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Thirty novel microsatellite markers for the coastal pelagic fish, *Scomber japonicus* (Scombridae)

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[Zeng L. and Cheng Q. 2012 Thirty novel microsatellite markers for the coastal pelagic fish, *Scomber japonicus* (Scombridae). *J. Genet.* **91**, e64–e68. Online only: http://www.ias.ac.in/jgenet/OnlineResources/91/e64.pdf]

Introduction

Scomber japonicus (Scombridae: *Scomber*) is a wide-spread pelagic fish in the warm and temperate transition coastal areas and adjacent seas of Atlantic, Pacific and northwest Indian oceans (Collette and Nauen 1983). Although there are few studies on development of microsatellite markers that provide useful tool to evaluate population genetic diversity and structure of *S. japonicus*, the number of primer sets was relatively small and more microsatellite markers are needed for performing the comprehensive studies such as evolutionary biology and reproductive ecology, as *S. japonicus* is the most widely distributed and morphologically divergent among its populations in coastal regions and adjacent seas. In this study, we isolated and characterized 30 new microsatellite primer sets in *S. japonicus* using the combined biotin capture method. These loci included dinucleotide, trinucleotide and teranucleotide repeat motifs. All markers showed polymorphism when assessed in 60 individuals from two distant populations (Taizhou and Sanya) in the East China Sea and South China Sea, respectively. The numbers of alleles per locus for a single population ranged from 1 to 25, and the polymorphism information content (PIC) is from 0 to 0.946. The observed and expected heterozygosities (H_O and H_E) ranged from 0 to 0.933 and from 0 to 0.964, respectively. After the sequential Bonferroni correction, five out of 30 loci from Taizhou population and 12 loci from Sanya population (minimum adjusted alpha = 0.00167) showed significant deviation from Hardy–Weinberg equilibrium (HWE), while no significant linkage disequilibrium was detected for any pairs of loci. Results of cross-species amplification showed that all microsatellite markers were successfully amplified in 28 individuals of *S. australasicus*, a closely related species of *S. japonicus*, and clearly indicated polymorphisms. These markers will provide a useful tool for investigating the genetic structure, gene flow, and mating system of *S. japonicus* and closely related species.

As one of the most economically important fishes in the East China Sea and South China Sea, the total capture production of this species in the East China Sea during the period 1980–2002 had experienced a rapid growth with the fluctuations, which ranged from about 52,000 to 194,000 tonnes (Cheng and Lin 2004). *S. japonicus* also served as the global marine capture fisheries production and provided stability for coastal systems (FAO 2010). Additionally, *S. japonicus* are moderately exploited in the eastern Pacific, while the stock was estimated to be recovering in the northwest Pacific (FAO 2010). Therefore, *S. japonicus* are more vulnerable to the impact of marine environment and strong fishing pressure.

Despite the great importance of economic value and resource protection of *S. japonicus*, we know little about its ecological genetics and evolutionary biology. Previous studies had reported some microsatellite markers for *S. japonicus* from the Pacific ocean of Japan and the south coast of Korea (Yagishita and Kobayashi 2008; Cha *et al.* 2010). However, *S. japonicus* is widely distributed over warm and temperate transition waters of the northern hemisphere region, and there may be a large amount of morphologically divergent and genetic differences within and among the populations of the species. Thus, a more suitable set of microsatellite loci is still needed as a supplement of genetic markers for further studies such as the evaluation of population genetic structure and mating pattern.

Here, we developed 30 new microsatellite markers for *S. japonicus* collected from two distant locations in the East

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Keywords. microsatellite marker; SSR; genetic variation; *Scomber japonicus*.

China Sea and South China Sea, which will facilitate characterization of its genetic structure and evolutionary responses to the ongoing change in coastal systems.

Material and methods

DNA extraction and enrichment of microsatellites

In order to check polymorphisms of the identified microsatellite loci, 60 individuals of *S. japonicus* from two distant populations were selected for test, among which 30 were from Taizhou (28.6613°N, 121.8025°E) in the East China Sea, and 30 from Sanya (17.8912°N, 109.5789°E) in the South China Sea. In order to perform cross-priming tests in the congeneric species, 28 *S. australasicus* individuals were collected from Qinglan (19.5048°N, 111.0227°E) in the South China Sea. The vouchers of the sampled population were deposited in herbarium of East China Sea Fisheries Research Institute. Muscle tissues of specimens were preserved in 100% ethanol at room temperature until DNA extraction. Genomic DNA was extracted from muscle tissue of *S. japonicus* and *S. australasicus* using the standard proteinase K/phenol/chloroform procedure (Sambrook *et al*. 1989). Extracted DNA was checked using 0.8% agarose gel electrophoresis, then stored at −20°C for PCR amplification.

