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Characterization of microsatellites in white croaker (*Pennahia argentata*) through cross species amplification of *Miichthys miiuy*

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Introduction

White croaker (*Pennahia argentata*), family Sciaenidae, is a demersal fish that inhabits sandy or muddy bottoms in coastal inlets to a depth of 40–100 m. It is widely distributed in northwest Pacific along the coastal seas of China, Japan and Korea (FishBase 2011, http://www.fishbase.org). As the major component of demersal fish in the coasts of China and Japan, white croaker supports an important commercial fishery (Taki 2000). But in the past decades, the resource of white croaker has been brought to the risk of exhaustion because of overfishing and heavy marine pollution (Seikai National Fisheries Research Institute 2001). Thus, the availability of molecular tools for population genetic studies is highly important to obtain the information of its genetic background for effective conservation and use.

Microsatellites are one of the most powerful molecular markers to estimate population genetic parameters because of its high genetic information (Morgante and Olivieri 1993). It has been successfully used in genetic mapping, population genetics, and marker assisted selection (Rehem *et al.* 2010; Afanas'ev *et al.* 2011; Sun *et al.* 2010). However, no microsatellite marker is available for genetic variation in white croaker. Previous researches were mainly focussed on phylogeny (Kojima 1967; Hu and Qian 1989; Higuchi *et al.* 2003), geographic variation and evolutionary taxonomy (Han *et al.* 2008; Li *et al.* 2010). A very few information related on genetic structure of white croaker was available among four populations between China and Japan coastal waters (Han *et al.* 2009).

Development of polymorphic molecular markers is the first step for genetic studies, but the technology of isolating and characterizing molecular markers generally requires a lot of time and resource for each target species. When cross-amplified PCR isolated polymorphic loci from the previously developed markers in related species, it has been frequently used in microsatellites isolation (Fraser *et al.* 2005; Kim *et al.* 2009). In this study, the polymorphic microsatellite loci of white croaker were isolated from the markers initially developed in *Miichthys miiuy* and *Collichthys lucidus* by cross-amplification.

Materials and methods

A total of 30 wild individuals of *P. argentatus* were collected from Zhoushan fishing ground, Zhejiang Province, China, and were identified in morphology. Genomic DNA was extracted from the fin clips using traditional proteinase K and phenol–chloroform method (Arai and Mukaino 1997) with some modification. The quality and concentration of DNA were examined on agarose gel electrophoresis and GeneQuant pro RNA/DNA spectrophotometer (Amersham, Cambridge, UK). DNA was finally adjusted to 100 ng/μL and stored at -20°C for use.

Microsatellites in white croaker were identified by cross-amplification using microsatellite markers initially developed in related species. Fifty polymorphic loci were used in this study, of which 31 were isolated from ESTs of *M. miiuy*, 13 were from genomic library of *M. miiuy* and six loci were developed in *C. lucidus* (Wang *et al.* 2010; Sun *et al.* 2011; Xu *et al.* 2011). Polymerase chain reaction (PCR) was performed in 10 μL reaction mixture containing 1× PCR buffer (Tiangen, Beijing, China), 0.2 mM dNTPs, 0.4 μM of each primer, and 0.5 U of *Taq* polymerase (Tiangen, 5 U/μL). The PCR was conducted using the following program: 95°C for 5 min followed by 30 cycles of 95°C for 30 s, annealing temperature for 30 s, and extension for 30 s at 72°C followed by a final extension for 5 min at 72°C, then holding at 10°C. PCR amplification was performed on S1000^{TM} thermal

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Keywords. microsatellites; cross-amplified; *Pennahia argentata*.

Table 1. Characteristics of 12 polymorphic microsatellite loci in white croaker, *Pennahia argentata*.

