local people catch the shrimp during the rainy season with traps of bamboo basket. The species is sold at a high price for domestic consumption, and forms a precious source of cash income for local farmers. However, the recent decrease of the shrimp catch by overfishing and deterioration of aquatic environments has damaged the cash income of the farmers. Stocking, farming and stock management measures are expected to protect the shrimp stock (Ito, 2008). Preliminary survey on the genetic diversity and differentiation among localities of this species was reported by Imai *et al*. (2012). Importance of the genetic differentiation among localities is essential for considering the best measures for stock enhancement.

This study intends to assess the amount of genetic diversity of the shrimp *M. yui* in each locality with nucleotide sequence analysis of mitochondrial DNA control region and to infer the population structure in northern Laos. On the basis of the results, we have some suggestions for stock enhancement in order to use the shrimp stock sustainably.

Material and methods

In total, 355 adult *M. yui* individuals were sampled in 2008; 50 individuals were collected from each of the Ken-sakan (B1) and Ken-bo (B2) Cave Streams pouring into the Xuang River. 51 individuals and 50 individuals were collected from the Gun Stream (H) and the Kok-dua Cave Stream (K) joining the Ou River, respectively. 50 individuals from the Ken-gun Cave Stream (XK) of the Khan River, 51 individuals from the Et Cave Stream (ET) of the Et River and 53 individuals from the Xay Stream (NS) of the Houng River were sampled (Table 1; Fig. 1). The captured shrimp had been in kept in ice soon after the sampling. Within 10 minutes of sampling, 50 mg of muscle tissue of the shrimp was extracted from the dorsal side of the body with scissors and tweezers and was preserved in a 1.5 -ml plastic test tube containing 0.5 ml TNES-8M urea buffer (Asahida *et al*., 1996) until DNA extraction using the modified procedure of Imai *et al*. (2004).

Amplification and Sequencing

The mtDNA control region was amplified by polymerase chain reaction (PCR) using primers Panulirus-12s(5'-TATAGCAAGAATCAAAC-TATAG-3') (Abdullah *et al*., 2014) and mac-tRNA (5'-CATTATTCGCCCTATCAAGACG-3') (Imai *et al*., unpublished data). These primers amplify a segment, approximately 1,000 base pairs (bp) in length, of the variable region of the mtDNA control region spanning from the 12S rRNA conserved region to the transfer RNA gene. The following reagents were added to each PCR microtube: 1 µl of template DNA, 12.5 pmol of each primer, 2.5 µl of 10 reaction buffer, 2.5 µl of deoxyribonucleotide triphosphate (dNTP) mixture and 1.25 unit of ExTaq™ DNA

Table 1. River basins, sampling sites, coordinates, dates, and sample size for *Macrobrachium yui*.

River basin	Sampling site	Coordinates	Date	n
Xuang River	Ken-saken cave (B1)	20° 07' 14" N, 102° 30' 57" E	July 2008	50
Xuang River	Ken-bo cave (B2)	20° 07' 31" N, 102° 32' 35" E	July 2008	50
Ou River	Gun stream (H)	20° 27' 27" N, 102° 33' 04" E	July 2008	51
Ou River	Kok-dua cave (K)	20° 34' 47" N, 102° 37' 29" E	July 2008	50
Khan River	Ken-gun cave (XK)	19° 40' 18" N, 102° 18' 52" E	July 2008	50
Ma River	Et cave (ET)	20° 44' 60" N, 103° 57' 29" E	Jan. 2009	51
Mekong River	Xay stream (NS)	19° 11' 42" N, 101° 46' 33" E	June 2009	53

polymerase (Takara Bio, Japan). Each sample was adjusted to 25 µl with distilled H_2O. PCR conditions consisted of plate heating (94 ºC, 120s) and 30 cycles of denaturation (94 ºC, 30s), annealing (45-51.5 ºC, 30s), and extension (72 ºC, 90s), and final extention (72 ºC, 90s) using the thermal cycler GeneAmp 9700 (Applied Biosystems, USA). PCR products were purified using a PCR Product Pre-sequencing kit (USB Co., USA). Amplified DNA was sequenced on the ABI 3700 genetic analyzer (Applied Biosystems, USA) using the Big Dye Terminator Cycle Sequencing kit ver. 3.1 (Applied Biosystems, USA).

