# Isolation and characterization of twenty-three microsatellite loci for the black rockfish, *Sebastes schlegelii*

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**ABSTRACT.** Twenty-three polymorphic microsatellite loci were developed from the black rockfish, *Sebastes schlegelii*, with an enriched partial genomic library by magnetic beads and polymorphism of these loci was assessed in 32 individuals from a wild population. The loci yielded 2-19 alleles per locus, the observed, expected heterozygosity and polymorphic information content ranged from 0.063 to 1.000, 0.091 to 0.945 and 0.085 to 0.926, respectively. Twenty loci confirmed to Hardy-Weinberg equilibrium and only one pairs of loci show significant linkage disequilibrium after Bonferroni's correction. The availability of these markers will facilitate studies of the conservation genetics of S. schlegelii.

**Key words:** *Sebastes schlegelii*; Microsatellite loci; Polymorphism

## INTRODUCTION

The black rockfish (*S. schlegelii*) is an important commercial fish distributed in Northwest Pacific enjoying high popularity in China, Korea and Japan (Yoshida *et al*., 2005). In recent decades, commercial exploitation and environmental changes have caused a decline in its population. Thus, demand for hypervariable molecular markers to provide a population-genetic perspective on conservation and management efforts of the spices (FAO, 1993) becomes urgent. Although few microsatellite loci have been isolated from *S. schlegelii*, more microsatellite markers are needed for further work on population structure within and among populations. In this paper, we describe twenty-three more polymorphic microsatellite loci that would be useful to facilitate further conservation genetics studies in *S. schlegelii*.

## MATERIAL AND METHODS

Thirty-two individuals of *S. schlegeli* were collected from the northern coastal waters of the Yellow Sea and preserved in alcohol until DNA extraction. Genomic DNA was extracted from muscle using a regular phenol-chloroform procedure (Sambrook *et al*., 2000), and simultaneously digested with restriction enzyme *Sau*3AI (Promega). DNA fragments ranging in size from 400 to 1000bp were collected and consequently ligated to adaptors (OligoA: 5'-GATCGTCGACGGTACCGAATTCT-3' and OligoB: 5'-GTCAAGAATTCGGTACCGTCGAC-3'). DNA fragments were amplified in a 25-μL reaction mix using the adaptor-specific primer (OligoA). Subsequently, the amplified DNA fragments were hybridized to biotinylated $(CA)_{16}$ and $(GA)_{16}$ probes，then captured by streptavidin-coated magnetic beads (Promega). Unhybridized DNA was washed away, and the remaining DNA was eluted from the beads. The enriched DNA was then ligated to pGEM-T easy vectors (Promega) and transformed into competent Escherichia coli DH5α cells to construct an enriched microsatellite sequence library. Positive clones were identified by PCR with SP6, T7 and $(CA)_{16}$ or $(GA)_{16}$ primers and sequenced on an ABI 3730 XL DNA sequencer (Applied Biosystems).

Primers were designed using Primer Primer 5.0 (Premier Biosoft) and subsequently tested at standard PCR conditions with six specimens. Successfully amplified loci with expected sizes were further evaluated with a sample of thirty-two individuals collected from Changdao, Shandong Province. PCR reactions were performed in a 25-μL reaction volume containing 0.4μM of each primer, 0.2mM dNTPs, 2mM $MgCl_2$, 1×PCR buffer, 1U Taq polymerase (Tiangen, China) and 50–100ng DNA. Amplification was performed under the following conditions: 94°C for 5 min, followed by 32 cycles of 94°C for 45 s, optimal annealing temperature (Table 1) for 45 s, and 72°C for 1 min, and a final extension step at 72°C for 10 min. Amplified products were separated by electrophoresis on 8% non-denaturing polyacrylamide gels, and visualized with silver staining. Allele sizes were estimated according to a 50-bp DNA ladder (Tiangen, China) with software Gel-Pro Analyzer4.5 (Media Cybernetics).

The number of alleles ($N_a$), observed and expected heterozygosity ($H_o$, $H_e$) were calculated using POPGENE 1.31 software (Yeh *et al*., 1999). Polymorphism information content (PIC) was estimated with PIC-CALC v0.6. Tests of Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were analyzed using GENEPOP v3.4 (Raymond and Rousset, 1995). The value for all diversity tests of significance was corrected by Bonferroni's correction (Rice, 1989).