Microsatellites were isolated from the total genomic DNA using an enrichment procedure as suggested by Hauswaldt and Glenn (2003). About 400–500 ng genomic DNA was completely digested with a restriction enzyme *Rsa*I (New England BioLabs, Beijing, China) and then ligated to SuperSNX24 double-stranded adaptors (mixture of equal volumes of equal molar amounts of SuperSNX24-F: 5′-GTTTAAGGCCTAGCTAGCAGAATC-3′ and SuperSNX24+4P-R: 5′-GATTCTGCTAGCTAGGCCTTAAACAAAA-3′). For enrichment, the ligation DNA were hybridized with an oligonucleotide combination of 5′-biotinylated probe $(AG)_{15}$, $(CG)_{15}$, and $(CT)_{12}$ in the 50 μL hybridization solution (2× SSC, 1 μmol/L probe and 10 μL ligation products) following the procedure: an initial denaturation at 95°C for 3 min, then quickly ramped down to 70°C and followed by −0.2°C temperature increment per cycle for the first 99 cycles, until 50°C for 10 min has been attained, then ramped down 0.5°C for every 5 s for 20 cycles. Hybridized DNA was then mixed with Streptavidin-coated magnetic beads (Dynabeads M-280, Invitrogen, California, USA) at 37°C for 1 h, and magnetism was used to selectively retain microsatellite-containing fragments. Two washing steps followed: twice with washing solution I (2× SSC, 0.1% SDS) for 1.5 min at room temperature and four times with washing solution II (1× SSC, 0.1% SDS) for 1.5 min at 40°C, 50°C, 45°C, 45°C in turn. Captured DNA was recovered by polymerase chain reaction (PCR) with SuperSNX-F. The PCR products were purified with TIANquick Mini Purification kit (Tiangen, Beijing, China). These fragments enriched with microsatellite loci were cloned using pMD18-T vector (Takara, Shiga, Japan) and transformed into *E. coli* competent cells (JM109, Takara, Shiga, Japan).

Primer design and PCR amplification

Positive colonies were amplified using universal M13 primers. PCR products of 300–700 base-pairs were sequenced using ABI3730XL sequencer (Applied Biosystems, Frederick, USA) using both universal sequencing primers in two directions. A total of 84 sequences were identified out of 267 and primer pairs for amplification of the microsatellite regions were designed using the Primer 5.0 (Clarke and Gorley 2001). The PCR was performed in a total volume of 15 μL containing approximately 10–50 ng of genomic DNA, 0.6 μM of each primer, 7.5 μL 2× Taq PCR MasterMix (TIANGEN). The amplifications were carried out by an initial denaturation at 94°C for 3 min, and then followed by 94°C for 30 s for 32 cycles, annealing at 50–60°C (annealing temperature is listed in table 1) for 30 s, 72°C for 30 s plus a final extension of 72°C for 7 min. PCR products were initially checked for PCR amplification on 1.5% agarose gels. The final PCR products were separated on a 6% polyacrylamide denaturing gel and visualized by silver staining using a 10-bp DNA ladder (Invitrogen, California, USA) as the reference.

Data analysis

Across these populations, the number of alleles per locus (N_A), mean values of observed (H_O) and expected (H_E) heterozygosities, and polymorphism information content (PIC) were calculated using CERVUS version 3.0.3 (Marshall *et al*. 1998). Deviations from HWE and linkage disequilibrium of each locus within each site were checked using GENEPOP v3.4 (Raymond and Rousset 1995). Significances of all test statistics were assessed using an adjusted alpha by the sequential Bonferroni procedure (Rice 1989).

Results and discussions

A total of 30 out of the 84 primers pairs successfully amplified the target regions, and showed clearly polymorphic banding patterns with a maximum of two alleles for each locus per individual. Among the 30 microsatellite motifs amplified, 28 were dinucleotide repeats, one was a trinucleotide repeat and one was a tetranucleotide repeat (table 1). Across the two populations of *S. japonicus*, no genotypic linkage disequilibrium for multiple comparisons of 30 loci within populations was detected in 870 tests after sequential Bonferroni correction (minimum adjusted alpha = 0.000057). Thus all microsatellite loci were thought to be genetically independent for further study.

The number of alleles per locus varied from 3 to 28, with a total of 479 alleles scored in 60 *S. japonicus* individuals. Across the two populations, the number of alleles per locus

Table 1. Characteristics of 30 polymorphic microsatellite markers developed for *Scomber japonicus*.

