



Isolation and multiplex genotyping of polymorphic microsatellite DNA markers in the snakehead murrel, *Channa striata*

Amirul Firdaus Jamaluddin Jamsari¹, Tan Min-Pau¹ and Mohd Nor Siti-Azizah^{1,2}

¹School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia.

²Centre for Marine and Coastal Studies, Universiti Sains Malaysia, Penang, Malaysia.

Abstract

Seven polymorphic microsatellite loci were isolated and characterized for the snakehead murrel, *Channa striata* (Channidae), a valuable tropical freshwater fish species. Among 25 specimens collected from Kedah state in Malaysia, the number of alleles per locus ranged from 2 to 7. Observed and expected heterozygosities ranged from 0.120 to 0.880 and 0.117 to 0.698, respectively. A single locus (CS1-C07) was significantly deviated from Hardy-Weinberg equilibrium after Bonferroni correction. These novel markers would be useful for population genetic studies of the *C. striata*.

Key words: *Channa striata*, population genetics, microsatellite, multiplex PCR.

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The snakehead murrel, *Channa striata* (Perciformes, Channoidei, Channidae) or haruan as it is locally known in Malaysia, is an indigenous tropical freshwater fish that is widely distributed across Southeast Asia, South Asia, and Southern China (Talwar and Jhingran, 1992). This sub-cylindrical fish can grow up to 100 cm in length and is found in rice fields, rivers, swamps, ponds, canals and lakes especially in areas infested by thick aquatic vegetation (Ali, 1999; Froese and Pauly, 2010). This carnivorous air-breather is renowned as a food fish and proven to have medical and pharmaceutical properties especially for wound healing (Mat Jais *et al.*, 1994; Michelle *et al.*, 2004). The demand for the species is increasing, and it is usually marketed alive, fetching a price of around USD 6/kg. Because of its commercial value, it is highly exploited in the wild. Aquaculture activity of this species is still low in Malaysia probably due to its presumed abundance in the natural habitats. However, anthropogenic effects such as overfishing, development, river flow regulation and pollution are expected to introduce pressure to the population and lead to population fragmentation and decline. Therefore, monitoring the population genetic status is essential to provide crucial information in stock evaluation of the species for both wild and cultivated population management and conservation. In the present study, we developed seven microsatellite markers for *C. striata* using the protocol by Edwards *et al.* (1996) modified according to Jones *et al.* (2001).

Genomic DNA extraction from fin-clip tissue of a single snakehead murrel was performed using standard phenol-chloroform method modified from Taggart *et al.* (1992). The extract was then digested with *RsaI* and fragments were ligated to a *MluI* adaptor. Amplification reactions were conducted in a 50 µL reaction mixture containing 5 ng of ligated DNA, 250 ng of one of the primers corresponding to the *MluI* adaptor, 1X PCR buffer, 2.5 mM MgCl₂, 0.2 mM dNTPs, 0.02 µM each primer, and 2U Ampli Taq Gold (Applied Biosystems). Polymerase chain reactions (PCR) were performed with the following profile: initial denaturation at 95 °C for 10 min followed by 30 cycles consisting of 94 °C for 15 s, 60 °C for 1 min, 72 °C for 3 min, and a final extension at 72 °C for 15 min. Microsatellite enrichment was performed by hybridization to a Hybond-N+ membrane containing the microsatellite probes (GACA)₉, (GATA)₉, (AAAT)₉, (GATA)₉, (CAA)₁₄, (CA)₂₀, (GT)₁₅, (AAT)₁₄, (CT)₁₅, and (AAG)₁₄. The eluted microsatellite-enriched DNA was amplified using the same PCR conditions as above and purified using a MicroSpin S-300 column (Pharmacia). The PCR product was then ligated into pGEM T-Easy Vector (Promega) and transformed into *Escherichia coli* JM109 competent cells (Promega). 96 recombinant colonies were picked from LB agar plates containing ampicillin, IPTG, and X-gal. Subsequently, their plasmids were amplified using TempliPhi (GE Healthcare) and inserts were sequenced using the automated sequencer (ABI PRISM 3730XL) and an ABI PRISM BigDye terminator cycle sequencing kit version 3.1 (Applied Biosystems).