## RESULTS AND DISCUSSION

Sixty-eight positive clones were obtained after amplifying. Fifty-six of them contained microsatellites and Fifty-two sequences provided sufficient quality for primer pairs to be designed after sequencing. Of the primers designed, 48 generated clear and specific products consistently, and 23 primers present polymorphic. A total of 151 alleles were detected in all 32 individuals. The allele number of the 23 polymorphic loci ranges from 2 to19 with an average of 6.565 per locus. Polymorphism information content ranges from 0.085 to 0.926. 8 and 12 loci presented a medium or high level of polymorphism according to standard of Botstein *et al.* (1980), respectively. The observed and expected heterozygosities ranged from 0.063 to 1.000 (mean 0.503) and 0.091 to 0.945 (mean 0.599), respectively (Table 1).

**Table 1.** Characteristics of twenty-three microsatellite loci developed from *S. schlegelii*.

| Locus/ Accession No. | Primer sequence (5′-3′) | Ta (℃) | Repeat motif | Size range (bp) | *A* | *Ho* | *He* | *PIC* | HWE *P* value |
|---|---|---|---|---|---|---|---|---|---|
| **HJ3-3/ KJ472145** | F:TGAGACTGAATTATGGGAGA R:AAATGCTTACAGGGCTTC | 60 | $(AG)_7(TC)_{10}(AC)_5$ | 122–134 | 8 | 0.188 | 0.867 | 0.836 | 0.000* |
| **HJ5-1/ KJ472146** | F:ATACGCTCTGTATTCAACG R:ACTTCCACATCAAATGTCCT | 63 | $(AG)_7C(GA)_{19}$ | 215–267 | 12 | 0.688 | 0.859 | 0.828 | 0.062 |
| **HJ5-6/ KJ472147** | F:CTGCCGAATCAGAGGTGT R:GCCAGCGTAGGAAGGAAT | 60 | $(CT)_{10}$ | 302–311 | 4 | 0.156 | 0.205 | 0.193 | 0.030 |
| **HJ5-7/ KJ472148** | F:CCCTTAGGCTGGACAACT R:GTGAATCTGCACCCGTGA | 64 | $(CA)_{12}GA(CA)_7\ldots(AC)_5\ldots(TG)_5$ | 352–363 | 6 | 0.375 | 0.445 | 0.414 | 0.029 |
| **HJ5-24/ KJ472149** | F:AAATGAACTACCAAAGGAAGCG R:GGGAAATCTCAAAGCAAACACT | 64 | $(TTC)_4...(GCT)_5$ | 457–468 | 4 | 0.406 | 0.622 | 0.547 | 0.000* |
| **HJ5-26/ KJ472150** | F:GACTCACCCTGCAAACAC R:TACCACCCAGACACCACA | 64 | $(AC)_{13}$ | 206–232 | 9 | 1.000 | 0.869 | 0.839 | 0.010 |
| **HJ5-29/ KJ472151** | F:ATGGCGTCTTGCCCTTGT R:CGCGGCCTTTTCATCTCA | 60 | $(AC)_8$ | 187–201 | 9 | 1.000 | 0.881 | 0.853 | 0.007 |
| **HJ5-31/ KJ472152** | F:AATTGGTTGTTAATTCCGGTCC R:GCCTTTCTTTCCAAATTCAGTG | 50 | $(CT)_{26}\ldots(TC)_5$ | 138–145 | 2 | 0.094 | 0.091 | 0.085 | 1.000 |
| **HJ5-34/ KJ472153** | F:TTCTGCTGAATGAGGGAG R:CAGCGGGAGTTTGTATTG | 60 | $(AG)_5$ | 490–498 | 4 | 0.500 | 0.538 | 0.494 | 0.014 |
| **HJ5-44/ KJ472154** | F:GCACCAATCTGTACCATCA R:TAAGAGCCCAGTCACCAT | 60 | $(TGCG)_3\ldots(TGCG)_3$ | 144–166 | 6 | 0.750 | 0.766 | 0.716 | 0.179 |
| **HJ5-46/ KJ472155** | F:CCACTGGCAGATAAACGA R:TTTTAACGGGCAGTTGTG | 63 | $(TG)_{15}\ldots(TG)_{10}$ | 197–219 | 13 | 0.656 | 0.856 | 0.829 | 0.049 |
| **HJ5-47/ KJ472156** | F:GGCCTCAAATGACAAGGT R:CCAGGGTGCTCTGAAGAA | 63 | $(AC)_5G(CA)_{20}$ | 362–496 | 19 | 1.000 | 0.945 | 0.926 | 0.114 |
| **HJ5-58/ KJ472157** | F:CGCTCGGTGTTGATTTCG R:CGCCACTTCAGCTCCTTT | 60 | $(CA)_5$ | 210–239 | 5 | 0.375 | 0.397 | 0.364 | 0.241 |
| **HJ5-64/ KJ472158** | F:GTCCACTTGGTGAGGGGTTT R:TGGAGCAGAACAGGGCGTAG | 60 | $(CT)_6\ldots(CT)_5$ | 496–500 | 3 | 0.438 | 0.673 | 0.588 | 0.007 |
| **HJ5-65/ KJ472159** | F:TTTACAGGTCTTGAAGCAGG R:GAAGTTGAAGTTTTGAGGG | 60 | $(CT)_{10}$ | 176–188 | 4 | 0.500 | 0.663 | 0.586 | 0.093 |
| **HJ5-75/ KJ472160** | F:CACCTGGATTTATTTGGG R:AAGAGGCAGTATGGATGG | 60 | $(CT)_9$ | 168–180 | 6 | 0.375 | 0.473 | 0.446 | 0.010 |
| **HJ5-84/ KJ472161** | F:AGCATCAGGGATAAAGGA R:GACGATTAGCCAGGTTGT | 60 | $(CA)_6$ | 188–199 | 5 | 0.313 | 0.379 | 0.353 | 0.027 |
| **HJ5-103/ KJ472162** | F:ACAGGAGAACAGGCAAGG R:TCAGCACGAGTACCGAAG | 62 | $(AC)_{27}\ldots(AC)_{12}$ | 335–411 | 10 | 0.906 | 0.895 | 0.869 | 0.118 |
| **HJ5-120/ KJ472163** | F:GCCCGACTCACCAAAACA R:TCCACAAATCGCCGTCTC | 52 | $(GA)_6$ | 280–285 | 3 | 0.063 | 0.121 | 0.116 | 0.031 |
| **HJ5-134/ KJ472164** | F:AGTGAAGCCACTGTGAGA R:AGTGAAGCCACTGTGAGA | 63 | $(TTAGGG)_6GTT(GGGTTA)_6\ldots(GGGTTA)_5$ | 126–182 | 7 | 0.719 | 0.824 | 0.788 | 0.010 |
| **HJ5-140/ KJ472165** | F:AAGAGCCCAGTCACCATG R:TGACCTCGGGTAGAAACG | 60 | $(GCAC)_4$ | 162–186 | 4 | 0.438 | 0.534 | 0.417 | 0.323 |
| **HJ5-153/ KJ472166** | F:TGCTGGATCGGTAAGAAG R:CCCGCATCAGTGAGAAAA | 60 | $(TCCTC)_3\ldots(CT)_7$ | 190–200 | 4 | 0.500 | 0.411 | 0.368 | 0.774 |
| **HJ5-154/ KJ472167** | F:GGGTGAGTGAGGAAGGAT R:GCACAACTCTGCCAAAAC | 60 | $(CCTCT)_4$ | 158–178 | 4 | 0.125 | 0.455 | 0.372 | 0.000* |

