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Genetic Characterization of Two Mahseer Species (*Tor douronensis* and *Tor tambroides*) Using Microsatellite Markers from Other Cyprinids

(Pencirian Genetik dua Spesies Mahseer (*Tor douronensis* dan *Tor tambroides*) Menggunakan Penanda Mikrosatelit daripada Siprinid yang Berbeza)

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ABSTRACT

*This study examined the genetic characteristics of twenty-six microsatellite primers developed from three cyprinid fishes (*Cyprinus carpio *Linnaeus,* Barbus barbus *Linnaeus and* Barbonymus gonionotus *Bleeker) in two indigenous mahseer. The* Tor douronensis *Valenciennes were randomly collected from two locations in Sarawak (N=52), while* Tor tambroides *Bleeker were obtained from Peninsular Malaysia (N=56). A total of ten and twelve primers were successfully amplified producing four and five polymorphic loci in* T. douronensis *and* T. tambroides*, respectively. The number of alleles per locus ranging from 2 to 5 in* T. douronensis *and 2 to 7 in* T. tambroides. *A significant deviation from Hardy-Weinberg equilibrium (HWE) was observed at three loci (Barb37, Barb59 and Barb62) in one or more populations in* T. tambroides *while two loci (Barb37 and Barb62) were deviated in* T. douronensis *population of Batang Ai. Population structure analysis showed low level of inter-population genetic differentiation in both mahseer. Overall, the identified microsatellite loci should be useful in analysing* T. douronensis *and* T. tambroides *natural populations.*

Keywords: Cross-species study; genetic characterization; mahseer; microsatellites

ABSTRAK

*Kajian ini meneliti pencirian genetik dua puluh enam primer mikrosatelit yang dibentuk daripada tiga ikan siprinid (*Cyprinus carpio *Linnaeus,* Barbus barbus *Linnaeus and* Barbonymus gonionotus *Bleeker) ke atas dua ikan kelah indigenus. Ikan kelah* Tor douronensis *Valenciennes telah dipilih secara rawak dari dua tempat yang berbeza di Sarawak (N=52), manakala* Tor tambroides *Bleeker pula telah dikumpul secara rawak dari Semenanjung Malaysia (N=56). Sejumlah sepuluh primer berjaya diamplifikasi menghasilkan empat lokus polimorfik pada* Tor douronensis*; dan dua belas primer pada* Tor tambroides *dengan lima lokasi polimorfik. Nombor alel per lokus berjulat di antara dua hingga lima dalam* Tor douronensis *dan dua hingga tujuh dalam* Tor tambroides. *Penyimpangan daripada keseimbangan Hardy-Weinberg (HWE) yang signifikan telah dijumpai pada tiga lokus (Barb37, Barb59 dan Barb62) di dalam satu atau lebih populasi* T. tambroides *manakala dua lokus (Barb37 dan Barb62) mengalami penyimpangan dalam populasi* T. douronensis *dari Batang Ai. Analisis struktur populasi menunjukkan tahap perbezaan genetik yang rendah di peringkat inter-populasi dalam kedua-dua ikan kelah. Keseluruhannya, lokus mikrosatelit yang dikenalpasti berguna untuk menganalisis secara mendalam populasi semulajadi ikan* T. douronensis *dan* T. tambroides.

Kata kunci: Kajian spesies-silang; kelah; mikrosatelit; pencirian genetik

INTRODUCTION

The mahseer from the genus *Tor* Gray such as *Tor tambroides* Bleeker and *Tor douronensis* Valenciennes, are among the most valuable and highly priced cyprinid fish in Malaysia (Litis et al. 1997). The market price for mahseer is one of the highest due to their great taste, for example, the price of *T. douronensis* can reach above RM100/kg while *T. tambroides* reaches above RM400 (USD100)/ kg in the open market in Kapit, Sarawak (Ingram et al. 2005). Thus, fishes of the genus *Tor* have great potential for freshwater aquaculture industry (Ingram et al. 2005). In addition, the *Tor* fishes are also recognized as an excellent game fish, and have high demand in the ornamental fish industry due to their attractive colourations (Ng 2004).

Therefore, realizing the economic importance of the two mahseer and given their limited distributions and population size, studies on the population structure and level of genetic variations throughout their distribution range are required for effective management and conservation strategy of this important freshwater resource. Populations of these species are declining due to degrading environmental conditions by deforestation, logging and over fishing that may have disturb their natural habitat. In India matured male and female are very difficult to find for several species of mahseer such as *Tor putitora* Hamilton due to over fishing by sport anglers that crave only for large fishes (Patil & Lakra 2005).

Microsatellites or simple sequence repeats (SSRs) are short tandem repeat motifs (1-6 bases) with high levels of allelic polymorphism and co-dominant inheritance (DeWoody & Avise 2000; Jarne & Lagorda 1996; Zane et al. 2002). They are present in both coding and noncoding regions characterized by a high degree of length polymorphism (Zane et al. 2002), useful for direct assessment of pattern and distribution of genetic variability at the intraspecific level (O'Connell & Wright 1997; Primmer et al. 2006).