Locus	GenBank	Primer sequence (5′-3′)	T_m (°C)	Repeat motif	Size range (bp)	N	PIC	HWE (P)	H_e/H_o
Mimi-19	GU084254	F: GGAGGAAAAGGGTAGAA R: CAAAAGGCCTGTCTCAT	54	$(GA)_9$	158–166	4	0.698	0.031	0.738/0.429
Mimi-16A03	GW668869	F:TGGAGAACCCAAAGAAAT R:CCACAAAGGAGCGTCATA	54	$(T)_{15}$	292–298	8	0.574	0.003*	0.604/0.429
Mimi-23B09	GW669343	F: CAGTCGCTTCCTGTCATA R: AGGGAGATTATCGGGTGT	54	$(ACT)_7$	139–157	8	0.577	0.000*	0.645/0.286
Mimi-32A10	GW669955	F: GAACCACCCATCCTTTA R:CTTTGCCCTTCTGTCTA	52	$(A)_{14}n_{12}(T)_{17}$	242–274	7	0.672	0.036	0.712/0.368
Mimi-32B08	GW669962	F:CGTCGCACCAAGAATGAG R:TGAAACCTACCGTCTACAAAT	54	$(A)_{14}\ldots(T)_{14}$	246–248	2	0.297	0.020	0.363/0.191
Mimi-34A09	GW670103	F:TTTGGGTCACTAAATGGT R:CGTCTGTAAAGCAGGTAA	52	$(A)_{13}$	211–213	6	0.697	0.022	0.745/0.454
Mimi-36C02	GW670261	F:AATATCCCTGCCCTGCTA R:TGTTCGCCATTGTCTTGC	52	$(TTTTC)_3$	228–333	3	0.419	0.600	0.434/0.546
Mimi-40C05	GW670563	F:GTGTAACAAATAACCCTCG R:TGCTGCTCGTCACAATAA	52	$(A)_{13}$	144–153	6	0.571	0.000*	0.643/0.191
Mimi-49C10	GW671186	F:CGGCTTTACTTCAGTGGTT R:TCTCCTCCTCGGTTGTCG	50	$(A)_{26}$	186–194	5	0.373	0.150	0.459/0.619
Mimi-52H10	GW671455	F:ACGCATTTGTTTACTTTCTC R:CACCACCATTCAGTTTCT	50	$(GA)_9(CTGT)_4\ldots(T)_{14}$	187–217	3	0.685	0.073	0.724/0.632
Mimi-56G05	GW671751	F:AGACACCCGACCAGAACC R:ACAGCCTCCATCCACAAA	50	$(AGC)_5$	145–157	6	0.521	0.194	0.590/0.524
Mimi-57A05	GW671772	F:CTCCTGCCCTTCGTGATT R:TCTTTCCCTGCTTGTTGTA	50	$(T)_{14}$	239–242	2	0.340	0.269	0.434/0.546
Average						4.083	0.532		

Locus name; primer sequences; repeat sequence; allele size range; N, number of alleles; (H_e/H_o), expected and observed heterozygosity; PIC, the value of polymorphic information content; HWE, Hardy–Weinberg equilibrium; and GenBank accession number of 12 polymorphic microsatellite loci. * Show significant deviation from Hardy–Weinberg equilibrium ($P <$ 0.0042, adjusted value).

cycler (Bio-Rad, Hercules, USA). The optimal special annealing temperatures were adjusted and listed in table 1.

The characterization of loci was performed by PCR basing on genomic DNA of 30 samples. PCR products were genotyped by 6% denatured-polyacrylamide electrophoresis (19 : 1 acrylamide : bis-acrylamide) using silver staining. Allele size was identified according to pBR322 DNA/*Msp*I molecular weight marker (Tiangen).

Polymorphism parameters, number of alleles per locus (N), expected (H_e) and observed (H_o) heterozygosity and Hardy–Weinberg equilibrium (HWE) were analysed using POPGENE software (Yeh and Boyle 1997). Null alleles were examined by MICRO-CHECKER (Van *et al.* 2004). Polymorphism information content (PIC) was calculated by the formula: $\mathrm{PIC} = 1 - \sum_{i=1}^{n} p_i^2 - 2\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2 p_j^2$, where P_i and P_j are frequencies of the i and j allele, respectively (Botstein *et al.* 1980). ARLEQUIN 3.11 software (Schneider *et al.* 2000) was used to calculate genotypic linkage disequilibrium between these loci. All of the results for multiple tests were adjusted using Bonferroni correction (Rice 1989).

Results and discussion

Among the 50 polymorphic loci cross-amplified in white croaker, 33 (66%) could be successfully amplified, with 12 (24%) of these loci were shown polymorphic. All of the 12 polymorphic loci were isolated from the primers developed in *M. miiuy*. Details of the polymorphic microsatellite loci in white croaker and variability were measured across 30 samples, which were summarized in table 1. N for each locus ranged from 2 to 6, with an average of 4.083. H_e and H_o ranged from 0.297 to 0.745 and from 0.191 to 0.632, respectively. Usual values of PIC of microsatellites ranged from 0.427 to 0.698, in which eight loci were highly polymorphic (PIC > 0.5). Among the 12 novel loci, three loci (Mimi-16A03, Mimi-23B09 and Mimi-40C05) were found significantly deviated from HWE in the sampled population after Bonferroni correction ($P < 0.0045$, adjusted value), possibly due to the presence of null alleles. The loci Mimi-16A03, Mimi-23B09 and Mimi-40C05 were found null alleles examined by Micro-Checker. There were no stuttering errors and no evidence of allelic dropout in any of the loci analysed by Micro-Checker (Bonferroni correction). No significant linkage disequilibrium was found between all pairs of these 12 loci after Bonferroni correction ($P < 0.0045$, adjusted value).

Overall, the 12 microsatellite loci developed in white croaker were highly polymorphic. This is the first time to develop microsatellites loci in white croaker. The 12 polymorphic loci developed in this study will be helpful in the studies of genetic variation, population structure, conservation genetics and molecular-assisted selective breeding of white croaker in the future.

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