GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Short Communication

Polymorphic microsatellite markers in the false kelpfish *Sebastiscus marmoratus*: isolation, characterization, and cross-species amplification

H.B. Liu[1,2]*, S.F. Liu[2]*, J.B. Ye[2], Y.J. Yuan[2,3], S.X. Ding[4] and Z.M. Zhuang[2]

[1]Key Laboratory of Mariculture, Ministry of Agriculture, Dalian Ocean University, Dalian, China
[2]Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China
[3]College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, China
[4]State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, China

*These authors contributed equally to this study.
Corresponding author: Z.M. Zhuang
E-mail: zhuangzm@ysfri.ac.cn

Genet. Mol. Res. 13 (1): 134-138 (2014)
Received November 23, 2012
Accepted May 2, 2013
Published January 10, 2014
DOI http://dx.doi.org/10.4238/2014.January.10.4

ABSTRACT. A $(GT/CA)_{13}$-microsatellite-enriched genomic library of the false kelpfish *Sebastiscus marmoratus* was constructed, and 20 polymorphic microsatellite loci were isolated and characterized. The polymorphisms were investigated in 48 wild individuals from a single population collected from the northern Yellow Sea. The numbers of alleles per locus varied from 4-22 with an average of 9. The observed and

expected heterozygosities of each locus ranged from 0.196-0.958 and from 0.487-0.942, with an average of 0.693 and 0.765, respectively. One locus significantly deviated from Hardy-Weinberg equilibrium, and one pair of loci was in linkage disequilibrium determined by Bonferroni's correction. Cross-species amplification was also conducted in the related species *Inimicus japonicus*, collected from East China Sea. The result showed that six loci could be amplified from *I. japonicus* DNAs. These polymorphic markers would be useful for assessment of genetic variation and population structure of scorpionfish.

Key words: *Sebastiscus marmoratus*; *Inimicus japonicus*; Microsatellite markers; Cross-species amplification

INTRODUCTION

The false kelpfish *Sebastiscus marmoratus* (Scorpaeniformes: Sebastidae), a common scorpaenid, is widely distributed in the western Pacific, south of the Philippines and north of Japan (Barsukov and Chen, 1979; Chen and Lee, 1980; Shen, 1993). *S. marmoratus* is an ovoviviparous fish inhabiting littoral rocky bottoms (Kita et al., 1996). As a highly valuable marine food and game fish, it is an important fishery object in China, Japan, and South Korea (Kita et al., 1996). In recent years, because of overfishing and marine environment deterioration, wild populations of *S. marmoratus* have sharply declined (Shen, 1993). To effectively protect and sustainably develop *S. marmoratus* as a resource, it is necessary to conduct studies on the molecular phylogeny, population structure, and conservation genetics of this species.

Microsatellite markers have been proven to be an extremely valuable tool for genetic studies and for conservation and management of genetic resources (Bai et al., 2011; Lin et al., 2012). At present, just 11 polymorphic microsatellite DNA markers have been reported for *S. marmoratus* (Xu et al., 2010). In the present study, we have developed a new batch of genomic microsatellite markers for *S. marmoratus* and characterized these markers by genotyping 48 individuals sampled from a wild population. In addition, cross-species amplifications of these new loci were carried out in 10 individuals of *Inimicus japonicas* (Scorpaeniformes: Synanceiidae) to determine their transferability to other related species. These microsatellite markers would facilitate studies of the population genetics of scorpaenid fish.

MATERIAL AND METHODS

Forty-eight *S. marmoratus* individuals used in this study were collected from China seas. Samples were soaked in alcohol and stored at -20°C. Genomic DNA was extracted from muscle and pterygiophore tissue using a standard traditional phenol-chloroform procedure. The purified DNA template was stored at -20°C until genotyping. A microsatellite-enriched library was constructed using the FIASCO (Fast Isolation by AFLP of Sequences Containing Repeats) method (Zane et al., 2002), with minor modifications (Liu et al., 2012). Genomic DNA was digested with *Mse*I (New England Biolabs, USA) at 37°C for 3 h. The digested DNA (10 µL) was ligated with synthesized *Mse*I adaptors MA-A (5'-TAC TCA GGA CTC

AT-3') and MA-B (5'-GAC GAT GAG TCC TGA G-3') at 16°C overnight. The adaptor-ligated DNA was amplified using the adaptor-specific primer MA (5'-GAT GAG TCC TGA GTA A-3'). PCR products were purified using DNAmate (TaKaRa, Japan) and hybridized to a biotin-labeled $(GT)_{13}$ probe. Then, the purified microsatellite-containing DNAs were ligated with pMD18-T vector (TaKaRa, Dalian, China) and transformed into *Escherichia coli* DH5α competent cells to construct an enriched microsatellite-containing library.