The flanking sequences of microsatellites within related taxa are found to be highly conserved (Scribner et al. 1996) including in fish (Rico et al. 1996), allowing cross-amplification from species that diverged as long as 470 million years ago (Zane et al. 2002). Thus, the potential of developing microsatellite markers through cross-species amplification is enhanced when primers designed for one species amplify homologous loci in other species (Zardoya et al. 1996; Zheng et al. 1995). A few cross-species amplification studies had identified polymorphic microsatellite loci from other fishes useful for population genetic structure analysis of the genus *Tor* e.g. *Tor putitora* from three other cyprinids (Mohindra et al. 2004) and *Tor tambroides* from a catfish, *Mystus nemurus* (Keong et al. 2008)).

The present study examines cross-species amplification of primers, developed for three cyprinids (*Cyprinus carpio*, *Barbus barbus* and *Barbonymus gonionotus*), in two indigenous mahseers, *T. douronensis* and *T. tambroides*. The objectives were to identify polymorphic microsatellite loci and to evaluate the suitability of the identified loci in population structure analysis of both mahseer in Malaysia.

Materials and Methods

Mahseer samples were difficult to obtain due to their reduced numbers in most of the major rivers (Ng 2004) and their natural populations are currently confined only to the upper streams of rivers or protected areas such as national parks. *Tor douronensis* samples were selected randomly from two locations in Sarawak; the Batang Ai River (N=37) and the Limbang River (N=15). The Batang Ai River is a tributary of the Batang Lupar River located in the southern part of Sarawak, while the Limbang River is located in northern part of Sarawak. Meanwhile, *T. tambroides* samples used in this study were obtained from three locations in Peninsular Malaysia; the Sia River (N=17), the Kampung Esok River (N=20) and the Perak River (N=19). The Sia River, Pahang and the Kampung Esok River, Negeri Sembilan, both served as tributaries of the Pahang River that drained to the South China Sea while the Perak River flow west into the Straits of Malacca. The mahseer samples were morphologically identified by using the keys provided by Mohsin and Ambak (1983), Kottelat et al. (1993), and Inger and Chin (2002).

Microsatellite primers from three freshwater cyprinids: *Cyprinus carpio* (Crooijmans et al. 1997), *Barbus barbus* (Chenuil et al. 1999) and *Barbonymus gonionotus* (Kamonrat et al. 2002; McConnell et al. 2001) were tested for amplification of homologous loci (Table 1). The initial cross-species amplification standardization including optimization of annealing temperature for each primer pair was carried out using eight random samples of *T. douronensis* and *T. tambroides* each. The primers yielding scoreable amplified products were further evaluated using larger sample sizes to assess their suitability in the population structure analysis of both mahseer.

The total DNA was extracted using the modified Cetyl Trimethylammonium Bromide (CTAB) method (Grewe et al. 1993) in the presence of Proteinase K. Polymerase chain reaction (PCR) amplifications were performed (Eppendorf) in a final volume of 10 µL, containing 25–50 ng of genomic DNA, 1X PCR buffer (10 mM Tris-HCl, pH 9.0; 50 mM KCl; 0.01% gelatin), 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 5 pmol of each primer and 1.5 units of *Taq* DNA polymerase. Amplification conditions were 94°C for 5 min followed by 25 cycles at 94°C for 30 s, T_a for 30 seconds and 72°C for 1 min, with a final extension of 72°C for 4 min. The optimum annealing temperature (T_a), was determined through experimental standardization for each primer pair.

After amplification, 10 µL of PCR products were electrophoresed on 4% high resolution MetaPhore agarose gels for 2 h at 78V/cm. The gels were stained using ethidium bromide (0.1 µL/mL) and photographed under UV light using an Alpha Imager 2200. The alleles were designated according to PCR product size and calculated relative to a standard molecular marker (20 bp and 100 bp; Cambrex).

Microsatellite genetic diversity was quantified as the number of alleles (A), the allelic richness A_R (the measure of the number of alleles per locus independent of the sample size), observed (H_o) and expected (H_e) heterozygosity values, and inbreeding coefficient (F_{IS} or f) as a measure of heterozygote deficiency or excess (Weir & Cockerham 1984) using FSTAT version 2.9.3.2 (Goudet 2001). GENEPOP version 3.3 (Raymond & Rousset 1995) was used to test genotypic distributions for conformance to Hardy–Weinberg expectations (HWE) and to test for genotypic disequilibria at each locus for each population using the Markov chain method (Guo & Thompson 1992). Genetic homogeneity tests of genotype frequency distribution at each locus were determined through an exact G- test (Goudet et al. 1996) in order to test the null hypothesis of no genetic differentiation between populations, also using FSTAT. Sequential Bonferroni adjustments (Rice 1989) were applied to correct for the effect of multiple tests.

