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# Isolation and characterization of thirteen polymorphic microsatellite loci from black porgy (*Acanthopagrus schlegeli*)

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[Yang Y., Cao X., Feng R., Chen S., Zhang Z., Ren W. and Xu S. 2014 Isolation and characterization of thirteen polymorphic microsatellite loci from black porgy (*Acanthopagrus schlegeli*). *J. Genet.* **93**, e97–e99. Online only: http://www.ias.ac.in/jgenet/OnlineResources/93/e97.pdf]

## Introduction

Black porgy, *Acanthopagrus schlegeli* (Bleeker 1854), is an important commercial species in the coastal waters of western Pacific Ocean, including Japan, Korea and China (Sadovy and Cornish 2000). Due to its fast growth, low mortality and good performance the interest of this species in aquaculture is increasing (Gwo *et al.* 2005). This species has been cultured successfully in China (Hsu *et al.* 2008), as well as in Japan (Blanco Gonzalez *et al.* 2008). In recent years, juveniles of black porgies are heavily fished in the southern waters of China and neighbouring countries (Wilson 1997). Overfishing has led to sharp decline in wild populations (Sadovy and Cornish 2000). In the present study, we have developed and characterized 13 microsatellite markers for *A. schlegeli* which will be useful for the future genetic studies.

## Materials and methods

Genomic DNA from 30 individuals of the *A. schlegeli* was extracted using a standard proteinase K / phenol–chloroform extraction protocol (Sambrook and Russell 2006). A microsatellite dinucleotide-enriched library was constructed using the fast isolation by AFLP of sequences containing repeats (FIASCO) protocol (Zane *et al.* 2002) with minor modifications. In brief, the whole genomic DNA was digested with *Mse*I enzyme (New England Biolabs, Massachusetts, USA) and ligated to the adapters (Oligo A: 5′-TACTCAGGACTCAT-3′ and Oligo B: 5′-GACGATGAGTCCTGAG-3′). Bio-labelled probes $(GT)_{13}$ were used to hybridize with the recovered products of preamplification (size ranged from 300 to 900 bp). Subsequently, the hybrids were captured by streptavidin-coated magnetic beads (Streptavidin Magnesphere Paramagnetic Particles, Promega, Shanghai, China). Nonspecific binding and unbound DNA was removed by several nonstringent and stringent washes. These microsatellite-enriched DNA fragments were amplified by polymerase chain reaction (PCR), and then ligated into pMD18-T plasmid vector (Takara, Dalian, China) and transformed into competent JM109 *Escherichia coli* cells (Takara, Dalian, China). Recombinant clones were identified using blue/white screening on Luria-Bertani agar plates. Insert positive bacterial clones were amplified using M13 primers and visualized by agarose gel electrophoresis. Sixty positive clones were identified and sequenced with ABI BigDye Terminators Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) in an ABI PRISM3100 automated sequencer. Microsatellites were detected using the Tandem Repeats Finder software (Benson 1999). A total of 42 sequences contained microsatellites with perfect repeat motifs, 36 of these were suitable for designing locus-specific primers using the Primer3 web interface program (Rozen and Skaletsky 2000).

The characterization of microsatellites was conducted using 30 unrelated individuals from a black porgy cultivation farm in Nantong, Jiangsu, China. Instead of a 5′-dye-labelled primer, a M13F (–29) sequence was added to the 5′-end of the primer. As a result, DNA fragments produced had the M13F (–29) sequence. To produce labelled DNA fragments, labelled M13F was added to the reaction. PCR amplifications

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**Keywords.** microsatellite loci; Hardy–Weinberg equilibrium; *Acanthopagrus schlegeli*.

