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# Fourteen polymorphic microsatellite markers isolated from big-head croaker (*Collichthys lucidus*)

DIAN-QIAO SUN†, BO ZHANG†, TIAN-JUN XU and RI-XIN WANG*

*Key Laboratory for Marine Living Resources and Molecular Engineering, College of Marine Science, Zhejiang Ocean University, Zhoushan 316000, People's Republic of China*

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## Introduction

The big-head croaker, *Collichthys lucidus*, is a near demersal fish species of family Sciaenidae. *C. lucidus* is an important commercial species widely distributed in the middle and western Pacific, but its numbers have decreased seriously because of overfishing (Wu and Wang 1991; Huang *et al.* 2010). In past decades, intensive studies were focussed on morphological classification, fish eggs and larvae, and size compositions (Zhu *et al.* 1963; Shan *et al.* 2007). Additionally, several researches have been conducted to investigate the genetic characteristics of the big-head croaker (Ma *et al.* 2010). Although 19 polymorphic loci of *C. lucidus* have been developed (Ma *et al.* 2011), molecular information in *C. lucidus* was still limited for genetic mapping or molecular marker-assisted selection (MAS) breeding of this species. Here, we describe the development of 14 polymorphic microsatellite markers of *C. lucidus* to provide more molecular information of this species.

## Materials and methods

Microsatellite-enriched genomic libraries were constructed following the process of fast isolation by amplified fragment length polymorphism of sequences containing repeats (FIASCO) method (Zane *et al.* 2002). Briefly, total genomic DNA was isolated from the fin clips of two different individuals to construct a DNA pool. DNA was digested with *Mse*I restriction enzyme (New England Biolabs, Beijing, China) and approximately 200–1000 bp fragments were ligated to adapters OligoA (5′-TAC TCA GGA CTC AT-3′) and OligoB (5′-GAC GAT GAG TCC TGA G-3′) and then amplified using *Mse*I-N primers (5′-GATGAGTCCTGAGTAAN-3′). Fragments with microsatellite repeats were hybridized with $(CA)_{15}$ biotin-labelled probes. Microsatellite enrichment was separated from the hybridization using streptavidin magnetic beads according to Glenn and Schable (2005) with minor modification. Separated fragments were amplified using *Mse*I-N and finally cloned into pMD19-T vectors (TaKaRa, Dalian, China) using ultra-competent *Escherichia coli* (DH5$\alpha$; Tiangen, Beijing, China).

Positive clones were PCR screened by universal primers M13-F and M13-R. Positive clones that appeared as single distinct bands on agarose gels were randomly selected for sequencing using M13-F primer on an ABI 3730 automated sequencer (PE Applied Biosystems, Foster City, USA). Of the 78 clones sequenced, 48 (61.5%) sequences contained sufficient flanking regions for primer designe and had $\geq 7$ dinucleotide repeats. A total of 28 pairs of primers were designed using Primer Premier 5.0 software (PREMIER Biosoft International, Palo Alto, USA). In addition, we cross-amplified 43 polymorphic loci initially developed in *Miichthys miiuy*, including all loci from Wang *et al.* (2010) and Xu *et al.* (2011). The annealing temperature of the previously published primer was reset for the big-head croaker. A total of 71 primer pairs were initially screened for polymorphism in 30 individuals collected from three different locations along the East Sea of China.

Amplification was carried out in 15 $\mu$L reaction mixture containing 1× PCR buffer (Tiangen), 1.2 $\mu$L dNTPs, 1 $\mu$L of the forward and reverse primers, and 0.3 $\mu$L of *Taq* polymerase (Tiangen, 5 U/$\mu$L). The protocol of amplification was performed as follows: 95°C for 5 min, 30 cycles of 95°C for 30 s, annealing for 30 s, and extension for 30 s

*For correspondence. E-mail: wangrixin1123@126.com.
†These authors contributed equally to this work.

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**Table 1.** Characteristics of 14 polymorphic microsatellite loci in *Collichthys lucidus*.