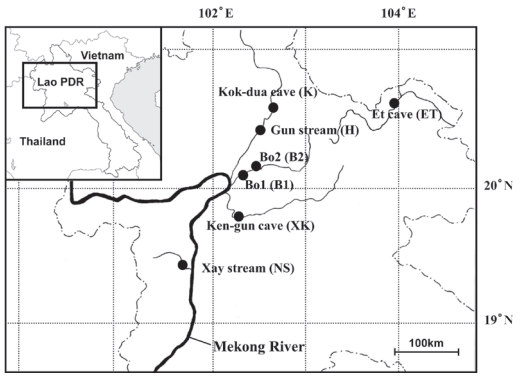

Fig. 1. Sampling sites of the shrimp *Macrobrachium yui*.

Data analysis

Nucleotide sequence data were aligned using the alignment software Clustal X version 1.83.1 (Thompson *et al*., 1997), followed by manual editing with MacClade version 4.08 (Maddison & Maddison, 2005). Haplotype diversity (*h*; Nei, 1987) and nucleotide diversity (π; Tajima, 1983) within populations were calculated using ARLEQUIN version 3.11 (Schneider *et al*., 2000). Population structure was investigated using the analysis of molecular variance (AMOVA) (Excoffier *et al*., 1992) package in ARLEQUIN. We also calculated the pairwise *F*st values and the significance test of the *F*st by 1,000 permutations to estimate the genetic divergence between paired populations using ARLEQUIN. The significance level of *F*st was tested using 10,000 permutations and the Bonferroni methods (Rice, 1989) were used to correct for multiple comparisons. To assess the relationships of haplotypes with the number of individuals, a minimum-spanning tree (MST) based on the number of nucleotide substitutions among haplotypes was constructed using ARLEQUIN and drawn by hand.

The population of historical demographic expansions were estimated by two different methods. Tajima's *D*-test (Tajima, 1989) and Fu's test (Fu & Li, 1993) were used to test if neutrality holds. The population demographic history was examined by calculating mismatch distributions over all haplotypes with ARLEQUIN. The parameter of the demographic expansion was τ estimated from mismatched data.

Results

In this study, 570 bp including mitochondria DNA control region were sequenced for a total of 355 specimens of the shrimp *M. yui* from seven sites and 58 variations in the base sequence were detected within the control region (Table 2). A total of 74 haplotypes was identified by comparison of base sequence among the individuals. The nucleotide sequences of the haplotypes were deposited in the DNA Data Bank of Japan (DDBJ) under accession numbers AB935252-AB935325. The number of haplotype detected in each locality are 17 haplotypes in the Ken-sakan Cave Stream (B1), 20 in Ken-bo Cave Stream (B2), 10 in the Gun Stream (H), 11 in the Kok-dua Cave Stream (K), 18 in the Ken-gun Cave Stream (XK), 6 in Et Cave Stream (ET) and 14 in the Xay Stream (NS). From our sampling of the shrimp *M. yui*, 13 haplotypes were shared between B1 and B2 in the Xuang River and 7 haplotypes between H and K in the Ou River. The remaining from six (Et River) to 22 (Xuang River) haplotypes were specific to one river. Genetic variability values in the seven localities were as follows: haplotype diversity h = 0.9061 and nucleotide diversity π = 0.0121 in B1, h = 0.9224 and π = 0.0086 in B2, h = 0.6824 and π = 0.0078 in H, h = 0.8433 and π = 0.0079 in K, h = 0.9910 and π = 0.0097 in XK, h = 0.4424 and π = 0.0008 in ET, and h = 0.8708 and π = 0.0039 in NS (Table 3). ET locality showed lower haplotype and nucleotide diversities than other localities.

Significant genetic differentiation was shown among five rivers (AMOVA: F_{CT} = 0.733, $P < 0.01$) and among populations (F_{ST} = 0.737, $P < 0.01$) but not among populations within the same river (F_{SC} = 0.013, P = 0.23; Table 4). An estimation of genetic homogeneity by AMOVA revealed that the genetic structure is not homogenous among five rivers (Table 4). Genetic differentiation was strongly significant between all populations except between B1 and B2 and between H and K when comparing all combinations (Bonferroni's correction methods: all combinations except B1 vs. B2 and H vs. K: $P < 0.001$, B1 vs. B2: P = 0.17, H vs. K: P = 0.34; Table5).

