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MICROSATELLITE LETTERS

Isolation and characterization of 21 polymorphic microstatellites in golden pompano *Trachinotus ovatus*

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Abstract The golden pompano *Trachinotus ovatus* is a most important marine fish in South China, and the wild population of *T. Ovatus* has been rapidly decreasing owing to overfishing recently, to understand the genetic status for the conservation, we isolated and characterized twenty-one polymorphic microsatellites from a $(GT)_{13}$ enriched genomic library. The number of alleles ranged from 2 to 10. The observed and expected heterozygosities ranged from 0.083 to 0.792 and from 0.081 to 0.886, respectively. The PIC value ranged from 0.0767 to 0.8623. Six loci deviated from Hardy-Weinberg equilibrium. Finally, the 21 novel informative microsatellite markers could be used in future population genetic study of *T. ovatus* that might be useful in a context of marine biodiversity conservation.

Keywords *Trachinotus ovatus* · Pompano · Genetic diversity · Microsatellite

The golden pompano *Trachinotus ovatus* belongs to the Carangidae family. It is distributed in tropical and subtropical areas of Southeast Asia and Mediterranean sea. Because of its delicious taste and rapid growth, it has been one of the most important marine fish commercially cultured in South China. However, the wild population of golden pompano has been rapidly decreasing due to overfishing in recent years, and the genetic status of its wild population is little known so far (Chen et al. 2007). Therefore, to facilitate a better understanding of genetic diversity and population structure of golden pompano for conservation, we have isolated and characterized 21 polymorphic microsatellites from *T. ovatus*, because microsatellites are the markers of choice for a variety of population genetic studies (Fernandez-Silva et al. 2013).

Total genomic DNA was extracted from one *T. ovatus*, using a phenol–chloroform extraction method (Ma and Chen 2009). Then the DNA was stored at −20 °C until used. All polymerase chain reactions (PCRs) were conducted on the Eppendorf PCR machine.

A $(GT)_n$ enriched genomic library of *T. ovatus* was constructed by employing the Fast Isolation by amplified fragment length polymorphism (AFLP) of Sequences Containing repeats (FIASCO) protocol which described detailed by Liao et al. (2007). Finally, 219 clones were sequenced successfully, but only 150 sequences contained the sufficient repeat motifs more than 5. 137 Primer pairs were designed using the PRIMER PREMIER 5.0 software, and forward primer of each pair was labeled with the FAM fluorescent dye.

Polymorphisms of each locus were assessed in the captive-bred population (48 individuals) of *T. ovatus*, which were collected from South China Sea, China. Total genomic DNA was isolated by phenol–chloroform extraction method. PCR amplifications were carried out in a 15-μL volume containing 1.5 μL 10 × PCR buffer (Mg^{2+} free), 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.2 μM of the forward and reverse primers, and 1.0 U Taq polymerase (Takara) and 50 ng template DNA. PCR programme consisted of an initial denaturation step of 5 min at 94 °C,

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Table 1 Characteristics of 21 polymorphic microsatellite loci from *T. ovatus*

