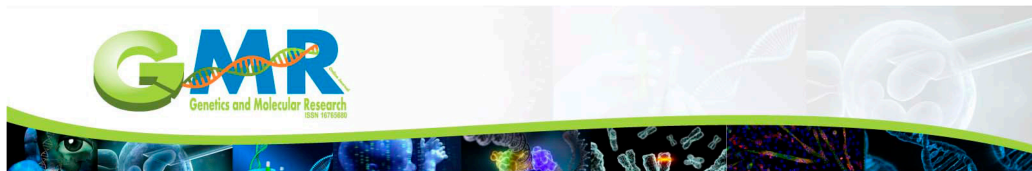

Development of polymorphic microsatellite loci for a new fish species, Chinese sillago (*Sillago sinica*)

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ABSTRACT. *Sillago sinica* is a newly identified species belonging to Sillaginidae, Perciforms, and was found along the coast of China in 2011. In the present study, 81 microsatellite loci were isolated from an enriched genomic library, and 24 positive clones containing microsatellite repeats had adequate flanking sequences for the development of PCR primers. Sixteen of these primers were monomorphic or would not amplify. Eight were polymorphic in an examined population with the number of alleles per locus ranging from 2 to 14. The number of observed and expected heterozygosities per locus varied from 0.125 to 0.958 and from 0.120 to 0.904, respectively. The polymorphism information content ranged from 0.110 to 0.721. All loci conformed to Hardy-Weinberg equilibrium (P > 0.05) after Bonferroni correction. There was no significant linkage disequilibrium between the eight polymorphic loci. These results suggest that these markers may be very useful for the characterization of natural populations of this species.

Key words: Microsatellite DNA; *Sillago sinica*; Polymorphic loci; New species

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INTRODUCTION

Sillago sinica, is a new marine fish species of China, which was found in the Bohai Sea (the Yellow River delta in Dongying), the Yellow Sea (the coastal region of Qingdao), and the East China Sea (the estuary of Feiyunjiang in Wenzhou) (Gao et al., 2011). *S. sinica* exhibits the same characteristics as other sillago species, which include the adults burying themselves in the sand when disturbed. Since it was first described, the morphological characteristics, sagittal otolith shape, and DNA barcoding of *S. sinica* have been described (Pan and Gao, 2010; Gao et al., 2011; Zhang et al., 2013). However, no genetic investigations based on microsatellite markers have been conducted until now because no suitable markers have been developed. Microsatellite markers are important tools that can be used to assess genetic diversity and develop molecular breeding techniques in fish because of their high level of polymorphism and co-dominant Mendelian inheritance (Chen et al., 2005). For the purpose of conserving the genetic resources and assessing the genetic diversity of *S. sinica*, it is essential to isolate polymorphic microsatellite markers for this species. In the present study, we developed eight polymorphic microsatellite markers isolated from a microsatellite enriched genomic library of *S. sinica*.

MATERIAL AND METHODS

A microsatellite-enriched partial library was constructed following the protocol of Hui et al. (2006) with a few modifications. Genomic DNA was extracted from muscle tissue using the standard phenol-chloroform method described by Sambrook et al. (1989), and then digested with Hae*III*. We used electrophoresis on a 1% low-melting-point agarose gel to isolate small fragments (500-1000 bp). A synthesized adaptor (21-mer: 5'-CTCTTGCTTGAATTCGGACTA-3' and a phosphorylated 25-mer: 5'-pTAGTCCGAATTCAAGC AAGAGCACA-3') was ligated to the size-selected DNA fragments using T4 DNA ligase. Fragments containing microsatellite DNA were captured by hybridization to nylon membranes fixed with oligonucleotide $(GA)_{15}$ and $(CA)_{15}$ probes. After washing to remove the non-simple sequence repeat fragments, the eluted single-stranded DNA contained the selected microsatellite DNA. The fragments were ligated into a pMD18-T vector, and the complexes were transformed into competent *Escherichia coli* DH5α cells. The colonies were transferred to a new plate and screened with $(GA)_{15}$ and $(CA)_{15}$ probes labeled with DIG Oligonucleotide 30-End labeling kit 2nd generation (Roche). Positive signals were detected using the DIG luminescent detection kit for nucleic acids according to the manufacturer instructions (Roche).

In total, 24 PCR primer pairs were designed using the PRIMER 5 software (http://www.premierbiosoft.com). Twenty-four *S. sinica* individuals were collected from Dongying, Shandong Province, and used to test the polymorphism of these primer pairs.