Mismatch Distribution Analysis showed bimodal distribution about frequency of nucleotide substitution (Fig. 2). The value for Tajima's D was not significant in all populations (B1: D = 0.209, P = 0.66, B2: D = -0.718, P = 0.25, H: D = 1.028, P = 0.87, K: D = 0.819, P = 0.85, XK: D = 0.585, P = 0.75, ET: D = -1.374, P = 0.067, NS: D = -0.468, P =0.37). MST for the shrimp *M. yui* showed the major two clades which are linked to each other by 13 base substitutions (Fig. 3). Haplotypes from H and K belong to Clade A, and the haplotypes from XK, ET, NS belongs to Clade B. Haplotypes from B1 and B2

Table 3. Haplotype diversity (h) and nucleotide diversity (π) in seven localities of *Macrobrachium yui* .

Sampling site	n	Number of haplotypes	h	π
Ken-sakan (B1)	50	17	0.9061 ± 0.020	0.0121 ± 0.006
Ken-bo (B2)	50	20	0.9224 ± 0.021	0.0086 ± 0.005
Gun stream (H)	51	10	0.6824 ± 0.062	0.0078 ± 0.004
Kok-dua cave (K)	50	11	0.8433 ± 0.036	0.0079 ± 0.004
Ken-gun cave (XK)	50	18	0.9110 ± 0.022	0.0097 ± 0.005
Et cave (ET)	51	6	0.4424 ± 0.074	0.0008 ± 0.001
Xay stream (NS)	53	14	0.8708 ± 0.028	0.0039 ± 0.002

Table 2. Variable sites within the mitochondrial DNA control region and haplotype distribution of *Macrobrachium yui* among seven localities.

Haplotype	Variable site	Localities						
	4681233356111111111222222333333333333344444444444445555555							
	8924542012333456234789000023444467901133445577790134666							
	0613797300202214567101348529303461967478318460 14	B1	B2	H	K	XK	ET	NS
1	GCAATAGTACGATCTATACTTGTACGGTTATGCCCATAACATAGTCTTTACTTGAGAG	1						
2	·····················A··	8	8					
3	·········A···········A····································		1					
4	·············T·······A····································	1	2					
5	·····················A······C·····························	1	1					
6	·····················A·······G····························	1						
7	·····················A·········A··························	9	3					
8	·····················A·········A···········A··············		1					
9	···············G·····A·········A···········A··············		1					
10	···············G·····A·····················A··············	6	2					
11	···············G·····A······C··············A··············	1	2					
12	···············G·····A·······G··············A··············	1	3					
13	···············G·····A·······G·············A·T············		1					
14	·T·G···········G·····A·····················A··············	2	2					
15	·T·············G·····A·····················A··············	8	10					
16	·T············GG·····A·····················A··············			1	3			
17	··············GG·····A·····················A··············			10	4			
18	··············GG·····A·····················A··············			1				
19	··············GG·····A·····················A········C·····			3	4			
20	·T·············G··G··A·········A···········A·····G········	2	5					
21	·T·············G·····A·········A···········A·····G········		1					
22	·T·············G··G··A·········A···········A··············		1					
23	·T·············G·····A·········A···········A··············		2					
24	··············GG·····A·········A··T········A··············	1	1					
25	··············GG·····A··T······A··T········A··············	1						
26	··············AG··G··A·······G·AA·T······C·A········C·····			27	17			
27	A·············AG··G··A·······G·AA·T······C·A········C··A··				7			
28	A·············AG··G··A·······G·AA·T········A········C·····			1	2			
29	A·············AG··G··A·······G·AA·T·C····C·A········C··A··				1			
30	A·············AG··G··A·······G·A··T······C·A········C··A··				1			
31	A·······G·····AG··G··A·········A··T······C·A········C····A			3	3			
32	A·······G·····AG··G··A·G·······A··T······C·A········C····A				2			
33	A·············AG··G··A·········A··T······C·A·······AC·····			1				
34	A·············AG··G·GA·········A··T······C·A········C·····			3	6			
35	··············AG··G·GA·········A··T········A········C·····			1				
36	A···AGAG·····TAG···G·A·G·······A··T·C··T···AAT····A·CT····	1				4		
37	A···AGAG·····TAG···G·A·G·······A··T·C··T···AAT··C·A·CT····					1		
38	A···AGAG·····TAG···G·A·G·······A··T·C··T···AGT····A·CT····					11		
39	A···AGAG·····TAG···G·A·G···G···A··T·C··T···AAT····A·CT····	3	1			1		

B1, Ken-sakan cave; B2, Ken-bo cave; H, Gun stream; K, Kok-dua cave; XK, Ken-gun cave; ET, Et cave; NS, Xay stream.

