GMR

Isolation and characterization of polymorphic microsatellite markers for the chub mackerel (*Scomber japonicus*) and cross-species amplification in the blue mackerel (*S. australasicus*)

F.F. Chen[1,2], C.Y. Ma[1], L.P. Yan[1], H. Zhang[1], W. Wang[1], Y. Zhang[1] and L.B. Ma[1]

[1]Key Laboratory of the East China Sea and Oceanic Fishery Resources Exploitation, Ministry of Agriculture, East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai, China
[2]College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, China

Corresponding authors: C.Y. Ma / L.B. Ma
E-mail: mcy0527@126.com / malingbo@vip.sina.com

Genet. Mol. Res. 16 (3): gmr16039712
Received May 4, 2017
Accepted July 21, 2017
Published August 17, 2017
DOI http://dx.doi.org/10.4238/gmr16039712

ABSTRACT. In this study, 10 polymorphic microsatellite markers were developed in *Scomber japonicus* and were examined on 30 individuals collected from the North Pacific. The number of alleles per locus ranged from 4 to 17. The observed and expected heterozygosities per locus ranged from 0.2759 to 0.8621 and from 0.43071 to 0.9177, respectively. The polymorphism information content (PIC) was from 0.3931 to 0.8939. One locus showed moderate polymorphism ($0.25 < PIC < 0.5$), while the rest were highly polymorphic ($PIC > 0.5$). Two loci showed significant deviation from Hardy-Weinberg equilibrium after Bonferroni corrections ($P < 0.005$). No linkage disequilibrium was detected among the loci. Results of cross-species amplification showed that 10 microsatellite markers were successfully amplified in 29 individuals of *S. australasicus* and 9 indicated polymorphisms. These markers will be useful for investigating the genetic structure, gene

flow, and species identification of *S. japonicus* and *S. australasicus*, its closely related species.

Key words: Microsatellite markers; Genetic diversity; Cross-species amplification; *Scomber japonicus*

INTRODUCTION

The chub mackerel (*Scomber japonicus*) is a widespread pelagic fish in the warm and temperate transition coastal areas and the adjacent seas of Atlantic, Pacific, and Northwest Indian (Collette and Nauen, 1983; Zeng et al., 2012). *S. japonicus* served as the global marine capture fishery production and provided stability for coastal systems (FAO, 2010). As one of the most important fishery resources of China, the yield of *S. japonicus* maintained at a higher level. However, the population was already in overfishing situation during the period 1980-2002 (Hua and Shan, 2004). Therefore, *S. japonicus* are more vulnerable to the impact of marine environment and strong fishing pressure. To protect this important fish resource, it is necessary to take knowledge of its population structure, gene flow levels, and genetic diversity (Utter, 2006; Cha et al., 2010). However, we know little about its ecological genetics and evolutionary biology.

Microsatellites, widely existing along the eukaryotic genome (Weber and May, 1989; O'Reilly and Wright, 1995), are popular genetic markers because of high polymorphism, ease of genotyping, and co-dominant inheritance (Sun et al., 2009). Moreover, they have been widely developed and used in the study of population genetics of many species (Guo et al., 2016; Mohanty et al., 2016; Tan et al., 2016). Furthermore, some of these microsatellite markers developed for *Scomber* species have been successfully used for amplification in other related species (Tang et al., 2009; Divya et al., 2016). However, it is not very sufficient, and some locus-by-population tests exhibited significant departure from Hardy-Weinberg equilibrium (HWE) (Cheng et al., 2015). So it is necessary to isolate more microsatellite markers for the utility of the population genetic study.

Scomber australasicus is one of the close species of *S. japonicus* and differ in a series of morphologic characters, including sculpturing of the skull, the number of precaudal vertebrae, the arrangement of palatine teeth, and the number of first dorsal spines (Matsui, 1967). *S. australasicus* inhabits the west Pacific and the southeast Indian Oceans.

It is known that the RAPD fragments contain rich microsatellites (Cifarelli et al., 1995; Ender et al., 1996). In the present study, we isolated 10 novel polymorphic microsatellite markers in *S. japonicus* using the RAPD-PCR method (Lunt, 1999). These markers provided a useful tool for investigating the genetic structure and species identification of *S. japonicus* and its closely related species.

