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Development of microsatellite markers for the small yellow croaker *Larimichthys polyactis* (Sciaenidae) by cross-species amplification

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ABSTRACT. The small yellow croaker (*Larimichthys polyactis*) is a highly valued fish for human consumption found in the Western Pacific that was considered endangered until recently because of overfishing. We selected microsatellite markers for this species from markers developed for *Miichthys miiuy*, also of the family Sciaenidae. Among 43 markers polymorphic for *M. miiuy*, 11 were found to be polymorphic for *L. polyactis*. Characterization of these 11 loci was made based on 30 *L. polyactis* individuals collected by trawling in the Zhoushan Fishing Ground, Zhejiang Province, China. Total genomic DNA was isolated from fin clips. The number of alleles per locus ranged from 4 to 10, with a mean of 5.82, while the effective number of alleles ranged from 1.64 to 10.00, with a mean of 3.22. Observed and expected heterozygosities ranged from 0.17 to 0.72 and from 0.39 to 0.81, respectively. Significant deviation from Hardy-Weinberg equilibrium was found at four loci, after applying Bonferroni's correction. There was no significant association between any of the pairs of microsatellite loci, hence allelic variation at these loci was considered independent. These 11 polymorphic microsatellite loci

will be useful for genetic diversity analysis and molecular-assisted breeding for *L. polyactis*.

Key words: *Larimichthys polyactis*; Cross-species amplification; Microsatellite loci

INTRODUCTION

The small yellow croaker, *Larimichthys polyactis* (Bleeker) is a benthopelagic fish species of the family Sciaenidae, living in warm-temperate sea water (Lin et al., 2004). Extensively distributed in the Yellow Sea and the East China Sea of the Northwest Pacific Ocean (Froese and Pauly, 2011), the small yellow croaker has been considered an important fishery resource and important marine food fish species in Asian countries because of its wide distribution and high capture production. In 2000, the global landing of small yellow croaker reached 320 thousand metric tons (Seikai National Fisheries Research Institute, 2001), but on the other hand, overfishing will have a negative effect on the resource of the small yellow croaker. There is evidence demonstrating that the resource of small yellow croaker has sharply decreased under the effect of over-exploitation in the past decades (Jin, 2004; Lin et al., 2008). Thus, *L. polyactis* has recently been the target of conservation concern.

Intensive studies focused on the areas of catch statistics, size compositions, early life history, and feeding habits (Xue et al., 2004; Wan and Sun, 2006; Yan et al., 2006). Although several studies have been conducted to investigate the genetic characteristics of the small yellow croaker by using molecular markers in recent years (Meng et al., 2003; Lin et al., 2009; Xiao et al., 2009), the information is still limited. Lack of enough polymorphic molecular markers limited the development of molecular phylogeny, population structure and conservation genetics in this species.

Microsatellites or simple sequence repeats (SSRs) of two to six nucleotides in a tandem repeat pattern are abundant and spread throughout the eukaryotic genome. They are recognized as powerful and informative genetic markers in molecular genetic studies, such as genetic mapping, population genetics, and marker-assisted selection, since they are codominant and highly polymorphic even within populations, and show stable amplification by polymerase chain reaction (PCR) (Morgante and Olivieri, 1993; Byrne et al., 1996; Oliveira et al., 2006). Unusually high mutation rates for the nucleotide sequences result in a high level of polymorphism for microsatellite markers (Peakall et al., 1998). However, microsatellite development for each target species requires sequence information from DNA regions flanking the repeat motif to design primers, which is generally time-consuming and requires the investment of resources on the part of the researchers.

The DNA sequences flanking SSRs are generally conserved within individuals of the same species allowing the selection of PCR primers that will amplify the intervening microsatellite in all genotypes. Thus, using loci already developed in a related species may provide a cost-effective alternative to microsatellite isolation and development. Rico et al. (1996) examined the conservation of microsatellite regions across distantly related species using 18 microsatellite loci isolated from other fish species to test across diverse fish species of 470 million years, and they revealed a high level of conservation of the flanking and microsatellite sequences. Cross-amplification has been studied to exploit microsatellite loci in phylogeneti-

cally related species (Moore et al., 1991; Pepin et al., 1995; Engel et al., 1996; Kuhn et al., 1996; Wilson et al., 1997; Slate et al., 1998). Generally, the number of loci amplified tends to decrease with increasing divergence between species (Moore et al., 1991; Peakall et al., 1998). Wilson et al. (2004) found that the success of cross-amplification in species belonging to the same genus as the target species is high.

We have already isolated and characterized polymorphic microsatellite marker in *Miichthys miiuy*, derived from genomic DNA marker (Wang et al., 2010) and an expressed sequence tag (EST) library (Xu et al., 2011). Liu et al. (2006) reported the availability of microsatellite markers developed from an EST library of the olive flounder for other marine fishes. *M. miiuy* and *L. polyactis* both belong to the same fish species of the family Sciaenidae. In the present study, we attempted to develop microsatellites of the small yellow croaker using cross-amplification.

MATERIAL AND METHODS

Fish sample and DNA extraction

L. polyactis individuals were collected by trawling in the Zhoushan Fishing Ground, Zhejiang Province, China. They were identified by morphology. Fin clips were removed and immediately preserved in alcohol at -20°C. Total genomic DNA was isolated from the fin clips using the standard phenol-chloroform method with some modifications and subsequently dissolved in 100 µL ddH_2O. The quality and concentration of DNA were detected on agarose gel electrophoresis and GeneQuant pro RNA/DNA spectrophotometer. DNA were finally adjusted to 100 ng/µL and stored at -20°C for using.