From the total recombinant plasmids sequenced, 27 sequences contained good signal data with at least eight repeat microsatellite motifs. Vector sequences were detected using Chromas version 2.33 and removed. PRIMER 3 (Rozen and Skaletzky, 2000) was used to design the primers. Primers to the flanking regions of each microsatellite were designed for 11 microsatellite loci, with forward primers being labeled with fluorescent dye. However, based on preliminary assessment on several individuals, only seven loci produced good amplification with allele size polymorphism and were considered robust for subsequent analyses (Table 1). These sequences were deposited in GenBank (accession numbers GU321675, GU321677 to GU321682).

25 individuals of *C. striata* collected from the state of Kedah (Malaysia), with geographical location 6°14' N 100°36' E, were then assayed for the seven microsatellites. PCR amplifications were performed in a 6.25 µL reaction volume containing 10 ng of genomic DNA, 2X QIAGEN Multiplex PCR Master Mix and 0.2 µM of each forward and reverse primer. The PCR protocol consisted of an initial 95 °C for 15 min followed by 29 cycles of 94 °C for 30 s, 57 °C for 1.5 min, 72 °C for 1 min, and finally 30 min of final extension at 60 °C. Allelic data was analyzed using Peak Scanner v1.0 software (Applied Biosystems) with GS500 (Applied Biosystems) as the internal size standard. Arlequin version 3.1 (Excoffier *et al.*, 2005) was used to calculate the allele frequency, observed (H_o), and expected (H_e) heterozygosities, to test deviations from Hardy-Weinberg equilibrium (HWE) and pairwise comparisons of all loci for linkage disequilibrium (LD). Bonferroni corrections were applied with a global significance level of 0.05 to account for multiple testing in HWE and LD tests. The presence of null alleles, large allele dropout, and scoring errors due to stutter peaks were assessed using MicroChecker v2.2.3 (Van Oosterhout *et al.*, 2004).

Table 1 shows the characteristics of the seven novel *C. striata* microsatellite loci. No evidence for the presence of null alleles, large allele dropout, and scoring error due to stuttering was observed. The number of alleles per locus ranged from two to seven. The observed and expected heterozygosities ranged from 0.120 to 0.880 (with an average of 0.520) and from 0.117 to 0.698 (with an average of 0.482), respectively. Deviation from Hardy-Weinberg equilibrium was detected at a single locus (CS1-C07), which showed significant excess of heterozygosity. The significant heterozygosity excess in CS1-C07 suggests the possibility of ancient and recent demographic events, *i.e.*, bottleneck effect (Cornuet and Luikart, 1996; Riccioni *et al.*, 2010). Overall, markers developed in this study would be useful in assessing the level of genetic diversity and population structure for breeding strategy, fishery management, and conservation of this species.

Table 1 - Characterization of microsatellites for 25 individuals of snakehead murrel, *Channa striata*. Allele size range in base pairs (bp); number of alleles (N_A); observed heterozygosity (H_o), expected heterozygosity (H_e), significant departure from Hardy-Weinberg equilibrium (*) following sequential Bonferroni correction for simultaneous tests ($\alpha = 0.05$).

Locus	Repeat motif	Primer (5'-3')	size range (bp)	N_A	H_o	H_e	GenBank Accession no.
CS1-A05	(TG) ₁₀	F: NED-TTCAAACTCCCGGTGAAAAC R: CAGTGACTGTGGGACACTGC	126-138	3	0.120	0.117	GU321675
CS1-B07	(TG) ₁₃	F: NED-TCCCTTCTGGCTCCTAAAT R: CCACCTGGAAGAGAGGTGAGA	182-190	3	0.520	0.512	GU321677
CS1-C07	(GT) ₁₆	F: VIC-TGGCTAGGTCCTCTTGCTGT R: TCACACTCACCCAGCCATTA	96-126	4	0.600	0.556*	GU321678
CS1-E11	(CA) ₁₂	F: PET-TGGGCACAATAACCAACAATC R: ATTGAGTGAATGGGCAATCC	302-312	4	0.880	0.656	GU321679
CS1-E12	(GT) ₁₆	F: VIC-AAAGGACTGGGAAAAGGAAA R: GCATCCAGACAGCAGACAAA	237-277	7	0.720	0.698	GU321680
CS1-F05	(CA) ₁₀	F: 6FAM-ACCACATTTGTGCGGTGT R: AGGCAGCTGATGTGAGACCT	157-161	2	0.320	0.274	GU321681
CS1-H09	(GT) ₆ -(GT) ₈	F: VIC-GTGCAACAAAAGCCACTCT R: GTGAGTTATGGCAGGGCAGT	171-181	4	0.480	0.561	GU321682
Mean				3.9	0.520	0.482	

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