Genetic differentiation among populations was measured by the fixation index F_{ST}, calculated according to Weir and Cockerham (1984) using ARLEQUIN version 3.0 (Excoffier et al. 2005). Permutation tests (10,000 permutations) were performed in order to determine if estimates differed significantly from zero. The genetic distance between populations (rivers) in both mahseer was calculated based on an unbiased measure following

Nei (1978). An Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) dendrogram was constructed to illustrate the relations among geographic samples using POPGENE 1.32 (Yeh & Boyle 1997). Finally, a Bayesian approach was used to infer the number of clusters (*K*) in the data set without prior information of the sampling locations, available in STRUCTURE version 2.0 (Pritchard et al. 2000). A model where the allele frequencies were correlated within populations was assumed (λ was set at 1, the default value). The software was run with the option of admixture, allowing for some mixed ancestry within individuals, and α was allowed to vary. Five independent runs were done for each value of *K* (K= 1 to 5) with a burn–in period of 25,000 iterations and 25,000 replications.

RESULTS

Of the 26 heterologous primer pairs tested, only 10 (38%) and 12 (46%) primers produced successful amplification of homologous loci in *T. douronensis* and *T. tambroides*, respectively (Table 1). In *T. douronensis*, four primers (Bgon13, Barb37, Barb62 and MFW7) were polymorphic exhibiting 2-5 alleles while the other six primers (Bgon22, Bgon69, Bgon75, MFW1, MFW5 and MFW11) were monomorphic when tested using all 52 individuals. In *T. tambroides*, five primers (Bgon13, Barb37, Barb59, Barb62 and MFW7) were polymorphic exhibiting 2-7 alleles while primers Bgon22, Bgon69, Bgon75, MFW1, MFW5, MFW11 and MFW17 produced a monomorphic band in all the 56 individuals tested. The characteristics of the polymorphic primers of both mahseer are summarized in Table 2.

The observed heterozygosity (H_o) values over all loci in *T. douronensis* ranged from 0.0606 (Locus MFW7 of Batang Ai) to 0.3939 (locus Barb62 of Batang Ai) (Table 4). In *T. tambroides*, the H_o values ranged from 0.0000 (locus MFW7 of N. Sembilan) to 0.8421 (locus Barb59 of Perak) (Table 3). Test of linkage disequilibrium between loci found no significant disequilibrium among pairwise comparisons (data not shown).

A significant deviation from Hardy-Weinberg equilibrium (HWE) was observed at three loci (Barb37, Barb59 and Barb62) in one or more populations in *T. tambroides* (Table 3) while two loci (Barb37 and Barb62) deviated in the *T. douronensis* population of Batang Ai (Table 4). Homogeneity test found significant heterogeneity (p <0.05) in genotype proportions after Bonferonni correction observed at locus Barb37 in *T. douronensis* while three of five loci (MFW7, Barb59 and Barb62) were significant in *T. tambroides*. Combined probabilities over all loci were significant (p <0.05) (data not shown).

Pairwise estimates of F_{ST} over all loci between samples in both species are presented in Table 5. Within *T. tambroides*, only one (between the Pahang and the Perak populations) pairwise estimate of F_{ST} showed significant genetic differentiation (p< 0.05) while pairwise estimates of F_{ST} showed no significant differentiation between *T. douronensis* populations from Batang Ai and Ulu Limbang. Pairwise estimates of genetic distances computed by Nei (1978) among populations (Table 5) showed that the highest genetic distance was between *T. tambroides* population from Pahang and *T. douronensis* population from Ulu Limbang (F_{ST}=0.4187).

Bayesian cluster analysis performed with STRUCTURE showed that the most likely *K* value identified was *K* = 2, and results from other *K* values (*K*= 3 to *K*= 5) did not identify any formation of additional clusters (Figure 1). The two identified cluster correspond to the two mahseer studied; Cluster 1 consisted of the three *T. tambroides* populations while Cluster 2 consisted of the two *T. douronensis* populations. No evidence of population substructuring was found in either species based on the STRUCTURE analysis. The UPGMA dendrogram also generated two clusters corresponding to the two species studied (Figure 2), similar to those identified by STRUCTURE.

DISCUSSION

The results of this study showed the potential of finding polymorphic microsatellites loci through a rapid non-cloning method. Although only a small proportion of polymorphic loci (10% (four out of 26) in *T. douronensis* and 12% (five out of 26 loci) in *T. tambroides*) were found, the genetic diversity parameters were comparable to the results found in other cross-species amplification studies of mahseer. This includes a study by Keong et al. (2008)

TABLE 1. Primers of microsatellite loci tested for cross-species amplification in *Tor douronensis* and *Tor tambroides*

Source species	Number of primer pairs tested	Locus	References	Successful amplification (n(%))	
				T. douronensis	*T. tambroides*
Barbonymus gonionotus	10	Bgon2, 8, 12, 13, 17, 19, 22, 69, 75, 79	McConnell et al. 2001, Kamonrat et al. 2002	4 (40)	4(40)
Barbus barbus	4	Barb37, 54, 59, 62	Chenuil et al. 1999	2 (50)	3(75)
Cyprinus carpio	12	MFW1, 2, 5, 7, 11, 15, 17, 18, 19, 24, 26, 28	Crooijmans et al. 1997	4 (33)	5(42)
Total tested	26			10 (38)	12(46)

