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Short Communication

Isolation and characterization of microsatellite loci in the fish *Coilia mystus* (Clupeiformes: Engraulidae) using PCR-based isolation of microsatellite arrays

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ABSTRACT. *Coilia mystus* is the most important harvested fish species in China; it inhabits quite different water environments during the different life history stages. Populations of *C. mystus* have dropped sharply due to overharvesting and water pollution. We developed eight microsatellite loci in *C. mystus* for conservation genetics studies. These new markers were tested in 20 individuals from the Min River in ChangLe. The number of alleles ranged from 3 to 8, the expected heterozygosity from 0.621 to 0.853 and the observed heterozygosity from 0.473-0.800. Only two loci deviated significantly from Hardy-Weinberg expectations due to heterozygote deficiency. These primers may provide a tool for understanding demography and population structure of this economically important and threatened species.

Key words: Microsatellite; Conservation; PIMA; *Coilia mystus*; RAPD-PCR enrichment

Genus *Coilia* fishes with 14 species are small to moderate sized in the Family Engraulidae, Clupeiformes, and distributed mainly in the mid-western Pacific and the Indian Ocean (Whitehead et al., 1988). In the northwest Pacific, four species of *Coilia* were recorded, including *C. nasus*, *C. mystus*, *C. grayii*, and *C. brachygnathus* (Zhang, 2001). *C. mystus*, an estuarine migratory fish that generally distributes in shallow marine areas of the East-Southern China coast, is the most important harvested species in the China's estuary fishery economy. Its capture in the fisheries of Yangtze River estuary averaged 2500 tones/year in the 1990s (Ni, 1999). However, the total catch is declining due to human activity such as overfishing, pollution and recent habitat degradation (Liu et al., 2004; Yang et al., 2006). In the present study, we have developed and characterized microsatellite markers for *C. mystus* that will provide the genetic information to manage and conserve these important fishery species in the future.

Briefly, total genomic DNA was isolated from muscle tissue or fins preserved in 95% ethanol, by proteinase K digestion at 55°C. DNA was purified by traditional phenol-chloroform protocol and ethanol precipitation. The isolation of microsatellite markers began with a random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) enrichment (Lin et al., 2008; Chiang et al., 2008). This PIMA (PCR isolation of microsatellite arrays) approach has been proposed by Lunt (1999). It takes advantage of the fact that the RAPD fragments contain microsatellite repeats more frequently than random genomic clones (Cifarelli et al., 1995).

Amplification of 20-100 ng DNA was performer in a 15-μL final volume with 0.2 mM of each dNTP, 2 mM $MgCl_2$, 0.5 U *Taq* polymerase (Promega), and 5 pmol of one RAPD primer. Several RAPD primers were used to amplify fragments from the target species' genome in separate reactions. PCR amplifications were conducted on an MJ PTC-100 Thermal Cycler using the following conditions: initial denaturing at 94°C for 3 min, 40 cycles of 94°C denaturing for 50 s, 37°C annealing for 1 min, 72°C extension for 1 min, and 72°C for 10 min. RAPD-PCR products were size-selected to preferentially obtain small fragments (500-1200 bp). Approximately 100 ng PCR product was ligated into a pGEM-T vector (Promega) according to manufacturer instructions, and the ligation mixture was transformed into competent *Escherichia coli* cells. Clones were screened using repeat-specific and vector primers (Lunt et al., 1999).

In positive clones, the repeat-specific and vector primers amplified DNA fragments that contain microsatellites, whereas no amplification was found in negative clones. Plasmid DNA from positives was purified using the High-Speed Plasmid Mini Kit (Geneaid). Both strands of the DNA insert were sequenced. DNA sequencing in both directions was conducted with an Applied Biosystems Model 377A automated sequencer (Applied Biosystems). Primers for eight loci were designed using the PRIMER 3 software (Rozen and Skaletsky, 2000). Preliminary assessment of polymorphism was performed on a few individuals. Reactions were performed in a total volume of 15 μL containing 10 ng genomic DNA, 0.2 mM dNTP, 2 mM $MgCl_2$, and 0.12 μM of each primer. PCRs were as follows: 94°C for 4 min followed by 40 cycles at 94°C for 30, 30 and 50 s at primer-specific annealing temperature (Table 1), 72°C for 45 s and a final extension step at 72°C for 10 min. Electrophoresis was conducted on denaturing 6% polyacrylamide gels and were visualized using silver-staining (Creste et al., 2001).

Table 1. Primer sequence, repeat motif, size range, number of alleles, expected (H_E) and observed (H_O) heterozygosities, and significance of deviation from Hardy-Weinberg equilibrium (HWE) for eight microsatellite loci of *Coilia mystus*.