## CONCLUSION

After Bonferroni's correction, *p* values were set to 0.002 for HWE and 0.0002 for linkage disequilibrium. Three loci (HJ3-3, HJ5-24 and HJ5-154) showed significant deviations in the observed genotype frequencies from Hardy-Weinberg equilibrium and one pair of loci yielded a significant linkage disequilibrium. The microsatellite loci developed here should be particularly useful for genetic identification and population management of *S. schlegelii* in future conservation biology efforts.

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## REFERENCES

Botstein D, White RL, Skolnick M and Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet.* 32: 314-331. https://doi.org/10.1007/bf00266542

FAO (1993). Report of the expert consultation on utilization and conservation of aquatic genetic resources. *FAO Fish Rep.* 491: 1-58.

Raymond M and Rousset F (1995). GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *J Hered*. 86 (3): 248-249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Rice WR (1989). Analyzing tables of statistical tests. *Evolution.* 43(1): 223-225. https://doi.org/10.2307/2409177

Sambrook JD and Russell W (2000). Molecular cloning: A laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. https://doi.org/10.1002/abio.370050118

Yeh FC, Yang RC and Boyle T (1999). Microsoft Window-based freeware for population genetic analysis (POPGENE), Ver. 1.31. University of Alberta, Canada.

Yoshida K, Nakagawa M and Wada S (2005). Multiplex PCR system applied for analyzing microsatellite loci of Schlegel's black rockfish, *Sebastes schlegeli*. *Mol Ecol Res.* 5 (2): 416-418. https://doi.org/10.1111/j.1471-8286.2005.00945.x