PCR was performed in 25-μL volumes containing 10X PCR buffer, 200 μM each dNTP, approximately 50 ng genomic DNA, 200 μM primers (forward and reverse), 1.5 mM $MgCl_2$, and 1 U Taq DNA polymerase (TakaRa). The thermo-cycling conditions were as follows: 5 min at 94°C, followed by 35 cycles of 45 s at 94°C, 45s at the annealing temperature listed in Table 1, and 45 s at 72°C, with a final extension at 72°C for 10 min. After PCR amplification, the products were electrophoresed on 8% denaturing polyacrylamide gels at 12 W for 2-3 h. The target bands were visualized by silver staining, and a reference marker of an 8-bp DNA ladder was used to identify allele size.

Table 1. Levels of variability at eight polymorphic microsatellite loci in *Sillago sinica*.

Locus	Primer (5'-3')	Ta (°C)	Repeat motif	No. of alleles	Size range (bp)	H_O	H_E	P	PIC	Accession No.
A47	F:AACAAACACGGTGCCCATC R:AGACCCTGCTGCCCCTCAT	50.0	$(GA)_{23}$	2	189-195	0.125	0.120	1.000	0.110	KC489769
C64	F:CGACCAGGTGCGAAACGGACAA R:GAGGAAAGCCAAGTGGGAGTAAAC	57.4	$(GA)_7(GA)_7(GA)_6$	3	244-258	0.292	0.260	1.000	0.206	KC489773
B54	F:GGCATCGGTAGTGACAGG R:GGAGATTACTCGCCAAAG	47.8	$(GA)_8(GA)_{16}(CT)_3$	5	148-176	0.958	0.842	0.991	0.647	KC489770
C22	F:CGAAGCAACCACATAGATAC R:GGACCACCTGCCCTCA	60.0	$(CA)_{27}$	6	156-176	0.542	0.724	0.023	0.606	KC489772
C66	F:CTGACAGATAACCGAGTG R:CAACCAAACCTGACGAG	54.7	$(CA)_{16}(CA)_5(CA)_{10}$	14	124-162	0.792	0.904	0.069	0.721	KC489766
B55	F:TGAATGGTGGGGTAGGAC R:TTGGATAGGAGGATGAGG	50.0	$(GA)_9(GA)_{14}(GA)_{11}$	3	202-212	0.375	0.465	0.057	0.454	KC489771
B45	F:GAGTAGGTGGGAAGGGAC R:TTGGAGGACTGGGTTTAG	53.0	$(AC)_4TC(AC)_{10}$	6	190-196	0.375	0.55	0.077	0.434	KC489767
A13	F:AGACAGGGAAAGAGTGAGA R:TGGGAAATAGAAAGGCA	58.0	$(GA)_{22}$	4	127-139	0.625	0.738	0.260	0.681	KC489768

Ta = optimal annealing temperature; H_O = observed heterozygosity; H_E = expected heterozygosity; P = Hardy-Weinberg equilibrium; PIC = polymorphism information content.

RESULTS AND DISCUSSION

Of 130 positive clones, 81 were sequenced successfully using the M13 universal sequence primers with an ABI3730 genetic analyzer, and 81 sequences were found to contain microsatellite motifs. Twenty-four positive clones had an adequate flanking sequence that could be used to design primers. The hybrid clones, duplicates, and sequences with short unique regions flanking the microsatellite array were discarded. Of the 24 primer pairs, 16 were monomorphic or could not be amplified from the 24 *S. sinica* individuals from Dongying. Eight loci showed obvious polymorphism, with allele numbers ranging from 2 to 14 (Table 1). The number of observed and expected heterozygosities per locus varied from 0.125 to 0.958 and 0.12 to 0.904, respectively. The polymorphism information content ranged from 0.110 to 0.721. Hardy-Weinberg equilibrium and linkage disequilibrium (LD) tests for each locus were conducted using GENEPOP 4.0 (Rousset, 2008). All eight polymorphic loci conformed to Hardy-Weinberg equilibrium after sequential Bonferroni correction (Rice, 1989). No significant LD was found in any of the eight polymorphic loci. The highly polymorphic loci identified in this study could be used for future evaluations of genetic variation in *S. sinica* or its closely related species.

Conflicts of interest

The authors declare no conflict of interest.

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