TABLE 2. Characteristics of amplified microsatellite loci in details of *Tor douronensis* and *T. tambroides*

Resource species					*T. douronensis*		*T. tambroides*	
Species	Locus	Primer sequence (5' to 3')	Repeat motif	T_a (ºC)	T_a (ºC)	No of alleles	T_a (ºC)	No of alleles
Barbonymus gonionotus	Bgon13	F: CCCGTGCAATTCAATATG R: TAAGTAGCACAGATGTGAGG	GT	53	50	2	50	2
	Bgon22	F: TCTTGTTGATCACACGGACG R: GTGACTGTATCAATGAGTCTG	TCC	49	50	2	50	2
	Bgon69	F: GCAAAGGTTCTGTCAAGG R:GTATCCAGAAACATGTTCAG	TG	49	46	1	46	1
	Bgon75	F: CTGGTAAAGACTTCAGATGC R: GCATGCAAAATGAGAAAGGCT	AC	53	46	1	46	2
Barbus barbus	Barb37	F: AAATACGCTCTCCTCATTAC R: GTACAAAAGCAAAAATAAATTA	ATTT	50	50	3	50	3
	Barb59	F: CTGTATCCATCACATAGGCT R: CATGATTTAATAGAACACACAC	GATA	56	-	-	50	7
	Barb62	F: GGCACAAAAATGGATTCATATC R: GTACACGAGCATATGGACAA	ATTT	58	50	5	50	4
Cyprinus carpio	MFW1	F: GTCCAGATCGTTCATCAGGAG R: GAGGTGTACACTGAGTCACGC	CA	55	50	1	50	1
	MFW5	F: GAGATGCCTGGGGAAGTCAC R: AAAGAGAGCGGGGTAAAGGAG	CA	55	65	1	65	1
	MFW7	F: TACTTTGCTCAGGACGGATGC R: ATCACCTGCACATGGCCACTG	CA	55	65	2	65	2
	MFW11	F: GCATTTGCCTTGATGGTTGTG R: TCGTCTGGTTTAGAGTGCTGC	CA	55	50	1	50	1
	MFW17	F: CAACTACAGAGAAATTTCATG R: GAAATGGTACATGACCTCAAG	CA	55	-	-	50	1

TABLE 3. Parameters of genetic variability for polymorphic microsatellite locus in *T. tambroides* samples from three rivers. Given are number of alleles (A), allelic richness (A_R), inbreeding coefficient (F_{IS}), observed (H_o) and expected (H_e) heterozygosity values, the probability of Hardy-Weinberg equilibrium (HWE) and the probability of genotype homogeneity between samples

Locus	Population	A	A_R	H_o	H_e	F_{IS}	HWE (p-value)[1]	Genotype homogeneity (p-value)[2]
MFW7	Pahang	2	2.0000	0.3529	0.2907	-0.2143	0.4256	0.0100*
	N. Sembilan	1	1.0000	0.0000	0.0000	0.0000	-	
	Perak	2	2.0000	0.3158	0.2659	-0.1875	0.4606	
Barb37	Pahang	3	3.0000	0.2353	0.4792	0.5090	0.0011**	0.3620
	N. Sembilan	3	2.8000	0.5500	0.4712	-0.1671	0.4371	
	Perak	3	3.0000	0.2632	0.4806	0.4525	0.0000*	
Barb59	Pahang	7	7.0000	1.0000	0.6816	-0.4670	0.0009*	0.0000**
	N. Sembilan	4	3.7940	0.5000	0.5775	0.1342	0.0278*	
	Perak	7	6.526	0.8421	0.7604	-0.1705	0.0003*	
Barb62	Pahang	3	3.0000	0.5294	0.4862	0.1530	0.0001*	0.0000**
	N. Sembilan	4	3.6000	0.1500	0.3362	0.5539	0.0134*	
	Perak	3	2.9790	0.5263	0.4598	-0.1446	0.0000**	
Bgon13	Pahang	2	1.9980	0.4235	0.1107	-0.0625	0.8575	0.8100
	N. Sembilan	2	1.9640	0.1000	0.0950	-0.0526	0.8694	
	Perak	2	1.9980	0.1579	0.1454	-0.0857	0.7632	

(P-value)[1] **P<0.001 and *P<0.01
(P-value)[2] **P<0.001

TABLE 4. Parameters of genetic variability for polymorphic microsatellite locus in *T. douronensis* samples from three rivers. Given are number of alleles (A), allelic richness (A_R), inbreeding coefficient (F_{IS}), observed (H_o) and expected (H_e) heterozygosity values, the probability of Hardy-Weinberg equilibrium (HWE) and the probability of genotype homogeneity between samples