Recombinant clones were randomly selected and amplified with $(GT)_{13}$ and M13 primers. Positive clones were sequenced on an ABI 3730 automated DNA sequencer (Applied Biosystems, USA). The sequence data were scanned by using the SSRHunter V1.3 software (Li and Wan, 2005). Sequences with microsatellite motifs and flanking regions were selected for PCR primer design on PRIMER PREMIER5 (Premier Biosoft International, USA) and tested for polymorphism with 10 individuals of *I. japonicas*. The above-mentioned primers were tested for polymorphism with genomic DNAs from 48 *S. marmoratus* individuals. PCR for all loci was performed separately in a 25-µL reaction volume containing 0.4 µM of each primer, 0.2 mM dNTPs, 2 mM $MgCl_2$, 1X PCR buffer, 1 U Taq polymerase (Fermentas, Canada), and 50-100 ng DNA. Amplification was carried out with the following thermal profile: predenaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 45 s, optimal annealing temperature (Table 1) for 45 s, and 72°C for 45 s, and a final extension step at 72°C for 10 min.

PCR products were separated on 6% denaturing polyacrylamide gels and visualized by silver-staining. Allele sizes were estimated by using the pBR322/*Msp*I marker (TaKaRa, Dalian, China). PCR products of the primers were separated on 6% denaturing polyacrylamide gels. Electrophoresis photos were analyzed to obtain primer information. The variability at each locus was measured in terms of number of alleles and expected and observed heterozygosity. Hardy-Weinberg equilibrium (HWE) and linkage-disequilibrium tests were conducted on GENEPOP 4.0 (Raymond and Rousset, 1995). Null-allele frequencies were calculated using Micro-Checker2.2.3 (Van Oosterhout et al., 2004) following sequential Bonferroni's correction (Rice, 1989), and significance criteria of all multiple tests were corrected.

RESULTS AND DISCUSSION

In total, 20 microsatellite markers isolated from the microsatellite-enriched genomic libraries and characterized for *S. marmoratus* were shown to be polymorphic. The numbers of alleles per locus ranged from 4-22 with an average of 9. The observed and expected heterozygosities ranged from 0.196-0.958 and from 0.487-0.942, with an average of 0.693 and 0.765, respectively (Table 1). One locus (HCY-50) deviated from Hardy-Weinberg equilibrium in the sampled population determined by Bonferroni's correction (adjusted P value <0.002). Upon analysis, the following four loci HCY-46, HCY-50, HCY-72, and HCY-84 were predicted to have null alleles. Based on the analysis by Bonferroni's correction, one pair of loci (HCY-86/HCY-96) was in linkage disequilibrium (P value <0.002).

A cross-species amplification test showed that 6 of these 20 loci were successfully cross-amplified in a related species of scorpionfish, *I. japonicas* (Table 1). It confirmed that microsatellite markers developed in *S. marmoratus* could also be used for related scorpionfish species.

Table 1. Characterization of microsatellite loci developed for *Sebastiscus marmoratus* and *Inimicus japonicas*.