| Locus | Primer sequence (5′-3′) | Repeat motif | Size (bp) | $T_m$ (°C) | $N$ | $N_e$ | $H_o$ $H_e$ | HWE ($P$) | Null | GenBank |
|---|---|---|---|---|---|---|---|---|---|---|
| Mimi-3 | CATTTTCCACGAGGTTAAT<br>GAAGAAGACATTTGCTGA | $(CA)_9$ | 268–276 | 50 | 5 | 2.78 | 0.44<br>0.64 | 0.16 | 0 | GU084248 |
| Mimi-4 | CATCATAAATAAGCACAGGGAG<br>TCGGAGCAGGGCTAAAGT | $(CA)_{23}$ | 186–190 | 52 | 2 | 1.07 | 0.01<br>0.07 | 0.00* | 1 | GU084249 |
| Mimi-24 | TCGATACAGCTGACGAGA<br>GAGACTCAGGCCCAGAG | $(AG)_{21}GCA(G)_{12}N_{22}(C)_{12}$ | 183–207 | 50 | 4 | 2.19 | 0.48<br>0.54 | 0.85 | 0 | GU084255 |
| Mimi-4-C07 | TGAGGCACAATATGATGG<br>ACCGAGGACTTGGCTACT | $(GAA)_{15}$ | 138–141 | 54 | 2 | 1.99 | 0.82<br>0.50 | 0.01 | 0 | GW668081 |
| Mimi-32-A10 | GAACCACCCATCCTTTTA<br>CTTTGCCCCTTCTGTCTA | $(A)_{14}N_{12}(T)_{17}$ | 224–236 | 50 | 5 | 3.85 | 0.36<br>0.74 | 0.00* | 1 | GW669955 |
| Mimi-40-H12 | TCATCAGAACCAGCCTCT<br>CACATCCTCTTACCTCCTATCT | $(CCT)_5$ | 226–238 | 54 | 2 | 1.96 | 0.50<br>0.49 | 0.99 | 0 | GW670618 |
| Mimi-54-D06 | TCCTCCCATACAAACTAA<br>GGTGGAAGACCGAAAA | $(T)_{13}\ldots(A)_{15}$ | 217–231 | 52 | 8 | 4.66 | 0.50<br>0.79 | 0.00* | 1 | GW671772 |
| Mimi-57-A05 | CTCCTGCCCTTCGTGATT<br>TCTTTCCCTGCTTGTTGTA | $(T)_{14}$ | 160–164 | 50 | 2 | 1.29 | 0.19<br>0.23 | 0.28 | 0 | GW671772 |
| Colu-J-25 | GTGTCTGTTCGTATGTATGC<br>CCGTCTTGCCTGTGTATC | $(GT)_8\ldots AjiGTAj_9$ | 142–163 | 48 | 4 | 1.69 | 0.23<br>0.41 | 0.00* | 1 | JF502234 |
| Colu-J-61 | CTCACAGCTTCACAACAG<br>CAGCCAATCAAGAACCATT | $(GT)_{12}$ | 192–200 | 47 | 5 | 1.62 | 0.14<br>0.38 | 0.00* | 1 | JF502235 |
| Colu-JT-24 | TCACTGTCTTGCCTCTAATG<br>GCTGCTGTACTAATGGAACT | $(TG)_{16}$ | 209–252 | 48 | 5 | 2.16 | 0.35<br>0.54 | 0.02 | 0 | JF502236 |
| Colu-JT-25 | ACAGCAGATGGTAAATTGGT<br>GGAAGCAGCACATATAGACA | $(TG)_{26}$ | 159–176 | 48 | 4 | 2.43 | 0.21<br>0.59 | 0.00* | 1 | JF502237 |
| Colu-JT-26 | CTATCTCCGTCTTGCTTGT<br>ATCTTGTCCCACCGTCAT | $(CA)_{14}$ | 240–246 | 56 | 3 | 1.33 | 0.17<br>0.25 | 0.18 | 0 | JF502238 |
| Colu-JT-28 | AGCCGTGAAGTTGATGAC<br>ATCCTGACATTGTGAGACTAG | $(CA)_{10}$ | 222–234 | 52 | 4 | 1.61 | 0.33<br>0.38 | 0.89 | 0 | JF502239 |
| Average | | | | | 3.93 | 2.19 | 0.23<br>0.41 | | | |

$N$, number of alleles; $N_e$, effective number of alleles; $H_e/H_o$, expected and observed heterozyosity; HWE, Hardy–Weinberg equilibrium and GenBank accession number of 14 polymorphic microsatellite loci. * Show significant deviation from Hardy–Weinberg equilibrium ($P < 0.004$).

at 72°C, finally post-cycling extension for 5 min at 72°C. The specific annealing temperature per primer are listed in table 1. Denatured products were electrophoresed and separated on 6% polyacrylamide gels (19 : 1 acrylamide : bis-acrylamide) using silver staining. Characterization per locus was performed based on genomic DNA of 30 individuals, 10 individuals per location (Zhoushan, Xiangshan and Ninghai).