Haplotype	Variable site	Localities						
	4681233356111111111222222333333333333344444444444445555555							
	8924542012333456234789000023444467901133445577790134666							
	061379730020221456710134852930346196747831846014	B1	B2	H	K	XK	ET	NS
40	A···AGAG·····TAG···G·A·G···G···A··TCC··T···AAT····A·CT····					1		
41	A···AGAG···G·TAG···G·A·G···G···A··TCC··T···AAT····A·CT····					2		
42	A···AGAG·····TAG·····A·G···G···A··TCC··T···AAT····A·CT····					4		
43	A···AGAG·····TAG···G·A·G···G···A··T·C··T···AAT·CC·A·CT····					1		
44	A···AGAG·····TAG·····A·G···G·G·A··T·C··T···AAT····A·CT····	3	2					
45	A···AGAG·····TAG···G·A·G···G·G·A··T·C··T···AAT····A·CT····					1		
46	A···AGAG·····TAG··GG·A·G···G·G·A··T·C··T···AAT····A·CT···A					1		
47	A···AGAG·····TAG··GG·A·G···G·G·A··T·C··T···AAT····A·CT····					3		
48	A·C·AGAG·····TAG··GG·A·G···G·G·A··T·C··T···A·T····A·CT····					2		
49	A···AGAG·····TAG··GG·A·G·····G·A··T·C··T···AAT···GA·CT····					1		
50	·T··AGAG·····TAG·····A·G·······A··T·C··T···AAT··C·A·CT··G·					6		
51	·T··AGAG·····TAG·····A·G·······A··T·C··T··CAAT··C·A·CT··G·					1		
52	·T··AGAG·····TAGC··G·ACG······CA··T·C··TC··AAT·CC·A·CT····					7		
53	·T··AGAG·····TAGC··G·ACG·······A··T·C··TC··AAT·CC·A·CT····					1		
54	·T··AGAG·····TAGC··G·ACG·······A··T·CG·TC··AAT·CC·A·CT····					2		
55	AT··AGAG··A··TAG·····A·G·····G·AA······T···AAT····A·CT····							5
56	AT··AGAG··A··TAG·····A·G·····G·AA·····GT···AAT····A·CT····							1
57	AT··AGAG··A··TAG·····A·G··T··G·AA·T····T···AAT····A·CT····							8
58	AT··AGAG··A··TAG·····A·G·····G·AA·T····T···AAT····A·CT····							1
59	AT··AGAG··A··TAG·····A·G·····G·A··T····T···AAT····A·CT····							4
60	AT··AGAG··A··TAG·····A·G·····G·A·······T···AAT····A·CT····							1
61	AT··AGAG··A··TAG·····A·G·······A··T····T···AAT····A·CT····							7
62	AT··AGAG··A··TAG·····A·G·····G·AA·TC···T···AAT····A·CT····							1
63	AT··AGAG··A·CTAG·····A·G·····G·AA·TC···T···AAT····A·CT····							1
64	AT··AGAG··A··TAG·····A·······G·AA·T····T···AAT····A·CT····							4
65	AT··AGAG··A··TAG·····A·······G·AA·T·C··T···AAT····A·CT····							2
66	AT··AGAG··A··TAG·····A·G··T····AA·T····T···AAT····A·CT····							15
67	AT··AGAG··A··TAG·G···A·G··T····AA·T····T···AAT····A·CT····							1
68	AT·GAGAG··A··TA······A·G··T····AA·T····T···AAT····A·CT····							2
69	AT··AGAG·····TAG·····A·G·A·····A··TTC··T···AATC·C·A·CT····						37	
70	AT··AGAG·····TAG·····A·G·A·····A··TTC··T···AATC·C·A·CTG···						1	
71	AT··AGAG·····TAG·····A·G·A·····A··TTC··T···AATC·C·A·CT··G·						1	
72	AT··AGAG·····TAG·····A·G·A·····A··TTC··T···AATC·C·A·CT····						1	
73	·T··AGAG·····TAG·····A·G·A·····A··TTC··T···AATC·C·A·CT····						10	
74	AT··AGAG·····TAG·····A·G·A·····A··TTC··T···AAT··C·A·CT····						1	

B1, Ken-sakan cave; B2, Ken-bo cave; H, Gun stream; K, Kok-dua cave; XK, Ken-gun cave; ET, Et cave; NS, Xay stream.

