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Nineteen polymorphic microsatellite markers developed for *Trachinotus ovatus*

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ABSTRACT. To evaluate the population genetic diversity of the ovate pompano, we isolated and characterized 19 microsatellite markers using a $(CA)_{13}$-enriched genomic library. Polymorphism was assessed in 30 individuals from a single population collected from the Daya Bay Aquaculture Center, Guangdong, China. The number of alleles per locus ranged from 2 to 18 with an average of 7.8. The observed and expected heterozygosities varied from 0.2667 to 1.000 and from 0.3960 to 0.9435, respectively. Sixteen of 19 loci conformed to Hardy-Weinberg equilibrium, and no significant linkage disequilibrium was detected between any locus pairs. Our study supplies candidate microsatellite markers that can be useful for studying the population genetic structure of ovate pompano.

Key words: *Trachinotus ovatus*; Microsatellite markers; Genetic structure

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INTRODUCTION

The ovate pompano *Trachinotus ovatus* (Teleostei, Carangidae), an economically important marine fish species, is widely distributed in the tropical and subtropical areas of the Indian Ocean, the Pacific Ocean, and the Atlantic Ocean. Because of its delicious flavor, the ovate pompano has always faced strong market demand, which results in the decline of the wild resources of ovate pompano because of overfishing and promotes the artificial culture of the species. Indeed, the ovate pompano has now become one of most commonly cultured marine fish species in Asian-Pacific regions. However, there is little information about the genetic diversity and population structure of this species, which can provide important scientific support for the conservation and sustainable use of the ovate pompano.

In order to provide useful molecular markers to evaluate population genetic diversity and management of stocks of the ovate pompano, this study aimed to isolate and characterize polymorphic microsatellite loci in this species. It has been demonstrated that microsatellite markers are highly polymorphic and widely used in genome mapping and population genetic studies (Litt and Luty, 1989; Shao et al., 2009; Yang et al., 2010). Furthermore, microsatellite markers are also useful for linkage map construction and future molecular marker-assisted breeding of the ovate pompano.

MATERIAL AND METHODS

To detect the genetic polymorphism of these isolated microsatellite markers, we collected 30 individuals of *T. ovatus* from the Daya Bay Aquaculture Center, Guangdong, China. The fin of the fresh fish was preserved in 95% ethanol at -20°C before DNA extraction.

Genomic DNA was extracted from the fin tissues of two unrelated individuals using the standard proteinase K/phenol extraction method. The microsatellite library was constructed according to the protocol of Zane et al. (2002), with some modifications. A total of 500 ng genomic DNA was digested with *Mse*I restriction enzyme (New England Biolabs, USA) in a 25-µL volume. Fragments with a length of 400-1000 bp were isolated from an agarose gel and then ligated to *Mse*I adaptors, oligo A (5'-TACTCAGGACTCAG-3') and oligo B (5'-GACGATGAGTCCTGAG-3'), using T4 DNA ligase (New England Biolabs). The product was subsequently amplified with adaptor-specific primers (5'-GATGAGTCCTGAGTAAN-3', *Mse*I-N) in a total volume of 30 µL containing 10.4 µL Ex-Taq premix buffer (TaKaRa, Japan), 1 µL 0.4 µM *Mse*I-N, 10 µL diluted digestion-ligation DNA, and 8.6 µL H_2O. Polymerase chain reaction (PCR) amplification was performed as follows: 94°C for 5 min; 35 cycles of 94°C for 45 s, 55°C for 45 min, and 72°C for 45 s; and a final extension at 72°C for 5 min. The PCR product was purified with the Wizard PCR clean-up system (Promega, USA) and hybridized with 100 nM biotin-labeled $(CA)_{13}$ probe at 68°C for 1 h after 5 min of denaturation. Streptavidin-coated magnetic beads (Promega) were used to selectively capture sequences containing TG repeats, and later, specific DNA was eluted from the beads by denaturation at 95°C. The eluted DNA was amplified again