Locus	Repeat motif	Primer sequence (5′-3′)	T_a (°C)	No. of alleles	Allele size range (bp)	GeneBank accession no.
SJT5	$(CT)_{17}$	F: CCAGTGGGAATCAAATCA R: TGGGCATCCATACTACCT	55	12	82–106	JN656631
SJT18	$(CT)_6$-$(CT)_6$	F: TGCTCTGTTTCACCAATGT R: GAATCACACAGTGGGGCGA	55	16	132–180	JN656632
SJT25	$(CAG)_9$	F: GAATTTCCTCCGGTTTTC R: CTTGGTCATGGGTTTGTG	52	7	111–129	JN656633
SJT31	$(AG)_{11}$-$(GA)_{17}$	F: AATCACTGCTGGGAGTCCT R: TCTATCCGACCTGAGTGGC	60	16	102–150	JN656634
SJT36	$(TG)_5$	F: CTGCTCCTCATGCAATGTC R: ACAACTACAGCAGGCCCAT	58	6	126–140	JN656635
SJT49	$(GA)_{17}$	F: GTGTTGGGTGTAAAGGAGGA R: CAGAATCACGATGAAGAGCA	55	28	98–198	JN656636
SJT51	$(TG)_8$	F: TCGCCGTCAAAGCCCTCT R: CGCCTGCATGGAACAAAG	56	6	188–200	JN656637
SJT52	$(AG)_8$-$(AG)_5$-$(AG)_5$	F: AGTCCACGCAGGCAAACT R: TGTGGGTAAATAAAGGTGAG	56	16	190–250	JN656638
SJT53	$(AG)_5$-$(AG)_7$-$(AG)_8$-$(AG)_5$	F: ACAGTAAGCGAGACAGACA R: ATAATCAACAAACCCACAG	52	14	172–214	JN656639
SJT83	$(GA)_5$-$(GA)_5$-$(AG)_{12}$	F: CTCTATGTGCGGCAGGTG R: AGACAAACATCCCTCTTC	58	12	224–278	JN656640
SJT122	$(AG)_{29}$	F: GCACACAAGTCTTCTTCG R: GGATCGTGACGGTTCTATT	52	24	80–128	JN656641
SJT138	$(GA)_{23}$-$(GA)_9$	F: CATCTGGAATCATGGTCTCA R: CCTCAGTCTGTTGGTCTCT	55	16	106–238	JN656642
SJT152	$(TC)_{30}$	F: GCACAGACTGACAATCCA R: CAGGAGGAATGAGAATGTCT	58	20	152–260	JN656643
SJT153	$(GT)_5$-$(GA)_7$-$(GA)_8$-$(GA)_{16}$	F: CAGTCAGTATCACATTCACA R: TTCTCTGCTCTGCCATTC	58	26	122–214	JN656644
SJT175	$(GA)_8$	F: TTTGTCAGCGTTGGGGTT R: ATCATGGAAGTGTTGGAG	55	8	142–158	JN656645
SJT182	$(CT)_5$-$(CT)_{20}$-$(TC)_8$	F: GCTCCCTGAATGAATCACT R: ATCAGCAAGTCAGCAGAG	52	17	124–202	JN656646
SJT199	$(TC)_5$-$(GA)_5$-$(AG)_5$-$(AG)_{15}$	F: CCTCACTTCCACTCCTCTA R: CTGCCATCCTCCTCTCAT	58	17	230–360	JN656647
SJT205	$(AC)_8$	F: CCACATACTGACAGAAGAG R: GTTTGAATACACCAGAGAT	55	3	140–146	JN656648
SJT216	$(GT)_{21}$	F: GGCATCTGTCTGTGTCTT R: GCATCACTGGCTGTATATG	55	16	148–190	JN656649
SJT218	$(CAAA)_5$	F: GACCTTGGCAGCATAATG R: TCCTCTTGAGATGATGAATC	55	8	112–140	JN656650
SJNT19	$(GA)_{26}$	F: ACAGATCGGTCCAATCAAG R: TTGTCAACTCCAGCAAATG	56	19	150–260	JN656651
SJNT28	$(TC)_9$-$(TC)_5$-$(TC)_{12}$	F: CAGGCTCTGAGATTGTGTT R: AAGAGTGGTGGTCTGGGTG	60	20	98–150	JN656652
SJNT62	$(CT)_9$-$(CT)_7$-$(TC)_9$	F: GCAGTATGTGTGATGAGCA R: GAATCACCATGTCTCGCTT	58	28	96–188	JN656653
SJNT66	$(TC)_{12}$	F: GCCCTGTCACAAAATAATC R: CAAAAGAGCGTTTAACAGT	56	14	104–132	JN656654
SJNT74	$(TC)_5$-$(CT)_5$-$(CT)_6$-$(CT)_6$	F: TCAGGGAGTGTCTCAGCT R: GATGCCAGTAAATCTTCA	56	28	126–252	JN656655
SJNT77	$(CT)_7$-$(CT)_{13}$-$(CT)_{16}$-$(TG)_7$	F: GAATCACACAATGTAGTCC R: GGGGATAGAGAACAAAGAT	54	26	94–208	JN656656
SJNT86	$(CT)_{18}$	F: CAGATGTAAGCCTTTTGTC R: GTTTTATTGCCTCATGATG	56	17	110–174	JN656657
SJNT91	$(GA)_5$-$(AG)_{18}$	F:GAATCACCTGACATCTGGA R:TCCCACTCATTTTTTCAGA	50	18	122–172	JN656658
SJNT116	$(TC)_{21}$	F: TATCACTGCTGGGACAGA R: CTCAGAGGCAACACTAAC	58	14	146–176	JN656659
SJNT142	$(AG)_{15}$	F: AAATCGATCTTTCCGTTGG R: AATCACACTCATGTCCCCT	58	7	126–140	JN656660