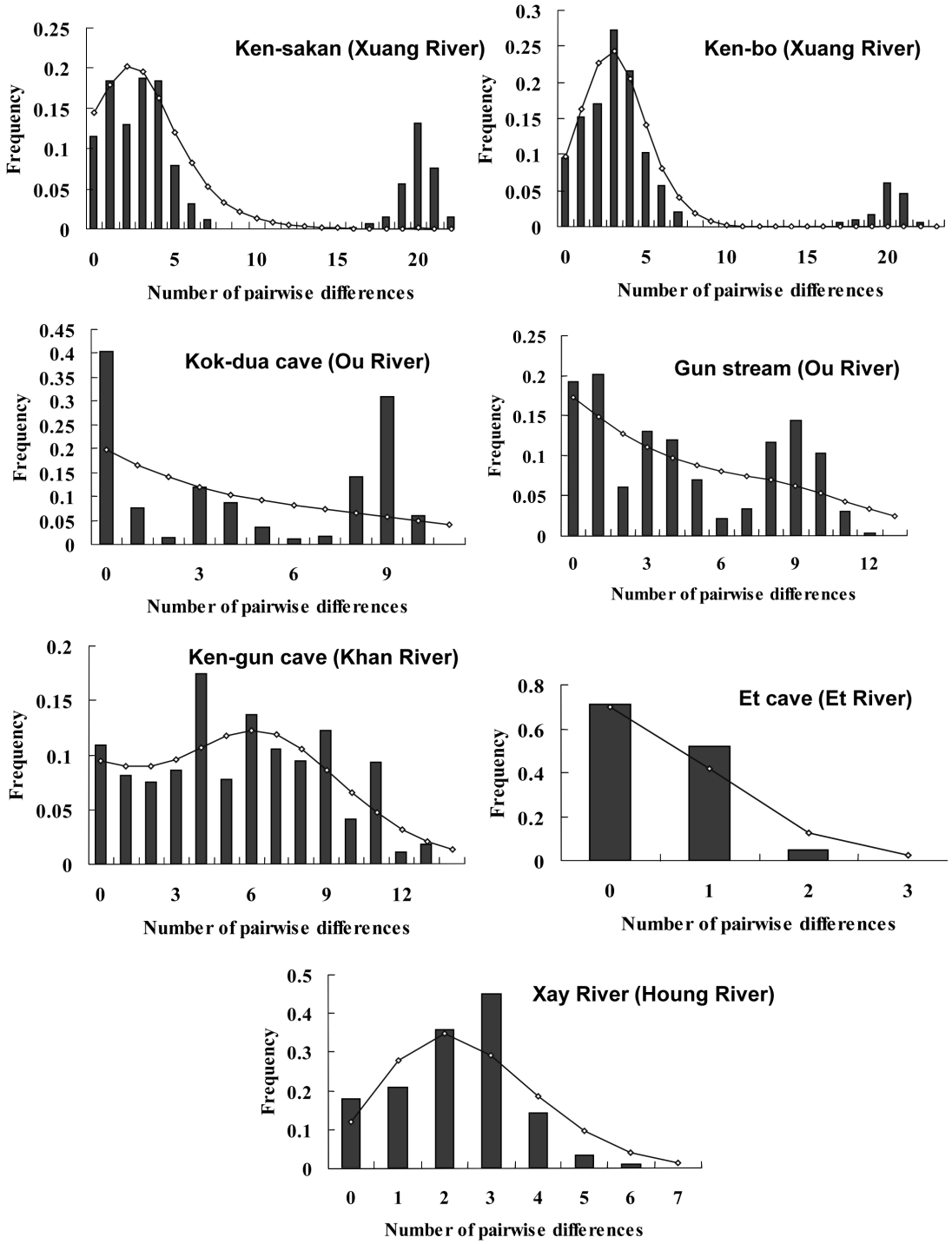

Fig. 2. The observed pairwise differences (bars), and the expected mismatch distributions under the expansion model (solid line) of control region haplotypes in the shrimp *Macrobrachium yui*.