Locus	Repeat motif	*Tm* (°C)	Primer sequence (5′–3′)	Accession no.	Size (bp)	N_a	H_o	H_E	*P*	*PIC*
TO12	$(CT)_{10}…(AC)_{14}$	55	F: ACCAAAGCACCGAACAGC	KC460674	309–323	3	0.511	0.569	0.217	0.4681
			R: CGCAGCCTTCAACCGATA							
TO24	$(TG)_{13}…(TG)_7$	55	F: ACGGATGGAGTGATGAAGTG	KC460675	246–272	4	0.787	0.611	0.014	0.5463
			R: CCTGAGATAGGCATGTAGGG							
TO28-2	$(TG)_{13}$	56	F: ATGTGGAGACAGCAGGTA	KC460676	209–211	2	0.333	0.333	1.000	0.2754
			R: GGAAGGTGTTTCGGGATA							
TO33	$(GT)_8…(GT)_6$	50	F: TTGGGTTTGTCCATCAGTT	KC460677	159–194	4	0.417	0.524	0.000	0.4216
			R: CAGGTGCTCAGCATTTCCT							
TO35	$(CA)_{10}$	55	F: CTCTTTGCTGCTCTGATG	KC460678	237–243	2	0.083	0.081	0.795	0.0767
			R: TTTGATGTGGTGGTATGG							
TO41	$(CT)_{12}$	55	F: CGTGGAGGGACTGTTGTT	KC460679	270–295	6	0.617	0.694	0.566	0.6524
			R: ACCATTTCCTGCCTTTCT							
TO47	$(TG)_{16}$	56	F: TCTGCCCGCAATGAAGCC	KC460680	188–199	6	0.739	0.804	0.004	0.7669
			R: TGCCCTGACGCCTGGGACTA							
TO67	$(CT)_{21}$	55	F: GCAGCCAGATGTTATTTC	KC460681	249–275	10	0.609	0.752	0.005	0.7188
			R:CCCTGTTTACCTCCACCC							
TO80	$(AG)_{14}$	54	F: CATAGCTTGCATCCGTGAA	KC460682	223–243	5	0.292	0.307	0.000	0.2781
			R: GGTTGTGCTCCATCTCCC							
TO87	$(CA)_8…(CA)_5…(CA)_{12}$	55	F: AGGCAGAAGTGACAGACG	KC460683	278–291	6	0.609	0.721	0.750	0.6630
			R: TTTCCCAGCAGGAGGTTT							
TO127-1	$(GA)_{21}$	55	F: GGCAGGAAATGCTTCAGTG	KC460684	222–242	6	0.814	0.813	0.188	0.7774
			R: GAGGCTTTGGCAGTCGTAA							
TO129	$(CA)_6…(TCCA)_9$	56	F: GGGATCATCTAACTTTTCATT	KC460685	235–255	4	0.792	0.706	0.193	0.6414
			R: CATCTACTCGACCATCACTCT							
TO132-1	$(CA)_{25}$	55	F: CCTGGATACTGCTGAATGCG	KC460686	248–296	9	0.349	0.886	0.000	0.8623
			R: CTGGGACCCTTACCAAGACA							
TO132-2	$(CA)_9$	55	F: CATTCTCCTATGTCCCTGTCTT	KC460686	193–202	5	0.792	0.744	0.178	0.6932
			R: GCTCCCTGCTTTAGTGCTAT							
TO139	$(CA)_8$	54	F: ATTATTGTTCTGCTCCCC	KC460687	278–280	2	0.227	0.329	0.035	0.2724
			R: ACTGTTATTTGCCTGCTT							
TO144	$(GA)_{10}$	55	F: CAGCAGGTTATGAATGTGACGC	KC460688	230–238	4	0.500	0.593	0.294	0.5309
			R: GTCTTCGCACTGTCTGTCCCTC							
TO145	$(CA)_{24}$	55	F: TCACTATCATTTATTTGTGGGTT	KC460689	246–254	5	0.426	0.777	0.000	0.7291
			R: ACTGTTTTCTGTCCGTCTGTT							

Table 1 continued

Locus	Repeat motif	*Tm* (°C)	Primer sequence (5′–3′)	Accession no.	Size (bp)	N_a	H_o	H_E	*P*	*PIC*
TO171	$(GT)_{13}$	55	F:AGGTGCTGCTGTGAAATA	KC460690	290–303	3	0.500	0.551	0.400	0.4714
			R: GAGTAATGACTGGTGCTGTT							
TO178	$(AG)_{19}$	56	F: AAATCCAGCGTTGGGAGAAC	KC460691	244–252	3	0.583	0.650	0.674	0.5704
			R: CCGCCATAACCATCCAGAGG							
TO203-1	$(GA)_5…(AG)_{15}$	54	F: TGTTCAGGGAAGAGGGAT	KC460692	201–203	2	0.583	0.434	0.016	0.3374
			R: AGCACGCTGTAGCATTAC							
TO204	$(AC)_7…(AC)_8…(AC)_7$	55	F:ACGGTTTTGTTTGTAAATACT	KC460693	177–185	3	0.583	0.497	0.175	0.4348
			R:ACTTGACGTTCTGCTTAGATC							
Average						4.48	0.585	0.589	0.262	0.5328

Number of alleles per locus (N_a), observed heterozygosity (H_O), expected heterozygosity (H_E), probabilities of deviation from Hardy–Weinberg equilibrium (*P*) and polymorphism information content (*PIC*)

followed by 35 cycles of denaturation at 94 °C for 45 s, annealing listed in Table 1 for 45 s and extension at 72 °C for 45 s, finally extension at 72 °C for 10 min. Finally PCR products were run on the 3130xl capillary DNA analyzer (Applied Biosystems), and the allele sizes were analyzed using GeneMapper V4.0.

POPGENE32 was used to calculate the allele numbers, the observed heterozygosities (H_O), expected heterozygosities (H_E) and Hardy–Weinberg equilibrium (HWE). Software Cervus 3.0 was used to assess polymorphism information content (*PIC*).

108 of the 137 selected loci were successfully amplified in the captive-bred population of *T. ovatus*, and only 21 loci were shown to be polymorphic. The number of alleles ranged from 2 to 10, with an average of 4.48. The H_O ranged from 0.083 to 0.792, and H_E ranged from 0.081 to 0.886, with an average of 0.585 and 0.589, respectively,it indicates that the genetic diversity of the captive-bred population are high. The *PIC* value ranged from 0.0767 to 0.8623 (Table 1), twelve of the 21 loci showed a high degree of polymorphism information content ($PIC > 0.5$) (Botstein et al. 1980). 6 loci (TO33, TO47, TO67, TO80, TO132-1, TO145) showed significant deviations from HEW in this population ($p < 0.01$), possibly for the existence of subpopulations, the small sample size, the presence of the null alleles or the inbreeding of the captive-bred population. The 21 novel informative and polymorphic microsatellite loci will provide useful information for the study of the population structure, genetic variation, conservation genetics of the *T. ovatus* in the future.

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