**Table 1.** Characteristics of 13 polymorphic microsatellite markers isolated from *A. schlegeli*.

| Locus | Repeat motif | Primer sequences (5′–3′) | Size range (bp) | $T_a$(°C) | $n$ | $N_A$ | $H_O$ | $H_E$ | $P$ value (HWE) | Accession no. |
|---|---|---|---|---|---|---|---|---|---|---|
| AC120 | $(GT)_{15}$ | F: CGTACTCAGTTACATTCCATCAC<br>R: TACTGCACAGCCATCAACC | 177–245 | 62 | 30 | 12 | 0.8222 | 0.7333 | 0.1760 | GU166141 |
| AC174 | $(GT)_{11}$ | F: TAAAGGAGCTGCGTGGTTG<br>R: AGACAGACTCCCAAACACC | 214–234 | 62 | 29 | 6 | 0.7438 | 0.7241 | 0.0120 | GU166142 |
| AC103 | $(CA)_{12}\ldots(CA)_5$ | F: AAATCCCATCTATGTCAAGCC<br>R: AGGGCATGCAAGGTAAGG | 249–287 | 63 | 29 | 14 | 0.8008 | 0.6897 | 0.0000* | GU166143 |
| AC229 | $(TG)_{11}$ | F: TGTCCGTTCTGCTTTGCTC<br>R: TGCGGTAGTGCCTTCTCTG | 292–310 | 65 | 30 | 9 | 0.8394 | 0.8000 | 0.2670 | GU166144 |
| AC110 | $(TG)_{16}$ | F: TGCTGAGTCTCAGAGGGAATAC<br>R: TTTCATAGATGAGGCAGGGAC | 227–237 | 63 | 29 | 6 | 0.7087 | 0.6552 | 0.2560 | GU166145 |
| AC35 | $(GT)_{10}$(gggttg)$(GT)_9$ | F: TCTGCGTGTCTTTGGGGT<br>R: CAACCAATAAAAGGAACTCGG | 121–263 | 64 | 30 | 10 | 0.7489 | 0.5667 | 0.0110 | GU166146 |
| AC230 | $(TG)_{13}$ | F: GGTGGTGTCGTCTGCTGTT<br>R: TCCGGCTGCTGTGAATC | 106–172 | 65 | 29 | 9 | 0.8050 | 0.7931 | 0.0470 | GU166147 |
| AC146 | $(CA)_{13}$ | F: CGTGGACTCTTATTCACCTGCT<br>R: AGAACGGAGGCAACAGACC | 107–203 | 65 | 29 | 19 | 0.8882 | 0.8276 | 0.5350 | GU166148 |
| AC234 | $(CA)_{17}$ | F: GATCTGCTCATGTTACGTTCTG<br>R: ATGTTTTGTTGCTTTTGACG | 179–321 | 64 | 30 | 8 | 0.7983 | 0.8333 | 0.1040 | GU166149 |
| AC221 | $(CA)_{14}$ | F: CACCATACACCCTCTTCATCC<br>R: GTTATTGGCTCTATGTGTGGG | 216–234 | 62 | 29 | 8 | 0.7122 | 0.2759 | 0.0000* | GU166150 |
| AC123 | $(GT)_{16}$ | F: CACTCTGTCCTACGTCCTCATC<br>R: GGTGGCTGTGGGTTTTATT | 285–343 | 65 | 30 | 18 | 0.9094 | 0.6333 | 0.0000* | GU166151 |
| AC176 | $(CA)_{13}$ | F: CTGACTTATTTGCCCTTTTCC<br>R: GACCTACTCCAGACCAAGCC | 191–213 | 64 | 30 | 7 | 0.7528 | 0.7667 | 0.3460 | GU166152 |
| AC87 | $(GT)_{12}$ | F: CATGTGGGCTCTCTGGAC<br>R: TTGTCCCAACACCTCCCT(tailed) | 187–205 | 65 | 29 | 6 | 0.6920 | 0.4138 | 0.0030* | GU166153 |

$T_a$, annealing temperature; $n$, number of individuals sampled; $N_A$, observed number of alleles; $H_O$, observed heterozygosity; $H_E$, expected heterozygosity; $P$ value, HWE probability test after Bonferroni correction; *significant deviation from HWE after Bonferroni correction (adjusted $P < 0.005$).

were conducted in 15 $\mu$L volumes containing 50 ng template DNA, Premix *Ex Taq* buffer 7.5 $\mu$L (Takara), 2.5 pmol of each primer and 0.5 pmol of fluorescently labelled M13 primer either IRD700 or IRD800 (LI-COR, Lincoln, USA). The conditions for amplification were 5 min at 95°C, followed by 30 cycles of 40 s at 94°C, 40 s at the annealing temperatures (table 1) and 40 s at 72°C, with a final extension time of 10 min at 72°C. Amplified products were separated on 6.5% polyacrylamide gels using a LI-COR 4300 automated DNA sequencer and analysed using LI-COR SagaGT software (LI-COR).