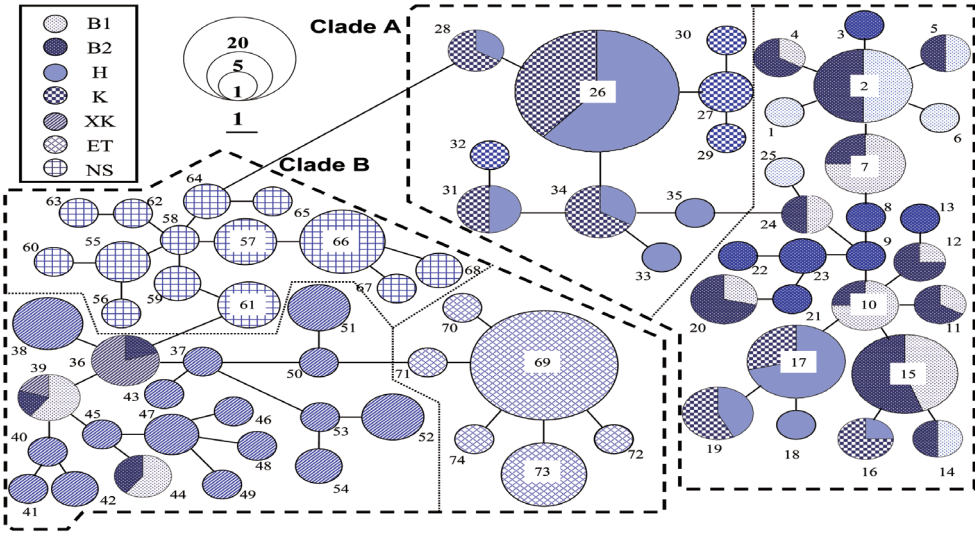

Fig. 3. Minimum spanning tree presenting relationships among 74 haplotypes in the shrimp *Macrobrachium yui*. Numerals indicate haplotype number. Scale bar indicates one substitution.

Table 5. Pairwise F_{ST} values (below the diagonal) and pairwise F_{ST} P values (above the diagonal) for the mitochondrial DNA control region among seven localities of *Macrobrachium yui*.

Localities	B1	B2	H	K	XK	ET	NS
B1	-	0.1738	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*
B2	0.0109	-	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*
H	0.3850	0.4411	-	0.3398	0.0000*	0.0000*	0.0000*
K	0.4230	0.4811	-0.0003	-	0.0000*	0.0000*	0.0000*
XK	0.6690	0.7389	0.7425	0.7406	-	0.0000*	0.0000*
ET	0.8199	0.8743	0.8837	0.8828	0.6159	-	0.0000*
NS	0.7550	0.8156	0.8116	0.8102	0.5438	0.8376	-

Bonferroni correction *$P < 0.001$.

belong to both Clade A and B.

Discussion

Six localities except ET showed the high haplotype diversity, the nucleotide diversity was lower in comparison with the general values for shrimps and prawns. This may be attributable to the genetic divergence of the locality at a river level as a result of the change in genetic frequency by founder effect. ET does not belong to the Mekong River system and is isolated from the other four rivers. This may explain the reason why the genetic diversity of ET is low.

The analysis of base sequence of the mitochondria DNA control region showed high haplotype diversity for the shrimp *M. yui* (mean h = 0.9591), as those for the giant river prawn *M. rosenbergii* in China (h = 1.000; Yao *et al*., 2007), the sakura shrimp *Sergia lucens* (h = 1.000; Imai *et al*., 2013), the pronghorn spiny lobster *Panulirus penicillatus* (h = 1.000; Abdullah *et al*., 2014), the pacific white shrimp *Litopenaeus vannamei* in the eastern Pacific Ocean (h = 0.8230; Jimenez *et al*., 2006) and the black tiger prawn *Penaeus monodon* in East China Sea (h = 0.9960; Zhou *et al*., 2009). On the other hand, the nucleotide diversity for *M. yui* (π = 0.0230) was at

the same level as that of *M. rosenbergii* (π = 0.022～0.032; Yao *et al*., 2007), *S. lucens* (π = 0.011～0.013; Imai *et al*., 2013) and the kuruma prawn *Marsupenaeus japonicus* (π = 0.0251; Tzeng *et al*., 2004), although the nucleotide diversity for *M. yui* is lower than those of *L. vannamei* (π = 0.0541; Jimenez *et al*., 2006), *P. monodon* (π = 0.0616; Zhou *et al*., 2009) and *P. penicillatus* (π = 0.03～0.05; Abdullah *et al*., 2014).