T_a, annealing temperature of primer pair.

Table 2. Results of initial primer screening for the 30 primer pairs in two populations of *Scomber japonicus* and the congeneric species, *S. australasicus*.

	Scomber japonicus from Taizhou ($n = 30$, N 28.6613°, E 121.8025°)					*Scomber japonicus* from Sanya ($n = 30$, N 17.8912°, E 109.5789°)					*Scomber australasicus* from Qinglan ($n = 28$, N 19.5048°, E 111.0227°)				
Locus	N_A	H_O	H_E	PIC	*P* value	N_A	H_O	H_E	PIC	*P* value	N_A	H_O	H_E	PIC	*P* value
SJT5	12	0.8	0.872	0.841	0.769	9	0.733	0.792	0.745	0.958	12	0.893	0.836	0.798	0.926
SJT18	6	0.633	0.525	0.481	0.987	13	0.667	0.892	0.866	0.002	15	0.571	0.898	0.872	0.002
SJT25	6	0.8	0.746	0.693	0.968	7	0.733	0.746	0.695	0.778	6	0.643	0.664	0.615	0.000*
SJT31	13	0.7	0.912	0.888	0.396	15	0.767	0.918	0.894	0.084	15	0.630	0.933	0.909	0.001
SJT36	4	0.133	0.188	0.177	0.364	2	0.067	0.066	0.062	0.850	4	0.143	0.284	0.256	0.016
SJT49	19	0.733	0.947	0.928	0.008	25	0.667	0.964	0.946	0.002	23	0.571	0.945	0.925	0.000*
SJT51	4	0.379	0.645	0.585	0.016	6	0.4	0.802	0.755	0.000*	7	0.192	0.801	0.756	0.000*
SJT52	8	0.533	0.773	0.729	0.009	14	0.767	0.914	0.89	0.094	15	0.630	0.924	0.9	0.008
SJT53	10	0.933	0.894	0.866	0.093	12	0.733	0.879	0.849	0.187	12	0.963	0.889	0.86	0.997
SJT83	9	0.733	0.834	0.799	0.560	9	0.5	0.889	0.861	0.000*	14	0.714	0.923	0.899	0.137
SJT122	20	0.667	0.945	0.925	0.000*	16	0.7	0.924	0.902	0.006	23	0.857	0.932	0.91	0.007
SJT138	10	0.8	0.812	0.778	0.141	6	0.357	0.782	0.737	0.000*	20	0.607	0.928	0.906	0.000*
SJT152	17	0.933	0.936	0.914	0.846	16	0.367	0.933	0.912	0.000*	22	0.607	0.962	0.942	0.000*
SJT153	19	0.633	0.937	0.916	0.020	22	0.667	0.95	0.93	0.006	18	0.444	0.939	0.916	0.000*
SJT175	6	0.7	0.757	0.706	0.416	7	0.083	0.816	0.772	0.000*	6	0.120	0.779	0.728	0.000*
SJT182	13	0.767	0.886	0.859	0.002	5	0.133	0.13	0.126	1.000	10	0.286	0.512	0.49	0.000*
SJT199	12	0.633	0.884	0.856	0.091	13	0.448	0.908	0.883	0.000*	15	0.500	0.917	0.892	0.000*
SJT205	3	0.333	0.315	0.278	0.450	2	0.2	0.183	0.164	0.543	3	0.214	0.366	0.33	0.000*
SJT216	12	0.567	0.908	0.883	0.000*	15	0.433	0.927	0.904	0.000*	17	0.786	0.932	0.909	0.480
SJT218	6	0.567	0.661	0.592	0.233	2	0.167	0.155	0.141	0.619	6	0.250	0.323	0.306	0.000*
SJNT19	11	0.5	0.896	0.87	0.001*	8	0.5	0.819	0.781	0.000*	17	0.714	0.914	0.891	0.000*
SJNT28	9	0.8	0.799	0.759	0.086	17	0.786	0.886	0.858	0.120	23	0.893	0.949	0.928	0.028
SJNT62	18	0.767	0.945	0.925	0.048	24	0.633	0.954	0.935	0.000*	26	0.821	0.965	0.945	0.374
SJNT66	14	0.8	0.923	0.901	0.134	1	0.000	0.000	0.000	–	5	0.143	0.203	0.195	0.000*
SJNT74	20	0.667	0.951	0.931	0.005	19	0.867	0.908	0.886	0.113	26	0.786	0.956	0.936	0.065
SJNT77	21	0.321	0.955	0.934	0.000*	19	0.633	0.942	0.922	0.005	20	0.625	0.943	0.919	0.004
SJNT86	14	0.867	0.913	0.89	0.520	6	0.16	0.824	0.782	0.000*	9	0.130	0.861	0.822	0.000*
SJNT91	12	0.6	0.904	0.879	0.001*	12	0.5	0.886	0.858	0.000*	17	0.852	0.923	0.899	0.969
SJNT116	12	0.6	0.904	0.879	0.039	8	0.633	0.718	0.669	0.847	7	0.607	0.673	0.608	0.969
SJNT142	7	0.5	0.662	0.604	0.247	4	0.3	0.373	0.344	0.000*	5	0.148	0.357	0.331	0.000*
Average	11.6	0.647	0.8076	0.7755	–	11.1	0.487	0.7294	0.7024		13.9	0.545	0.781	0.7531	–