Locus	Population	A	A_R	H_o	H_e	F_{IS}	HWE (p-value)[1]	Genotype homogeneity (p-value)[2]
MFW7	Batang Ai	2	1.6360	0.0606	0.0588	-0.0313	0.8997	0.5850
	Ulu Limbang	2	2.0000	0.0769	0.0740	-0.0400	1.0000	
Barb37	Batang Ai	3	2.9790	0.2424	0.4155	0.4166	0.0000**	0.0180*
	Ulu Limbang	2	2.0000	0.0769	0.0740	-0.0400	1.0000	
Barb62	Batang Ai	4	3.3810	0.3939	0.6028	0.3465	0.0012**	0.1980
	Ulu Limbang	3	3.0000	0.3846	0.5178	0.2571	0.5153	
Bgon13	Batang Ai	2	1.9260	0.1515	0.1400	-0.0820	0.6758	0.3640
	Ulu Limbang	2	2.0000	0.0769	0.0740	-0.0400	1.0000	

(*P*-value)[1]***P*<0.001 and **P*<0.01

(*P*-value)[2]***P*<0.001

TABLE 5. Estimates of pairwise genetic distances (Nei 1978; below diagonal) and F_{ST} (Weir & Cockerham 1984; upper diagonal) among populations of *T. tambroides* and *T. douronensis*

		T. tambroides			*T. douronensis*	
		Pahang	N. Sembilan	Perak	Batang Ai	Ulu Limbang
T. tambroides	Pahang	-	0.0409	0.0499*	0.3170*	0.4187*
	N. Sembilan	0.0134	-	0.0000	0.2238*	0.2724*
	Perak	0.0181	0.0000	-	0.2163*	0.2581*
T. douronensis	Batang Ai	0.1952	0.1490	0.1412	-	0.0361
	Ulu Limbang	0.2201	0.1526	0.1416	0.0128	-

**P*-value, significant level (*P*< 0.05)

in *T. tambroides* using five polymorphic microsatellites developed from a catfish (*Mystus nemurus*) (number of allele per locus ranged from 1 to 7 and observed heterozygosities ranged from 0.2400 to 1.0000), and a study by Mohindra et al. (2004) in *Tor putitora* using seven (22%) polymorphic (out of a total of 32 primers tested) microsatellites developed from *Catla catla*, *C. carpio* and *B. barbus* (number of alleles per locus ranged from 1 to 10 and observed heterozygosities ranged from 0.0000 to 0.9000).

However, the current genetic diversity results were lower than those found by Nguyen et al. (2006) in microsatellites developed for *T. tambroides* (number of alleles per locus ranged from 1 to 9 and observed heterozygosities ranged from 0.0390 to 0.7510) and subsequent cross-species study in *T. douronensis* by Nguyen (2007) (number of alleles per locus ranged from five to 21 and observed heterozygosities ranged from 0.0400 to 0.7850). Nevertheless, the results of this study supported the hypothesis that certain sequences flanking the microsatellite regions of the genome might be conserved among the cyprinids (Zane et al. 2002), thus potentially allowing primers to be used interspecifically among cyprinids (Lal et al. 2004; Mohindra et al. 2004; Nguyen 2007; Yue & Orban 2002).

The results of this study also showed that the optimum annealing temperature (T_a °C) observed in both mahseer differed from that reported in the source species for the respective primer pair, except in Barb37 similar to the findings by Mohindra et al. (2004). The fact that eight out of 12 (67%) of the successfully amplified primers in this study exhibited annealing temperature lower that