MATERIAL AND METHODS

Sample collection and DNA extraction

Our project was approved by the East China Sea Fisheries Research Institute. The 30 individuals of *S. japonicus* in the present study were collected from the North Pacific (145.383E, 41.083N). Moreover, 29 individuals of *S. australasicus* were collected from

the Indian Ocean for the performance of cross-priming tests. Muscle tissues of specimens were preserved in 100% ethanol at room temperature until DNA extraction. Total Genomic DNA was extracted from muscles using the Animal Genomic DNA Extraction Kit (TianGen, Beijing, China) and stored at -20°C until RAPD was conducted.

Isolation of microsatellite markers

First, we randomly selected 10 RAPD primers from the catalogue of Sangon Biotech (Shanghai) Co., Ltd. (Table 1) to amplify the target DNA fragments. The PCR amplification was performed in a 25-μL total volume containing 12.5 μL 2X Kod PCR MasterMix, 0.5 μM RAPD primer (10 μM), 11 μL sterilized distilled water and approximately 1 μL template DNA. After 5 min of denaturation at 94°C, amplification proceeded for 40 cycles (94°C for 30 s, 37°C for 40 s, 72°C for 45 s) and a final extension for 5 min at 72°C. Then, the PCR products were cloned using the pMD19-T vector (Takara). The connection system including 1 μL pMD19-T Vector, 4 μL of PCR product, and 5 μL solution was stored at 16°C for reaction for 3 h and then transferred to 4°C. Finally, 10 μL of fluid connection was transformed into *Escherichia coli* competent cells (TianGen) and cultivated in plate culture medium at 37°C for several hours. The cultivated competent cells were randomly selected for sequencing using the ABI Prism 3730 automated DNA sequencer (PE Corporation). Microsatellite sequences obtained were searched using the SSR Hunter 1.3 software (Qiang and Wan, 2005).

Table 1. Ten RAPD primers used for amplifying the target DNA fragments of *Scomber japonicus* (the primers were derived from the product catalogue of Sangon Biotechnology Company).

Primer name	Primer sequence (5'-3')
S1	GTTTCGCTCC
S2	TGATCCCTGG
S3	CATCCCCCTG
S4	GGACTGGAGT
S5	TGCGCCCTTC
S6	TGCTCTGCCC
S7	GGTGACGCAG
S8	GTCCACACGG
S9	TGGGGGACTC
S10	CTGCTGGGAC

Characteristics and polymorphism assessment

Firstly, seven individuals of *S. japonicus* were randomly selected to assess the preliminary polymorphism of the microsatellite markers. Moreover, we used 30 individuals of *S. japonicus* to quantify the levels of polymorphism of the 10 loci. The PCR amplification was performed on a Peltier Thermal Cycler in a 25-μL total volume, which included 0.75 μL of each primer (10 μM), 2.0 μL dNTP (2.5 μM), 2.5 μL 10X PCR buffer (Mg^{2+} plus), 2.5 U *Taq* polymerase, 17.5 μL sterilized distilled water, and approximately 1 μL template DNA under the following conditions: one cycle of denaturation at 94°C for 5 min; 30 cycles of 30 s at 94°C, 45 s at a primer-specific annealing temperature, and 1.5 min at 72°C. Finally, the products were extended for 7 min at 72°C (Table 1). The PCR products were separated on a 6% polyacrylamide denaturing gel and visualized by silver staining using a 10-bp DNA ladder marker pBR322/*Msp*I (TianGen).

Data analysis

Allele frequency, observed (H_O) and expected (H_E) heterozygosities, the chi-square test for HWE, and the linkage disequilibrium (LD) were performed using the Popgene 3.4 software (Raymond and Rousset, 1995). The null-allele frequency was estimated by the MICRO-CHECKER version 2.2.3 software (Oosterhout et al., 2004). Polymorphism information content (PIC) was calculated by CERVUS 3.0 (Marshall et al., 1998). Significance values for all multiple tests were corrected by sequential Bonferroni procedure (Rice, 1989).

RESULTS AND DISCUSSION

A total of 1000 clones were randomly selected to be tested by PCR with M13-47 (5'-GGG ATC CTC TAG AGA TT-3') and M13-48 (5'-GCC TGC AGG TCG ACG ATT-3'). Then, 148 positive clones were identified and sequenced using an ABI Prism 3730 automated DNA sequencer. In comparison to other species, the positive clone rate in this study was lower than that in fish (*Cynoglossus semilaevis*) (Liu et al., 2008) and crab (*Scylla paramamosain*) (Ma et al., 2010). Moreover, 75 pairs of primers were designed by using Primer Premier 5.0 and synthesized. The preliminary polymorphism assessment of the 75 pairs of microsatellite primers was evaluated by seven individuals, which were randomly picked out from the 30 individuals collected from the North Pacific (145.383E, 41.083N). All primers could lead to successfully PCR amplification, but only 10 of them showed polymorphism. Moreover, the levels of polymorphism of the polymorphic loci were evaluated by 30 individuals collected from the North Pacific (145.383E, 41.083N). Finally, we identified 10 polymorphic microsatellite loci (Table 2).