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using the same cycling program as before. After purification using the Wizard PCR clean-up system (Promega), the DNA products were cloned into the pMD18-T vector (TaKaRa) and transformed into *Escherichia coli* DH5α competent cells. Transformed cells were plated on lysogeny broth agar containing ampicillin, isopropyl β-D-1-thiogalactopyranoside, and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, which is used for blue/white selection, and incubated at 37°C for 12 h. Positive clones were randomly selected and sequenced using an ABI PRISM 3730 automated DNA sequencer (Applied Biosystems, USA).

Primer pairs were designed using the PRIMER 3 online software (Rozen and Skaletsky, 2000). These microsatellites were characterized in a sample of 30 individuals collected from Daya Bay Aquaculture Center, Guangdong, China. Genomic DNA of each individual was isolated using the Wizard Genomic DNA Purification kit (Promega). PCR amplification was performed in a 20-μL volume containing the following components: 10 μL Ex-Taq premix buffer (TaKaRa), 1 μM of each primer, and 50 ng template DNA. The PCR conditions were 5 min at 94°C; 35 cycles of 45 s at 94°C, 40 s at the annealing temperature for each locus (Table 1), and 40 s at 72°C; and a final extension of 5 min at 72°C. Amplified products were separated on an 8% polyacrylamide gel and visualized by silver staining. The size of alleles was identified according to a pBR322/*Msp*I marker (Tiangen, China). After screening all loci in the population tested, genotypes of polymorphic loci were scored. 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). The polymorphism information content (PIC) was calculated using PIC_CALC 0.6 (http://hi.baidu.com/luansheng1229/item/306815126d58e3a4feded5a4).

RESULTS AND DISCUSSION

In this study, 19 polymorphic microsatellite loci were successfully isolated from ovate pompano. The average number of alleles per locus was 7.8 and ranged from 2 to 18. The observed heterozygosity ranged from 0.2667 to 1.000, and the expected heterozygosity ranged from 0.3960 to 0.9435, with mean values of 0.6912 and 0.6941, respectively (Table 1). Of the 19 loci, three loci (EC-11, EC-18, and EC-27) deviated significantly from HWE ($P < 0.05$) after sequential Bonferroni's correction (adjusted P value <0.00158). The observed deviation from HWE was probably due to the presence of null alleles. Pairwise comparisons of loci revealed no linkage disequilibrium after Bonferroni's correction. Among the 19 microsatellite loci, 14 had PIC values that were higher than 0.5, while the PIC values of the other loci were between 0.25 and 0.50.

With respect to the decline of the wild resource and large-scale mariculture of ovate pompano, it is particularly important to study the population genetic diversity and population structure for its conservation and sustainable use. This study provided 19 novel highly polymorphic microsatellite markers that can be employed to analyze the population genetics of ovate pompano.

Table1. Characteristics of nineteen microsatellite loci in ovate pompano.