For each polymorphic locus, the allele number, size range, number of bands per individual, expected and observed heterozygosities were calculated using the program Genetix 4.05 (Belkhir *et al.* 2004). Deviation from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) at each locus was calculated using GenePop ver. 3.4 (Raymond and Rousset 1995).

## Results and discussion

A total of 42 sequences containing microsatellites with perfect repeat motifs of which 36 were suitable for designing locus-specific primers. Eighteen primer sets were designed and synthesized. Finally, 13 of 18 loci examined in *A. schlegeli* were found to be polymorphic, while the other six primer pairs amplified monomorphic loci or failed to amplify a consistent product.

The number of alleles per locus ranged from 6 to19 (table 1). The expected and observed heterozygosities ranged from 0.2759 to 0.8333 and 0.6920 to 0.9094, respectively. LD tests revealed significant linkage between loci AC174 and AC146 following sequential Bonferroni correction (Rice 1989). In addition, after sequential Bonferroni correction, four (AC103, AC221, AC123 and AC87) of the 13 loci showed significant departures from HWE ($P < 0.005$). These deviations from expectations may be due to insufficient sample size, the occurrence of null alleles or the sampling strategy with individuals from structured populations.

The microsatellite loci presented in this study will be useful for investigating the population structure and genetic diversity of the *A. schlegeli* and other related species.

**Acknowledgements**

This work was supported by the National Natural Science Foundation of China (NSFC) (grant no. 31000953 and 31370401), the Priority Academic Programme Development of Jiangsu Higher Education Institutions to S. X. Xu, NSFC for Talents Training in Basic Science (J1103507, J1210025). The authors would like to thank Shuzhen Li for her help during the research.

## References

Belkhir K., Borsa P., Chikhi L., Raufaste N. and Bonhomme F. 2004 GENETIX 4.0.5.2., software under Windows$^{TM}$ for the genetics of the populations. Laboratory Genome, Populations, Interactions, CNRS UMR 5000, University of Montpellier II, Montpellier, France.

Benson G. 1999 Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Res.* **27**, 573–580.

Blanco Gonzalez E., Nagasawa K. and Umino T. 2008 Stock enhancement program for black sea bream (*Acanthopagrus schlegeli*) in Hiroshima Bay: monitoring the genetic effects. *Aquaculture* **276**, 36–43.

Bleeker P. 1854 Faunae ichthyologicae Japonicae species novae. *Nat. Tijdschr. Ned-Indie* **6**, 395–462.

Gwo J. C., Chiu J. Y., Lin C. Y., Su Y. and Yu S. L. 2005 Spermatozoal ultrastructure of four sparidae fishes *Acanthopagrus berda*, *A. australis*, *Lagodon rhomboids* and *Archosargus probatocephus*. *Tissue Cell* **37**, 109–115.

Hsu T. H., Ning Y., Wang Z. Y., Lee Y. C., Chou C. C., Lin K. H. and Gwo J. C. 2008 The utilization of molecular marker to identify wild and cultured black sea bream (*Acanthopagrus schlegeli*) - a prelimary study. *J. Ecol. Environ. Sci.* **1**, 31–45.

Raymond M. and Rousset F. 1995 Genepop (version 1.2): Population genetics software for exact tests and ecumenicism. *J. Hered.* **86**, 248–249.

Rice W. R. 1989 Analysing tables of statistical tests. *Evolution* **43**, 223–225.

Rozen S. and Skaletsky H. 2000 Primer3 on the WWW for general users and for biologist programmers. In *Bioinformatics methods and protocols* (ed. S. Krawetz and S. Misener), pp. 365–386. Humana Press, Totowa, USA.

Sadovy Y. and Cornish A. S. 2000 *Reef fishes of Hong Kong*. Hong Kong University Press, Hong Kong.

Sambrook J. and Russell D. W. 2006 *Molecular cloning*, 4th edition. Cold Spring Harbor Laboratory Press, New York, USA.

Wilson K. D. P. 1997 The Hong Kong marine fish culture industry-challenges for sustainable development. Proceedings of the First International Symposium on Marine Conservation in Hong Kong, Hong Kong, China.

Zane L., Bargelloni L. and Patarnello T. 2002 Strategies for microsatellite isolation: a review. *Mol. Ecol.* **11**, 1–16.

Received 15 April 2014, in revised form 18 May 2014; accepted 21 May 2014
Unedited version published online: 24 June 2014
Final version published online:10 October 2014