Loci	GenBank accession No.	Repeat motif	Primer sequence (5'-3')	Size range (bp)	Ta (°C)	N_A	H_O	H_E	P_{HW}	Cross-amplified in *I. japonicus*
HCY-15	JX683382	$(CA)_{17}$	F: GGATGACGACCATTTACGC R: TGCCTCTTTCCGAGTTGAG	310-390	45	12	0.7917	0.8605	0.1981	+
HCY-30	JX683383	$(TCTG)_{12}$	F: TATCATTATTCATGGGTGGA R: AGAAGTCATTGCAGACAACA	260-330	57	13	0.8125	0.7925	0.5510	-
HCY-31	JX683384	$(AC)_6\ldots(TC)_6$	F: TCTTGCTATTTGTGGCTTTC R: TGTGCCGTTTGCTTTATC	260-310	55	7	0.7083	0.8230	0.0235	+
HCY-45	JX683385	$(CA)_5$	F: TGCTCTGCGTTTTACATA R: TTAGGGGTCAGTCTTTTG	260-310	55	4	0.4255	0.4866	0.0046	-
HCY-46	JX683366	$(CA)_8$	F: CTGCACATGAAAGGCTCTG R: AGGCGTTCTGTTACACTGACT	240-300	55	5	0.4375	0.6116	0.0390	+
HCY-48	JX683367	$(TG)_6\ldots(AC)_5\ldots(AC)_5$	F: GAGTCCTCGGTTTCACAG R: ATGGGCTCGTAACTCATC	200-250	55	6	0.7500	0.7610	0.2709	-
HCY-49	JX683368	$(GT)_9$	F: GAGTTTCAGGGATGGTCT R: AAGTTTGGGATCTGGATT	190-250	55	14	0.8936	0.8922	0.6155	-
HCY-50*	JX683369	$(CA)_8$	F: TTGTGGAACTAGGCTTTAT R: TGAGTCGGGTTATTATCTG	270-330	55	6	0.1957	0.7886	0.0000	-
HCY-52	JX683370	$(TG)_8$	F: CTGAATAAATACAGCGTCTC R: TTACCACCATCTGAAAGG	240-290	55	7	0.6667	0.7042	0.3554	-
HCY-61	JX683371	$(TG)_5$	F: GCAACCCTACCTCTGACA R: GAGACTTTCCATCCACGA	310-400	55	7	0.6875	0.7618	0.0133	-
HCY-72	JX683372	$(TG)_{10}$	F: GCAGGTATGCGTGCTAAG R: CTGCGATGCTGAATAAATAG	240-330	55	12	0.7083	0.8228	0.0097	-
HCY-80	JX683373	$(AC)_6$	F: GTGGGAGAAACAGAAATC R: GACTCACAGGAAACAACC	200-250	50	7	0.6042	0.7294	0.2299	+
HCY-84	JX683374	$(GT)_{11}$	F: TTCCTTGTAGCCCACTTC R: CCTTTACACGAGGGTCAG	290-330	55	7	0.5417	0.6785	0.0769	-
HCY-86	JX683375	$(CT)_{20}\ldots(TC)_5$	F: GTTGGACTGGAAGCTGAT R: TGGGAAACATGGTATTGA	180-300	55	22	0.9583	0.9423	0.8288	-
HCY-95	JX683376	$(TG)_5\ldots(TG)_{13}$	F: CTCTTCCTCCCTCCATCT R: TTCCCACGCCTATACATC	310-400	55	8	0.7391	0.8500	0.0161	+
HCY-96	JX683377	$(GT)_{12}$	F: AGCGTAATCCCAAACCTCA R:CAGCCATTCAACCAATAACAT	220-280	55	10	0.6667	0.6750	0.2041	-
HCY-105	JX683378	$(GT)_6\ldots(TG)_7$	F: TTTGGCTTTGAAACATTTAC R: CTTTACCCCGAAGGAGAT	180-270	55	17	0.9583	0.9235	0.8448	-
HCY-107	JX683379	$(GT)_9$	F: GATGAGCCATCAGGTGTA R: AAGTTTCTGGGAAGGTTT	230-280	55	6	0.6875	0.7419	0.0579	-
HCY-108	JX683380	$(TG)_{14}$	F: CAATATGGCTTCCCTCAC R: TGTCCCTGTATCTAATCTGC	260-320	57	7	0.6667	0.8226	0.0127	+
HCY-115	JX683381	$(TCC)_5$	F: GTATCTACACCACGAATGCT R: TTTTGAAAGGTAAACAAACC	220-280	55	5	0.6458	0.6340	0.6301	-

Ta = annealing temperature; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity. *Indicated deviation from Hardy-Weinberg equilibrium ($P < 0.002$) after Bonferroni's correction; P_{HW} = Hardy-Weinberg probability test; (+) = successful in cross-species amplification; (-) = unsuccessful in cross-species amplification.

CONCLUSIONS

In the present study, a microsatellite-enriched genomic library of false kelpfish (*S. marmoratus*) was constructed, and a total of 20 new genomic microsatellite loci were identified and characterized. Most of these markers could also be successfully amplified in another species, *I. japonicas*. These polymorphic markers represent a valuable microsatellite marker resource for *S. marmoratus* and may be used in studies to assess population genetic structures or in other studies on scorpionfish species.

ACKNOWLEDGMENTS

Research supported by Public Science and Technology Research Founds Projects of Ocean (Grant #201205024) and the National Natural Science Foundation of China (Grant #31061160187).

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