N_A, number of alleles per locus; H_O, observed heterozygosities; H_E, expected heterozygosities; PIC, polymorphism information content; *P* value, possibilities to fit to HWE using an exact *P* test. The vouchers of the sampled population were deposited in herbarium of East China Sea Fisheries Research Institute, and the accessions are Q.Q. Cheng *et al*. TZ2, Q.Q. Cheng *et al*. SY13 for *S. japonicus*, and Q.Q. Cheng *et al*. QL16 for *S. australasicus*. *Deviation from HWE after Bonferroni correction ($P < 0.05$, minimum adjusted alpha = 0.00167).

for a single population ranged from 1 to 25 with an average of 11.35 ± 0.35, and the PIC is from 0 to 0.946 (table 2). The H_O and H_E ranged from 0 to 0.933 and from 0 to 0.964, respectively (see table 2). Verification of the HWE revealed significant departure in five loci from Taizhou population and 12 loci from Sanya population after the sequential Bonferroni correction (minimum adjusted alpha = 0.00167).

Cross-priming tests were performed in the congeneric species, *S. australasicus* from Qinglan using 28 individuals (table 2). All 30 loci were successfully amplified and showed polymorphisms. No significant genotypic disequilibrium was detected for any pairs of loci in 435 tests after sequential Bonferroni correction (minimum adjusted alpha = 0.00011). Fifteen of the 30 loci were detected to deviate from HWE (minimum adjusted alpha = 0.00167). The number of alleles per locus ranged from 3 to 26 with an average of 13.9, and the PIC is from 0.195 to 0.945 (table 2). The H_O and H_E ranged from 0.12 to 0.963 and from 0.203 to 0.965, respectively (see table 2). These microsatellite markers will be useful for detecting population genetic diversity and structure of the cross-species.

In a previous study based on mtDNA sequence, Scoles *et al*. (1998) detected that greater geographical distance and isolation cause high level of genetic divergence among *S. japonicus* populations in the northern hemisphere region. A few works that had been done about development of microsatellite markers of *S. japonicus* in local areas were not enough to investigate the genetic structure, gene flow, and mating system of this wide-spread species. The microsatellite markers reported here will facilitate characterization of the gene flow in *S. japonicus*, which is essential for our understanding of the response of this pelagic fish to ongo-

ing coastal systems change in the East China Sea and South China Sea.

Acknowledgements

This work was supported by the Special Research Fund for national nonprofit institutes (East China Sea Fisheries Research Institute, no. 2008Z02) and the Fund of State Key Laboratory of Genetics Resources and Evolution (GREKF10–01).

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Received 30 October 2011, in final revised form 13 January 2012; accepted 20 February 2012
Published on the Web: 19 June 2012