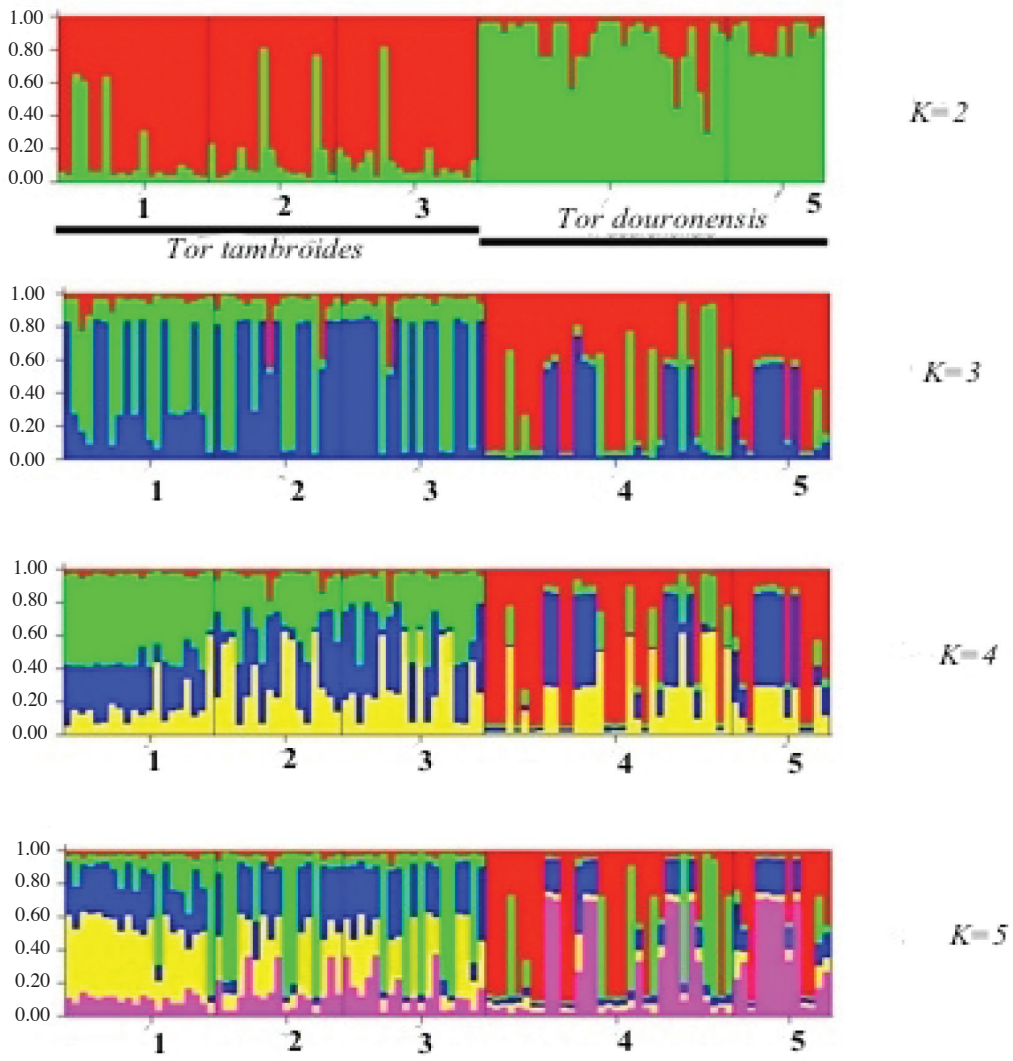

FIGURE 1. Proportional membership (Q) of each individual of *T. tambroides* and *T. douronensis* identified by STRUCTURE at *K*=2 to *K*=5. The numbers in theX-axis correspond to a specific population: 1-Negeri Sembilan, 2-Pahang, 3-Perak, 4-Batang Ai, 5-Ulu Limbang

those found in the source species supported the general assumption that cross-species amplification tends to have lower annealing temperature as compared with the species where the primer(s) were originally developed (Zane et al. 2002).

Two and three out of the five polymorphic loci were not in Hardy-Weinberg equilibrium (HWE) in *T. douronensis* and *T. tambroides* in one or more population, respectively. Departure from HWE may result from one or more of the followings reasons: (i) Sampling error because only a small sample size was studied, thus did not have a true representation of the population allele frequencies (Mohindra et al. 2004). (ii) Wahlund effect, i.e. presence of fewer heterozygotes in a population than predicted on account of a population subdivision (Kumar et al. 2006). (iii) presence of null alleles as suggested by excess of homozygotes for most allele size classes (Nguyen 2007) and (iv) Reduction in the effective breeding population size in *T. tambroides* as a result of overexploitation and/or anthropogenic disturbances (i.e. river pollution, deforestration, watershed erosion etc).

Our microsatellite analysis showed low levels of genetic differentiation among the three *T. tambroides* populations of Peninsular Malaysia (N. Sembilan, Pahang and Perak) similar to the results found using mitochondrial cytochrome c oxidase I sequences (Esa et al. 2008). In addition, the two *T. douronensis* populations (Batang Ai and Ulu Limbang) separated by high geographical distance (around 800 km) also showed very low genetic substructuring between them and was not concordant with the high population structuring results found by Nguyen (2008). Thus, the inclusion of more polymorphic microsatellite loci and of sample sizes in each population might provide a better resolution of the population genetic structure of the two mahseer species.

Overall, the cross-species amplification study identifies five polymorphic microsatellite loci with considerable genetic variation potentially useful in the fine