Locus	Accession No.	Repeat motif	Primer sequences (5'-3')	Size range	N_A	Ta (°C)	H_O	H_E	PIC	P_{HWE}
EC-1	KF623044	$(TG)_8$	F:TGCTTGAAAAATCAGGCAAG R: TGCCAGGGAAAAGAGAGAGA	134-144	4	56	0.6333	0.4938	0.4458	0.418
EC-4	KF623045	$(GT)_{13}(GT)_9$	F: TTTAGGACACCATCCCCTCA R: GTGGGTGAGGATGAAATGCT	157-175	6	56	0.6667	0.6605	0.5910	0.894
EC-7	KF623046	$(GT)_{18}$	F: ATATCAGCGTCCACCCAAAC R: GACGACACACATCCTGCACT	182-202	10	56	0.7667	0.8469	0.8116	0.494
EC-8	KF647747	$(CA)_{13}$	F: TGCTTGAAAAATCAGGCAAG R: TGCCAGGGAAAAGAGAGAGA	133-144	3	55	0.6333	0.4876	0.4317	0.109
EC-9	KF623047	$(CA)_{19}$	F: GCTTGTGGAGACCATGACG R: CTCCTGGAGGAACTGTGGAG	127-158	7	56	0.9333	0.7565	0.6901	0.781
EC-10	KF623048	$(CA)_{32}$	F: CGTCTGATCCCATCTCTGTG R: CTGGTCACTGGAGCTGTGTG	135-196	17	56	0.9000	0.9260	0.9051	0.421
EC-11*	KF647748	$(CA)_{13}$	F: TGGCACAGCCCTTTTAGGTA R: TTTCAATTGGGCAGTTTTCC	140-149	4	55	0.3333	0.3960	0.3590	0.001
EC-12	KF623049	$(CA)_{20}$	F: TGCTGTAAATCCACTCGCTCT R: TGTGATGGTAAGGGGCTAGG	153-190	7	56	0.7333	0.8395	0.7975	0.518
EC-13	KF623050	$(GT)_{14}$	F: TTGTTCCCCAAGAGCTCACT R: AGCGGAGCTGGATGTTGTAT	151-206	4	56	0.5667	0.5763	0.5152	0.300
EC-14	KF623051	$(GT)_{26}$	F: AGGGTAAGGGGCTAGGGAAT R: GTTCTGGTCCACGCCTCTTA	143-187	8	56	0.8667	0.7938	0.7503	0.880
EC-15	KF623052	$(TG)_{10}(TG)_{11}$	F: TGTCTGTCTGCTTTCCTGTGA R: GTGAGGACTGGCCAAAATGT	169-177	5	56	0.7000	0.6876	0.6286	0.964
EC-17	KF623053	$(CAT)_{27}$	F: GGTCTGTAGAGAACCAGAACAGTC R: GCTCCTGTGGAGGACAGAGA	154-203	14	56	1.0000	0.8757	0.8488	0.998
EC-18*	KF623054	$(GT)_{15}$	F: AGAGAGCAGGACGTCCAAAA R: CTTTTCCATCTCTCCCCTCA	168-202	12	56	0.6667	0.8333	0.8007	0.009
EC-20	KF623055	$(AC)_6(AC)_{10}$	F: CCACCATCAATCAGCTGTCA R: AGGTGCTCCACAGATGTTCC	171-201	8	56	0.8667	0.8136	0.7688	0.812
EC-23	KF647749	$(CA)_{11}$	F: ACCATGCAACTGTGGTGAAG R: TGCTGCTGTAGTCCACTGCT	184-192	2	53	0.2667	0.3977	0.3165	0.064
EC-24	KF623056	$(TG)_{15}$	F: GGCTAGCTTTGCATTGTGTG R: CAGCCCAGTCAGTCCCTACT	154-170	5	56	0.4667	0.4277	0.3808	0.999
EC-25	KF623057	$(AC)_{10}(AC)_{47}$	F: CCTTACTAAGTAGCAAGTACAAGTCCA R: TGACTTGTTGAGGGTGAAAGTG	143-213	18	56	0.9667	0.9435	0.9250	0.088
EC-27*	KF647750	$(TG)_{14}$	F: TGTGAGGTGTCTGCGACAGT R: GCACTAAAGGCACATGCAGA	142-160	7	54	0.5333	0.6299	0.5424	0.000
EC-28	KF623058	$(CA)_{21}$	F: GACGTGTTCCACAGCAAGAA R: AGGAATGGTCCCAAAGAATG	179-205	8	56	0.6333	0.8011	0.7514	0.489

Ta = optimized annealing temperature; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; P_{HWE} = Hardy-Weinberg probability; PIC = polymorphic information content. *Locus deviated from Hardy-Weinberg equilibrium (adjusted P value <0.00158).

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