PCR amplification and polymorphism screened

Forty-three loci of microsatellite markers developed in genomic DNA and EST library of *M. miiuy* were cross-amplified in DNA samples from *L. polyactis*. PCR was performed in a 15-µL reaction mixture containing 1X PCR buffer, 1.2 µL dNTPs, 0.4 µL forward and reverse primers, and 0.3 µL *Taq* polymerase (5 U/µL; Tiangen). The cycling conditions were 95°C for 5 min followed by 30 cycles of 95°C for 30 s, annealing for 30 s, and extension for 30 s at 72°C, finally followed with post-cycling extension for 5 min at 72°C, and then holding at 4°C. PCR amplification was performed on a BIO-RAD S1000™ thermal cycler.

Polymorphism at each locus was performed by PCR based on genomic DNA of 30 samples. The products of PCR amplifications were confirmed by 1.5% agarose gel electrophoresis and then denatured at 96°C for 8 min using denaturant (98% formamide, 10 mM EDTA, 0.25% FF). The denatured amplified products were separated on 6% denaturing polyacrylamide gels using silver staining. A denatured pBR322 DNA/*Msp*I molecular weight marker (Tiangen) was used as a size standard to identify alleles.

Polymorphism analysis

Genotypes were determined as approximate allele size (bp). Number of alleles per locus (N_A), effective number of alleles (N_E), expected (H_E) and observed (H_O) heterozygosities,

and Hardy-Weinberg equilibrium (HWE) were examined using a POPGENE software package (Yeh and Boyle, 1997). N_E was estimated based on the formula: $N_E = 1/\sum xi^2$, where xi is the frequency of the *i*th allele for each locus (Crow and Kimura, 1965). The ARLEQUIN 3.11 software was used to calculate genotypic linkage disequilibrium between these loci (Schneider et al., 2000). All results for multiple tests were adjusted using Bonferroni's correction (Rice, 1989).

RESULTS AND DISCUSSION

By the method of cross-species amplification, all of the 43 markers of *M. miiuy* were successfully amplified in the small yellow croaker except four pairs of primers. Further characterization of these markers in the small yellow croaker was tested based on 30 individuals. Among these 39 pairs of amplified primers, 11 loci were polymorphic for *L. polyactis* and the details of these loci in the small yellow croaker are listed in Table 1. N_A per locus for the small yellow croaker ranged from 4 to 10, with an average of 5.82, while N_E ranged from 1.64 to 10.00 with an average of 3.22. H_O and H_E ranged from 0.17 to 0.72 and from 0.39 to 0.81, respectively. Significant deviation from HWE was found at Mimi-15, Mimi-43-H04, Mimi-54-D06, and Mimi-57-A05 after Bonferroni's correction ($P < 0.0045$, adjusted value). There was no significant association between any pair of microsatellite loci, and hence, allelic variation at these loci was considered to be independent.

In our study, the size of alleles for some loci in the small yellow croaker showed nonconformity with *M. miiuy*. Several authors have reported intraspecific and interspecific allelic size variation at microsatellite loci, which was caused by insertions and deletions (indels) in the flanking regions (Angers and Bernatchez, 1997; Grimaldi and Crouau-Roy, 1997; Colson and Goldstein, 1999; Matsuoka et al., 2002). In all, these polymorphic microsatellite loci will be useful for genetic diversity analysis and molecular-assisted breeding for *L. polyactis*. The microsatellite markers from *M. miiuy* were available to develop microsatellite loci for other Sciaenidae species by the method of cross-species amplification, which is truly effective.

Table 1. Characteristics of 11 polymorphic microsatellite loci in small yellow croaker.

Locus	GenBank accession No.	Repeat motif	Tm (°C)	Size range (bp)	N_A	N_E	H_O	H_E	HWE (P)
Mimi-3	GU084248	$(CA)_9$	48	170-188	6	3.20	0.63	0.69	0.068
Mimi-6	GU084250	$(CA)_{23}$	50	174-184	5	5.00	0.40	0.64	0.103
Mimi-15	GU084252	$(CA)_{15}$	49	120-128	4	1.64	0.32	0.39	0.001*
Mimi-16-A03	GW668869	$(T)_{15}$	50	289-301	6	4.44	0.70	0.78	0.121
Mimi-28-G08	GW669768	$(A)_{14}$	47	242-249	6	6.00	0.70	0.81	0.009
Mimi-34-A09	GW670103	$(A)_{13}$	50	112-119	7	7.00	0.72	0.78	0.499
Mimi-35-E08	GW670215	$(T)_{12}$	51	139-162	7	7.00	0.67	0.62	0.998
Mimi-40-H12	GW670618	$(CCT)_5$	47	218-233	5	5.00	0.45	0.65	0.123
Mimi-43-H04	GW670839	$(TTTC)_6$	50	133-197	10	10.00	0.67	0.73	0.000*
Mimi-54-D06	GW671567	$(T)_{13}\ldots(A)_{15}$	50	163-173	4	4.00	0.17	0.54	0.000*
Mimi-57-A05	GW671772	$(T)_{14}$	50	174-184	4	4.00	0.26	0.55	0.003*
Average					5.82	3.22	0.52	0.65	

Tm = melting temperature; N_A = number of alleles; N_E = effective number of alleles; H_E and H_O = expected and observed heterozygosities, respectively; HWE = Hardy-Weinberg equilibrium. *Significant deviation from HWE ($P < 0.0045$, Bonferroni, adjusted value).

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