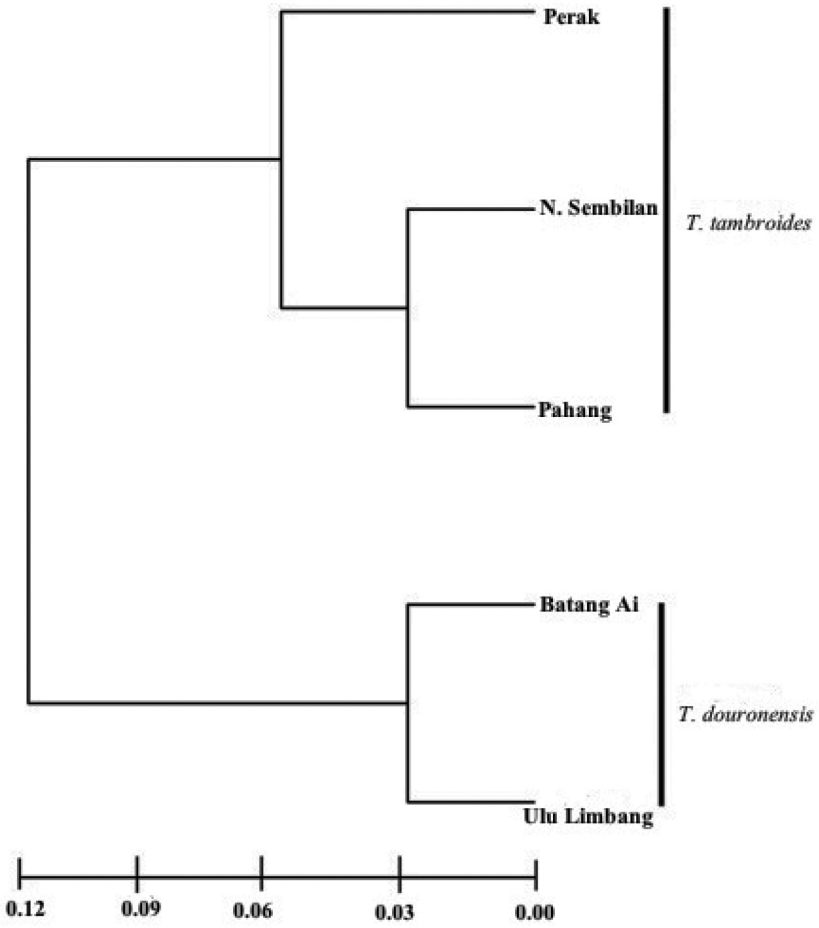

FIGURE 2. UPGMA dendrogram showing the genetic relationships among populations of *T. tambroides* and *T. douronensis*

scale population structure analysis of *T. douronensis* and *T. tambroides* natural populations.

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REFERENCES

Chenuil, A., Galtier N. & Berrebi, P. 1999. A test of the hypothesis of an autopolyploid vs allopolyploid origin of a tetraploid lineage. Application to the genus *Barbus* (Cyprinidae). *Heredity* 82: 373-380.

Crooijmans, R.P.M.A., Bierbooms, V.A.F., Komer, J., Van der Poel, J.J. & Groenen, M.E.M. 1997. Microsatellite markers in common carp (*Cyprinus carpio*). *Animal Genetics* 28: 129-134.

DeWoody, J.A. & Avise, J.C. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *Journal Fisheries Biology* 56: 461-473.

Esa, Y.B., Siraj, S.S., Daud, S.K., Khairuladha, A.R., Ryan, J.R.J. & Tan, S.G. 2008. Mitochondrial DNA diversity of *Tor tambroides* Valenciennes (Cyprinidae) from five natural populations in *Malaysian Zoology Study* 47(3): 360-367.

Excoffier, L., Laval, G. & Schneider, S. 2005. Arlequin Version 3.0: An integrated software package for population genetics data analysis. *Evolution Bioinformat Online* 1: 47-50.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). http://www.unil.ch/izea/softwares/fstat.html.

Goudet, J., Raymond, M., Demeeus, T. & Rousset, F. 1996. Testing differentiation in diploid populations. *Genetics* 144: 1933-1940.

Grewe, P.M., Krueger, C.C., Aquadro, C.F., Bermingham, E., Kincaid, H.L. & May, B. 1993. Mitochondrial variation among lake trout (*Salvenilus namaycush*) strains stocked into Lake Ontario. *Canadian Journal of Fisheries Aquatic Science* 50: 2397-2403.

Guo, S.W. & Thompson, E.A. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics* 48: 361-372.

Inger, F.R. & Chin, P.K. 2002. The freshwater fishes of North Borneo. (Revised edition with supplementary chapter by PK Chin). *Natural History Publications* (Borneo), Kota Kinabalu.

Ingram, B.A., Sungan, S., Gooley, G.J., Sim, Y.S., Tinggi, D. & De Silva, S.S. 2005. Induced spawning, larval development and rearing of two indigenous Malaysian Mahseer, *Tor tambroides* and *Tor douronensis*. *Aquaculture Research* 36: 1001-1014.

Jarne, P. & Lagoda, P.J. 1996. Microsatellites, from molecules to populations and back. *Trends Ecology Evolution* 11(10): 424-429.

Kamonrat, W., McConnell, S.K.J. & Cook, D.I. 2002. Polymorphic microsatellite loci from the Southeast Asian cyprinid, *Barbodes gonionotus* (Bleeker). *Molecular Ecology Note*s 2: 89-90.

Keong, R.B.P., Siraj, S.S. & Daud, S.K. 2008. Cross species amplification of ikan kelah, *Tor tambroides* by using *Mystus nemurus* microsatellite markers. *Pertanika Journal Tropical Agriculture Science* 31(2): 179-189.

Kottelat, M., Whitten, A.J., Kartikasari, S.N. & Wirjoatmodjo, S. 1993. *Freshwater fishes of Western Indonesia and Sulawesi*. Singapore: Periplus Edition.

