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*Short Communication*

# Seventeen polymorphic microsatellite markers developed for the Javelin goby, *Synechogobius hasta* (Gobiidae)

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**ABSTRACT.** *Synechogobius hasta* is an important commercial marine fish with distinctive features of rapid growth and short lifespan. We isolated and characterized 17 microsatellite markers for *S. hasta* using a $(GT)_{13}$-enriched genomic library. Polymorphism was assessed in 48 individuals from a single population collected from the northern coastal waters of the Yellow Sea. The number of alleles per locus ranged from 2 to 23, with a mean of 11.3. The observed and expected heterozygosities ranged from 0.130 to 1.000 and from 0.123 to 0.939, with means of 0.758 and 0.774, respectively. Fourteen of 17 loci conformed to Hardy-Weinberg equilibrium and no significant linkage disequilibrium between locus pairs was detected. These microsatellite markers will be useful for population genetic structure analyses.

**Key words:** Javelin goby; *Synechogobius hasta*; Microsatellite loci; Genetic structure

 

## INTRODUCTION

Javelin goby (*Synechogobius hasta*), which belongs to the family Gobiidae, is a warm-temperature, demersal, euryhaline, amphidromous, commercial fish inhabiting the inshore waters of the northwestern Pacific Ocean from Japan and China to Indonesia (Chen, 1978). Individuals grow rapidly and have a lifespan of just 1 year (Chen, 1978). With the decline in fishery resources in recent years, increasing attention has been paid to *S. hasta* breeding because the fish has good taste and fast growth (Feng et al., 2004; Luo et al., 2008). Most studies on *S. hasta* have focused mainly on morphology and genetics (Sun and Chen, 1993; Wang and Zhao, 1994; Feng et al., 2010). However, assessments of genetic diversity are also important for conservation and management of this species, because it has high economic and scientific value. Microsatellite markers are highly polymorphic and widely used in genome mapping and population genetic studies in artificially cultured species (Litt and Luty, 1989; Shao et al., 2009; Yang et al., 2010). However, specific microsatellite markers for *S. hasta* are currently unavailable. Our aim was to isolate and characterize polymorphic microsatellite loci in *S. hasta* to provide useful markers for conservation and management studies of the species.

## MATERIAL AND METHODS

Forty-eight samples of *S. hasta* were collected from the northern coastal waters of the Yellow Sea and preserved in alcohol until DNA extraction. A dinucleotide-enriched genomic library was constructed following the method of Ma and Chen (2009). Genomic DNA was extracted from muscle tissue using the phenol-chloroform procedure (Sambrook and Russell, 2001). Genomic DNA was then digested with *Mse*I restriction enzyme (New England Biolabs, USA). The fragments were ligated to the adapters (5'-TACTCAGGACTCAT-3'/ 5'-GACGATGAGTCCTGAG-3'). Linker-ligated DNA was pre-amplified in 25-µL reactions using the adapter-specific primer (5'-GATGAGTCCTGAGTAA-3'). Polymerase chain reaction (PCR) conditions included 20 cycles of denaturation (94°C, 30 s), annealing (55°C, 1 min), and extension (72°C, 1 min). After the PCR products were purified with DNAmate (TaKaRa, Japan), they were hybridized to a biotin-labeled $(GT)_{13}$ probe through denaturation at 94°C for 5 min, then at 53°C for 15 min.

Single-stranded DNA fragments containing microsatellite repeat sequences were captured using streptavidin-coated magnetic beads (Promega, USA). The captured DNA was eluted from the magnetic beads and amplified using the adaptor-specific primer and the above program. PCR products ranging from 500 to 1000 bp were selected through separation on 1.5% agarose gels. The fragments were cloned into pMD18-T vectors (TaKaRa) and then transformed into *Escherichia coli* DH5α competent cells to produce a microsatellite-enriched library. Positive clones were sequenced on an ABI 3730 automated DNA sequencer (Applied Biosystems, USA).

PCR primer pairs were designed in DNA sequence-containing microsatellite repeats using the PRIMER PREMIER 5 software (Premier Biosoft International, USA) and then tested for polymorphism from 6 *S. hasta*. After preliminary screening, only 17 polymorphic microsatellite loci were evaluated in a sample of 48 individuals. PCR was performed on a Veriti Thermal Cycler in a total volume of 25 µL containing 0.4 µM of each primer, 0.2 mM of each deoxyribonucleotide triphosphate, 1X PCR buffer, 2 mM $MgCl_2$, 1 U *Taq* polymerase (Fermentas, USA), and 10-100 ng DNA. Cycling conditions consisted of initial denaturation

at 94°C for 5 min, 35 cycles of 45 s at 94°C, 1 min at the locus-specific annealing temperature (Table 1), 45 s at 72°C, and a final cycle of 10 min at 72°C. Allele size was estimated according to the pBR322 DNA/*Msp*I marker (TianGen, China) after PCR products were separated on 6% denaturing polyacrylamide gel. The expected and observed heterozygosities together with an analysis of Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were calculated using GENEPOP 4.0 (Raymond and Rousset, 1995). Null allele frequencies were calculated with MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004). The significant values for all multiple tests were corrected by the sequential Bonferroni's procedure (Rice, 1989).