The result of Tajima's *D* showed that the neutrality of DNA variance was not significant for the seven localities of the shrimp *Macrobrachium yui*, implying that the mitochondrial DNA control region of the seven localities has accumulate neutral variance and selection pressure has not operated for a long time. The results of Fu's *Fs* tests did not deviate significantly from neutral evolution. The mismatch distribution analysis showed multimodal or unimodal distribution on frequency of nucleotide substitution except bimodal distribution of B1 and B2 (Xuang River). This result suggested a typical of demographically bottleneck population of Xuang River. Also, the bottleneck like shape of the MST distribution was observed for the Xuang River. As the result of MST for the shrimp *M. yui*, Clade A contained the haplotype of the northern populations (H, K) and Clade B contained that of the southern populations (XK, NS), indicating that the genetic lineages in northern Laos are divided into northern and southern lineages. However, the haplotype of B1 and B2 is present in both Clade A and B because the Xuang River, containing B1 and B2, is geographically on the boundary between the two genetic lineages. The haplotype of ET, belonging to Clade B, belongs to the southern genetic lineage even though ET is situated northeastern part of Laos and is out of the Mekong River system. The further data from other sampling sites would be required to draw the detailed distribution of the two Clade groups.

The evaluation of genetic homogeneity among different populations indicated that there are significantly genetic differences between all the sampling localities except between B1 and B2 and between H and K., suggesting that the gene flow is restricted among the five rivers and the population are structured within the five rivers almost without gene flow. The landlocked species generally has shorter larval period because a part of the development stages were abbreviated (Shokita, 1979; Jalihal *et al*., 1993). Therefore, the hatched larvae which have advanced developmental stage usually do not undergo migratory drifting to the downstream of the river, limiting the dispersal of the larvae. This leads to restricted distribution of the landlocked species (Cook *et al*., 2002; Murphy & Austin, 2004). Cook *et al*. (2002) reported that Australian landlocked species, *M. australiense* Holthuis, 1950 showed that population is genetically structured by water systems. The shrimp *M. yui* undergoes migratory drifting to the river after spending the planktonic larvae period in the cave stream and settling to the bottom (Ito, 2011). The cave water shows higher electric conductivity than the river water and contains salt slightly (Ito, 2011). Ito (2008) found that the survival rate of larvae until settling to the bottom becomes the highest at 3.5 ppt seawater though the seawater is not needed for rearing of the shrimp after settling to the bottom. Some substances contained in the cave water or 3.5 ppt seawater are essential for this species to develop into the juvenile healthy, restricting the spawning site of the shrimp. The specific ecological characteristics would promote genetic structure in this species.

The waters around the Mekong River have a rich aquatic fauna with more than 1,200 fish species inhabit there (Coates *et al*., 2003). Hurwood *et al*., (2008) showed the genetic differentiation among localities of the Mekong River and its tributaries in the silver mud carp *Henicorhynchus lobatus*. The giant catfish *Pangasianodon gigas* that the genetic diversity decreases recently is now designated as an

endangered species (Ngamsiri *et al*., 2003). One of the reasons of lower genetic diversity is attributed to smaller coefficient population size. If the population size is small, the partial hybridization of inbreeding coefficient increases, taking place loss of allele and inbreeding depression. This study proved that the six populations except ET population keep high genetic diversities. However, the catch of the shrimp has been decreasing year by year (Ito, 2011). Hereafter, the continuous monitoring on the health of the shrimp stock and genetic diversity is important for sustainable use of the shrimp stock. This study indicated that the gene flow of this species is restricted among tributaries. Therefore, we should avoid transferring shrimp among rivers, ie from one population to another, and for stocking seed using only broodstock from the target population so as to avert genetic contamination. The recent high fishing pressure would decrease effective population size of the shrimp within the river, suggesting that the shrimp stock management at a river population level is also important to maintain the genetic diversity of this species.

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