The program Popgene 32 (Yeh *et al.* 1999) was used to test the number of alleles per locus ($N$), effected number of alleles ($N_e$), expected ($H_e$) and observed ($H_o$) heterozygosity, and departures from Hardy–Weinberg equilibrium (HWE). ARLEQUIN 3.11 software was used to calculate genotypic linkage disequilibrium between these loci (Schneider *et al.* 2000). All results for multiple tests were corrected using Bonferroni correction (Rice 1989).

## Result and discussion

Of the 71 primer pairs, 14 loci were polymorphic in initial screens and were genotyped in the larger set of 30 individuals (table 1). The characterization of these loci in *C. lucidus* were summarized in table 1. $N$ for each locus ranged from 2 to 8, with an average of 3.93, while $N_e$ ranged from 1.07 to 4.66 with an average of 2.19. $H_e$ and $H_o$ ranged from 0.07 to 0.79 and from 0.01 to 0.82, respectively. Significant deviations from HWE were found at Mimi-4, Mimi-32-A10 Mimi-54-D06, Colu-J-25, Colu-J-61 and Colu-JT-25 after Bonferroni correction ($P < 0.004$, adjusted value), which possibly was due to the presence of null alleles. Null alleles were found in six loci (Mimi-4, Mimi-32-A10, Mimi-54-D06, Colu-J-25, Colu-J-61 and Colu-JT-25). Stuttering errors were found in two loci, Mimi-3 and Mimi-32-A10, but no evidence of allelic dropout was found in any of the loci analysed by the program MICRO-CHECKER (Van *et al.* 2004) with Bonferroni correction. Additionally, there was no significant linkage disequilibrium according to 91 pairwise tests ($P > 0.004$, adjusted value), hence allelic variations at these loci were considered independent. Overall, of the 14 developed microsatellite loci in *C. lucidus*, eight significant polymorphic loci will enable studies of the genetic variation, population structure, conservation genetics and molecular-assisted selective breeding of the big-head croaker in future.

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## References

Glenn T. C. and Schable N. A. 2005 Isolating microsatellite DNA loci. *Methods Enzymol.* **395**, 202–222.

Huang L. M., Li J., Xie Y. J. and Zhang Y. Z. 2010 Study of the *Collichthys lucidus* fisheries resources of the Minjiang Estuary and its adjacent waters. *J. Oceanogra Taiwan Strait* **29**, 250–256 (in Chinese).

Ma G. Q., Gao T. X. and Sun D. R. 2010 Discussion of relationship between *Collichthys lucidus* and *C. niveatus* based on 16S rRNA and *Cyt b* gene sequences. *South China Fish. Sci.* **6**, 13–20.

Ma C. Y., Ma H. Y., Ma L. B. and Cui H. Y. 2011 Development and characterization of 19 microsatellite markers for *Collichthys lucidus*. *Conserv. Genet. Res.* **3**, 503–506.

Rice W. E. 1989 Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Schneider S., Roessli D. and Excoffier L. 2000 ARLEQUIN: a software for population genetics date analysis, version 2.000. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Geneva, Switzerland.

Shan L. Z., Shao X. B. and Yan M. C. 2007 The study on biological charactersistics of *Collichthy lueidus* (Richardson). *J. Aquaculture* **28**, 4–5.

Van O. C., Hutchinson W. F., Wills D. P. M. and Shipley P. 2004 MICRO-CHECKER: software for identifying and correcting genotyping errors in mirosatellite data. *Mol. Ecol. Notes* **4**, 535–538.

Wang R. X., Xu T. J., Sun Y. N. and He G. Y. 2010 Polymorphic microsatellite loci from two enriched genomic libraries for the genetic analysis of the miiuy croaker, *Miichthys miiuy* (Sciaenidae). *Genet. Mol. Res.* **9**, 931–934.

Wu C. W. and Wang W. H. 1991 Analysis of the biological characteristics the distribution and resource change of *Collichthy lueidus*. *Mar. Fish.* **13**, 6–10 (in Chinese).

Xu T. J., Sun D. Q., Sun Y. N. and Wang R. X. 2011 Development of 30 novel polymorphic expressed sequence tags (EST)-derived microsatellite markers for the miiuy croaker, *Miichthys miiuy*. *Int. J. Mol. Sci.* **12**, 4021–4026.

Yeh F. C., Yang R. C. and Boyle T. 1999 POPGENE 32, Version 1.31. Population genetics software. Hyperlink http://www.ualberta.ca/~fyeh/fyeh/.

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

Zhu Y. D., Lo Y. L. and Wu H. L. 1963 *A study on the classification of the Sciaenoid fishes of China, with description of new genera and species*. Shanghai Science and Technology Press, Shanghai, China (in Chinese).

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