**Table 1.** Characteristics of microsatellite loci in *Synechogobius hasta*.

| Locus | Primer sequence (5'-3') | Repeat motif | Ta (°C) | Allele size range (bp) | $N_A$ | $H_O$ | $H_E$ | N | $P_{HWE}$ | Accession No. |
|---|---|---|---|---|---|---|---|---|---|---|
| Syn2†* | F: CCCCACCAAATGAAAGAA<br>R: AGCGCAGCATGTAAACAA | $(CAA)_6$ | 45 | 300-310 | 6 | 0.522 | 0.758 | 46 | 0.0002 | JQ398712 |
| Syn6 | F: CGTTCAGTGGAGGTGTAGT<br>R: CAAATTATCATCCTGGGTC | $(GT)_{10}$ | 57 | 255-265 | 7 | 1.000 | 0.791 | 46 | 1.000 | JQ398713 |
| Syn13†* | F: AGAGCAAACTGACAAAACC<br>R: CAGGGACAAAAGTACAAGG | $(CA)_{18}$ | 55 | 215-225 | 6 | 0.422 | 0.649 | 45 | 0.000 | JQ398714 |
| Syn15 | F: ACCACTAATACCTTCCCCAAAC<br>R: GTAGCCCCGAGGAACAGC | $(AC)_{15}$ | 57 | 200-250 | 9 | 0.700 | 0.798 | 40 | 0.0084 | JQ398715 |
| Syn23 | F: AACGGCTGACTGAAGAAG<br>R: CGTACTAACCATAGCTGGAA | $(TG)_5(GT)_{12}$ | 57 | 252-300 | 17 | 0.792 | 0.833 | 48 | 0.0061 | JQ398716 |
| Syn24 | F: TTTTACCCGTGGATAAGTG<br>R: TTGAACGCAGCAAACATA | $(CTT)_7$ | 55 | 290-295 | 4 | 0.708 | 0.687 | 48 | 0.5986 | JQ398717 |
| Syn26†* | F: TGGATCTGTCACCGAAAT<br>R: GTTGATACCTGGAAGAAGAGC | $(AC)_{19}$ | 55 | 335-385 | 15 | 0.729 | 0.909 | 48 | 0.000 | JQ398718 |
| Syn28 | F: CACCTGACCTTGACCCTT<br>R: TGCCTGCGTGTAGTCTATT | $(AC)_9(CA)_7.(CA)_5\ldots(CA)_{12}$ | 55 | 240-300 | 17 | 1.000 | 0.891 | 44 | 1.000 | JQ398719 |
| Syn34 | F: CCATTGAACTGCGTGACTA<br>R: TGAAGACTGGCATCTCCTT | $(TG)_{14}$ | 50 | 240-270 | 11 | 0.917 | 0.799 | 48 | 0.9923 | JQ398720 |
| Syn35 | F: TTGTTTTGTTATTGGAGC<br>R: TGTTTGGTTGTAGTTGTATT | $(TG)_{14}\ldots(GT)_{20}$ | 50 | 180-250 | 23 | 1.000 | 0.939 | 47 | 1.000 | JQ398721 |
| Syn36 | F: GGCTGTCCTGTGGATTGT<br>R: CGCAGATGTAAGCGAAAC | $(GT)_{14}$ | 50 | 180-200 | 12 | 0.957 | 0.823 | 47 | 0.8770 | JQ398722 |
| Syn37 | F: GTGGCAGTTCCTTATTGTG<br>R: AGGCGTTGTGAGTTTCAG | $(GT)_9GA(GT)_5\ldots(GT)_{12}$ | 55 | 260-315 | 15 | 1.000 | 0.898 | 48 | 1.000 | JQ398723 |
| Syn41 | F: GCTGTTGTACGGATGGGAATT<br>R: TGCCGCCTCAACCTCTTT | $(AC)_{11}..(AC)_5..(AC)_{19}$ | 55 | 340-440 | 15 | 0.809 | 0.851 | 47 | 0.0608 | JQ398724 |
| Syn51 | F: TCCGACACCAACAAACTCC<br>R: TTCCACCAACCGTATCTCC | $(AC)_{23}$ | 52 | 220-265 | 12 | 0.938 | 0.797 | 48 | 0.9739 | JQ398725 |
| Syn53 | F: TACATTAGAGCCCTCTGCA<br>R: TCATCACCAAAACCAACC | $(CA)_8$ | 58 | 180-200 | 10 | 0.841 | 0.816 | 44 | 0.3956 | JQ398726 |
| Syn61 | F: GAGGTCGTTCCACTTGTC<br>R: GCTGAGTTTAGGGTTTATGT | $(AC)_{10}..(CT)_8\ldots(CA)_9$ | 57 | 205-210 | 2 | 0.130 | 0.123 | 46 | 1.000 | JQ398727 |
| Syn68 | F: AAACATCAGCTCCAGAACA<br>R: GAATATGGCAAAAGACAAAA | $(TG)_5..(GT)_{20}..(TG)_{13}$ | 55 | 185-240 | 11 | 0.417 | 0.750 | 47 | 0.004 | JQ398711 |

Ta = optimized annealing temperature; $N_A$ = number of alleles; $H_O$ = observed heterozygosity; $H_E$ = expected heterozygosity; N = number of individuals genotyped; $P_{HWE}$ = Hardy-Weinberg probability; †Locus deviated from Hardy-Weinberg equilibrium (adjusted P value <0.00294); *Locus may harbor null alleles (null allele frequency >5%).

## RESULTS AND DISCUSSION

The 17 loci that were successfully amplified in this study showed polymorphism, and the number of alleles per locus ranged from 2 to 23, with an average of 11.3. The observed and expected heterozygosities ranged from 0.130 to 1.000 and from 0.123 to 0.939, with an average of 0.758 and 0.774, respectively (see Table 1). Three loci departed significantly from HWE

($P < 0.05$) after sequential Bonferroni's correction (adjusted P value <0.00294). The observed departure from HWE is likely due to the presence evidence of "null" alleles (syn2, syn13, and syn26). No significant linkage disequilibrium was found between any pair of loci after Bonferroni's correction. These developed loci are highly polymorphic and powerful enough to assess the population genetic structure and analyze the kinship analysis of *S. hasta* and related species.

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