Table 2. Characteristics of the 10 microsatellite loci in the chub mackerel *Scomber japonicus*.

Loci	GenBank accession No.	Primer sequence (5'-3')	Repeat motif	Ta (°C)	Allele size range (bp)
SJ-23	KY042106	F: GACGACTGACGGAGACGCTT R: TGCTCAGGGTGTCGCAGTTT	$(AG)_{11}$	55	169-187
SJ-28	KY042107	F: GATCTAAGAGCAATCAGTCC R: CAAACCTGACCCAGAATGAT	$(TG)_8$	56	182-200
SJ-30	KY0421078	F: GTTTCCCATCTGTCCACCCA R: AGCAACTTCCTCCGCTTCTG	$(TC)_6G(C T)_6\ldots(CT)_8$	55	199-245
SJ-31	KY042109	F: AGAACCTCCATCCTGAAGCC R: AAAGCCTAATCCCAAACCGA	$(GT)_5$	56	286-300
SJ-32	KY042110	F: CTCCCCTAACACTCCTTCCC R: TTTCCCATCTGTCCACCCAG	$(GA)_5\ldots (GA)_{7\ldots} (GA)_6GC(GA)_6$	55	291-325
SJ-42	KY042111	F: CGTGACAGCAGGCTTAGAGG R: TGACAGACGGCCTGACAGAT	$(GA)_7\ldots(AG)_7$	55	222-262
SJ-45	KY042112	F: TAAAATCATCAAAGACACGCTC R: CACATCCTCCATTCGGTAAA	$(TC)_5$	54	135-163
SJ-65	KY042113	F: TAGACAGGCAGTCAGATTTCAAC R: TCATCCCTTCATTTATACAACCC	$(AG)_9(AC)_5$	57	149-157
SJ-76	KY042114	F: GCTCAGGGTGTCACAGTTTT R: TAATCCATGTAATTGCGTTG	$(CT)_{13}$	55	137-213
SJ-78	KY042115	F: GCATTGAGATGATTTTGGTA R: GCAGAATTGGGATGGAAATA	$(TC)_5\ldots(CT)_7$	53	191-227

Ta = annealing temperature.

The levels of polymorphism of the 10 loci were qualified in 30 individuals of *S. japonicus* collected from the North Pacific (145.383E, 41.083N). A total of 99 alleles with size ranging from 135 to 325 bp were identified in 30 individuals. The N_A per locus ranged from 4 to 17, with an average of 9.9. The H_O and H_E per locus ranged from 0.2759 to 0.8621

and from 0.4307 to 0.9177, with an average of 0.6226 and 0.7250, respectively. The PIC of these molecular markers was calculated to access their usefulness (Botstein et al., 1980). One locus (SJ-45) showed moderately polymorphic (0.25 < PIC < 0.5) and nine loci were highly polymorphic (PIC > 0.5).

Two loci (SJ-31 and SJ-65) showed significant departure from HWE after Bonferroni corrections (P < 0.005) (Rice, 1989), which may result from the small sample size or the presence of null alleles. The MICRO-CHECKER analysis showed no evidence for scoring error or technical or statistical artifacts (Oosterhout et al., 2004). There was no significant genotypic LD between all pairs of these loci after Bonferroni correction (P > 0.005). These 10 sequences were further searched in GenBank using the BLASTn program, and no similar sequence was found. The information about these pairs of primers is listed in Table 2.

The applicability of these markers in a closely related species, *S. australasicus*, was evaluated. All 10 loci were successfully amplified, and nine showed polymorphisms. The H_O and H_E ranged from 0.4286 to 1.0000 and from 0.5792 to 0.9273, respectively. The N_A per locus ranged from 5 to 16 with an average of 9.4, and the PIC was from 0.4853 to 0.9039. Two loci (SJ-31and SJ-78) showed to be moderately polymorphic (0.25 < PIC < 0.5) and seven loci were highly polymorphic (PIC > 0.5) (Table 3). Six of the nine loci were detected to deviate from HWE. These microsatellite markers might be useful for detecting population genetic diversity and structure of the cross-species.