Locus	Primer sequence (5' to 3')	Repeat motif	Size range (bp)	Total No. of alleles	Tm (°C)	H_E	H_O	HWE P value
MICM 01	F: TGCAATGGGAAATTCCTCTC R: GTGAGGAGCTGTGGAGGATG	$(TG)_{14}$	176-184	3	60	0.633	0.473	0.416
MICM 02	F: GACATCAGTCAGCAGCTCCA R: AACAGAGGCAGGGAGTGAAA	$(CT)_{10}$	180-216	6	60	0.784	0.777	0.816
MICM 03	F: CTGGATACCCCGAACTCTGA R: ATTGTGAGGCGTCAGAGAGG	$(CT)_{18}$	210-244	6	54	0.621	0.800	0.923
MICM 04	F: AGCCAACTTATTGTGTATGGAGA R: GAGTGCAAGACCCATCACTG	$(TG)_8$	208-232	8	59	0.780	0.600	0.023*
MICM 05	F: TGTCACATGACGCTGCAGTA R: TTAGCGCCATTGATCAACCT	$(CAA)_8$	208-216	5	58	0.698	0.800	0.950
MICM 06	F: TGGCTCCCTGTTTTAACGTC R: ACCCGCTTTGTGTTAGCAGT	$(CT)_{10}N(CA)_{10}$	256-270	7	58	0.853	0.631	0.020*
MICM 07	F: CGTTTCTCTGGGAAATTGGA R: ACGCTGCACCTACCAAACTT	$(AC)_8$	228-260	7	59	0.771	0.700	0.401
MICM 08	F: GGTTGAAATCCTCGTCCTCA R: TCCATTACACATCTGGCTCA	$(TG)_6(AG)_{17}$	240-268	8	59	0.851	0.800	0.421

*Significant deviation from HWE. Tm = melting temperature.

Allele sizes were estimated using a 10-bp ladder molecular size standard (Invitrogen). Allele frequency, observed (H_O) and expected (H_E) heterozygosities were calculated. All loci were tested for fitness to the Hardy-Weinberg equilibrium (HWE), and all pairwise combinations of loci were tested for linkage disequilibrium. All these parameters and tests were computed using Arlequin version 3.1 (Excoffier et al., 2005).

We estimated the level of genetic diversity by genotyping 20 *C. mystus* individuals collected from ChangLe in Min River. The number of alleles for the eight loci ranged from 3 to 8 (average 6.25). As shown in Table 1, the H_E and H_O ranged from 0.621-0.853 (average of 0.749) and 0.473-0.800 (average of 0.697), respectively. Only two loci deviated significantly from HWE (Table 1), due to the heterozygote deficiency. These deviations may have resulted from small population size associated with human disturbances and habitat loss. No significant linkage disequilibrium was detected between the comparisons of these loci. Microsatellite makers described here should be useful to monitor population size, and to determine dispersal patterns and genetic diversity within and between populations of this species.

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REFERENCES

Chiang TY, Lee TW, Lin FJ, Huang KH, et al. (2008). Isolation and characterization of microsatellite loci in the endangered freshwater fish *Varicorhinus alticorpus* (Cyprinidae). *Conserv. Genet.* 9: 1399-1401.

Cifarelli RA, Gallitelli M and Cellini F (1995). Random amplified hybridization microsatellites (Rahm) - isolation of a new class of microsatellite-containing DNA clones. *Nucleic Acids Res.* 23: 3802-3803.

Creste S, Neto AT and Figueira A (2001). Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. *Plant Mol. Biol. Rep.* 19: 299-306.

Excoffier L, Laval G and Schneider S (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinform. Online* 1: 47-50.

Lin HD, Lee TW, Lin FJ, Lin CJ, et al. (2008). Isolation and characterization of microsatellite loci in the endangered freshwater fish *Pararasbora moltrechti* (Cyprinidae) using PCR-based isolation of microsatellite arrays (PIMA). *Conserv. Genet.* 9: 945-947.

Liu K, Zhang M-Y, Xu D-P and Shi W-G (2004). Studies on resource change and MSY of *Coilia mystus* in the Yangtze River estuary. *Shanghai Fish.* 13: 298-303.

Lunt DH, Hutchinson WF and Carvalho GR (1999). An efficient method for PCR-based isolation of microsatellite arrays (PIMA). *Mol. Ecol.* 8: 891-893.

Ni Y (1999). Fishery resources conservation for *Coilia mystus* in the Changjiang estuary. *J. Fish. Sci.* 6: 75-77.

Rozen S and Skaletsky HJ (2000). Primer3 on the WWW for General Users and for Biologist Programmers. In: Bioinformatics Methods and Protocols: Methods in Molecular Biology (Krawetz S and Misener S, eds.). Humana Press, Totowa, 365-386.

Whitehead PJP and Nelson GJ (1988). FAO Species Catalogue. Vol. 7. Clupeoid Fishes of the World (Suborder Clupeoidei). An Annotated and Illustrated Catalogue of the Herrings, Sardines, Pilchards, Sprats, Shads, Anchovies and Wolf-herrings. Part 2 - Engraulidae. FAO, Rome.

Yang J, Arai T, Liu H, Miyazaki N, et al. (2006). Reconstructing habitat use of *Coilia mystus* and *Coilia ectenes* of the Yangtze River estuary, and of *Coilia ectenes* of Taihu Lake, based on otolith strontium and calcium. *J. Fish Biol.* 69: 1120-1135.

Zhang SY (2001). Fauna Sinica Osteichthyes: Acipenseriformes, Elopiformes, Clupeiformes and Gonorhynchiformes. Science Press, Beijing.