Kumar, S., Gupta, J., Kumar, N., Dikshit. K., Navani, N., Jain, P. & Nagarajan, M. 2006. Genetic variation and relationships among eight Indian riverine buffalo breed. Mol*ecular Ecolog*y 15: 593-600.

Lal, K.K., Chauhan, T., Mandal, A., Singh, R.K., Khulbe, L., Ponniah, A.G. & Mohindra, V. 2004. Identification of microsatellite DNA markers for population structure analysis in Indian major carp, *Cirrhinus mrigala* (Hamilton-Buchanan, 1882). *Journal Applied Ichthyology* 20: 87-97.

Litis, B.A., Sungan, S., Jugang, K., Ibrahim, M. & Bin, H.A. 1997. *Features of Indigenous Fish Species having Potential for Aquaculture*. Inland Fisheries Division, Department of Agriculture, Sarawak, Kuching.

McConnell, S.K.J., Skibinski, D.O.F. & Mair, G.C. 2001. Microsatelite markers from a commercially important South-east Asian cyprinid, the silver barb (*Barbodes gonionotus* Bleeker). *Molecular Ecology Notes* 1: 135-136.

Mohindra, V., Ranjuna, K. L., Ponnial, A.G. & Lal, K.K. 2004. Microsatellite loci to access genetic variations in *Tor putitora*. *Journal Applied Ichthyology* 20: 466-469.

Mohsin, A.K.M. & Ambak, M.A. 1983. *Freshwater fishes of Peninsular Malaysia*. Serdang: Universiti Pertanian Malaysia Publication.

Nei, M. 1978. Estimation of heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.

Ng, C.K. 2004. *Kings of the Rivers: Mahseer in Malaysia and the Region*. Inter Sea Fishery (M), Kuala Lumpur.

Nguyen. T., Baranski, M., Rourke, M. & McPartlan, H. 2006. Characterization of microsatellite DNA markers for a mahseer species, *Tor tambroides* (Cyprinidae) and cross-species amplification in four congeners. *Molecular Ecology Notes* 7: 109-112.

Nguyen, T.T.T. 2007. Population structure in the highly fragmented range of *Tor douronensis* (Cyprinidae) in Sarawak, Malaysia revealed by microsatellite markers. *Freshwater Biology* 53: 924-934.

O'Connell, M. & Wright, J.M. 1997. Microsatellite DNA in fish. *Review of Fish Biology and fisheries* 7: 331-363.

Patil, R. & Lakra, W.S. 2005. Effect of cryoprotectants, equilibration periods and freezing rates on cryopreservation of spermatozoa of mahseer, *Tor khudree* (Sykes) and *T. putitora* (Hamilton). *Aquaculture Rese*arch 36: 1465-1472.

Primmer, C.R., Veselov, A.J., Zubchenko, A., Poututkin, A., Bakhmet, I. & Koskinen, M.T. 2006. Isolation by distance within a river system: genetic population structuring of Atlantic salmon, *Salmo salar,* in tributaries of the Varzuga River in northwest Russia. *Molecular Ecolog*y 15: 653-666.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

Raymond, M. & Rousset, F. 1995. GENEPOP: population genetic software for exact test ecumenicism. *Journal Heredity* 86: 248-249.

Rice, W.R. 1989. Analysing tables of statistical tests. *Evolution* 43: 223-225

Rico, C., Rico, I. & Hewitt, G. 1996. 470 million years of conservation evolution-evidence for directionality and variation in rate between species. *Natural Genetics* 10: 337-343.

Scribner, K.T., Gust, J.R. & Field, R.L. 1996. Isolation and characterization of novel salmon microsatellite loci: cross species amplification and population genetics application. Can. J. Fish. A*quatic Science* 53: 833-841.

Weir, B.S. & Cockerham, C.C. 1984. Estimating F-statistics from the analysis of population structure. *Evolution* 38: 1358-1370.

Yeh, F.C. & Boyle, T.H.B. 1997. Population genetic analysis of codominant and dominant markers and quantitative traits. *Belgian Journal of Botany* 129: 157.

Yue, G.H. & Orban, L. 2002. Polymorphic microsatellites from silver crucian carp (*Carassius auratus gibelio* Bloch) and cross amplification in common carp (*Cyprinus carpio* L.). *Molecular Ecology Notes* 2: 534-536.

Zane, L., Bargelloni, L. & Patarnello, T. 2002. Strategies for microsatellite isolation: a review. *Molecular Ecology* 11: 1-16.

Zardoya, R., Vollmer, D.M., Craddock, C., Streetman, J.T., Karl, S. & Meyer, A. 1996. Evolutionary conservation of microsatellite flanking regions and their use in resolving the phylogeny of cichlid fishes (Pisces: Perciformes). *Proceeding Royal Society London* B 263: 1589-1598.

Zheng, W., Stacey, N.E., Coffin, J. & Strobeck, C. 1995. Isolation and characterization of microsatellite loci in goldfish *Carassius auratus*. *Molecular Ecology* 4: 791-792.

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