Table 3. Results of the 10 microsatellite loci in the *Scomber japonicus* population and the congeneric species, *Scomber australasicus*.

Locus	*Scomber japonicus*					*Scomber australasicus*				
	N_A	H_O	H_E	HWE (P value)	PIC	N_A	H_O	H_E	HWE (P value)	PIC
SJ-23	8	0.6207	0.7846	0.1678	0.7423	11	0.7500	0.8526	0.3627	0.8180
SJ-28	5	0.6552	0.6407	0.7021	0.5576	5	0.5714	0.6688	0.1496	0.5929
SJ-30	17	0.8276	0.9080	0.8294	0.8833	16	0.7143	0.8708	0.0000*	0.8412
SJ-31	6	0.5000	0.6156	0.0030*	0.5698	12	0.4286	0.5792	0.8051	0.4853
SJ-32	16	0.7586	0.8959	0.4965	0.8704	5	0.6400	0.8122	0.0000*	0.7805
SJ-42	7	0.5185	0.6024	0.9997	0.5152	14	0.4286	0.9273	0.0000*	0.9039
SJ-45	5	0.2759	0.4307	0.2903	0.3931	-	-	-	-	-
SJ-65	4	0.3793	0.5977	0.0000*	0.5193	6	1.0000	0.7747	0.0124*	0.7248
SJ-76	14	0.8621	0.8566	0.3894	0.8237	10	0.4286	0.8383	0.0000*	0.8011
SJ-78	17	0.8276	0.9177	0.0899	0.8939	6	0.6071	0.5896	0.0000*	0.4929

N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphism information content. *P < 0.005 for significant deviation from HWE after Bonferroni correction.

In conclusion, 10 polymorphic microsatellite markers were developed for *S. japonicus* by the RAPD-PCR method and amplified successfully in the related species *S. australasicus*. Most of these loci show high polymorphism in *S. japonicus* and *S. australasicus*. This set of microsatellite markers should be an important supplement of former microsatellite database and should also be used for accessing population genetic diversity and individual identification of *S. japonicus* and closely related species.

ACKNOWLEDGMENTS

Research supported by the National Basic Research Special Foundation of China (#2013FY110700), the National Science and Technology Support Plan (#2013BAD13B05), the Special Fund for Agro-Scientific Research in the Public Interest (#201303047), and the National Infrastructure of Fishery Germplasm Resources.

REFERENCES

Botstein D, White RL, Skolnick M and Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32: 314-331.

Cha H, An H, Choi J, Kang S, et al. (2010). Erratum to: Isolation and characterization of polymorphic microsatellite markers for genetic analysis of chub mackerel (*Scomber japonicus*). *Conserv. Genet. Resour.* 2: 7-9. https://doi.org/10.1007/s12686-009-9123-7

Cheng J, Yanagimoto T, Song N and Gao TX (2015). Population genetic structure of chub mackerel *Scomber japonicus* in the Northwestern Pacific inferred from microsatellite analysis. *Mol. Biol. Rep*. 42: 373-382.

Cifarelli RA, Gallitelli M and Cellini F (1995). Random amplified hybridization microsatellites (RAHM): isolation of a new class of microsatellite-containing DNA clones. *Nucleic Acids Res.* 23: 3802-3803. https://doi.org/10.1093/nar/23.18.3802

Collette BB and Nauen CE (1983). FAO species catalogue. Vol. 2. Scombrids of the world. An annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. *FAO Fish Synop.* 125: 137.

Divya PR, Vineesh N, Kathirvelpandian A, Mohitha C, et al. (2016). Identification and characterisation of microsatellite markers in narrow barred Spanish mackerel *Scomberomorous commerson* (Lacepede, 1800). *Indian J. Fish*. 63: 61-65.

Ender A, Schwenk K, Städler T, Streit B, et al. (1996). RAPD identification of microsatellites in Daphnia. *Mol. Ecol.* 5: 437-441. https://doi.org/10.1111/j.1365-294X.1996.tb00333.x

FAO (2010). The State of World Fisheries and Aquaculture. *State World Fish. Aquacult.* 49: 40-41.

Guo HP, Zhou SJ, Zhu XJ, Xu XJ, et al. (2016). Population genetic analysis of from the Qinling-Daba Mountain Areas based on microsatellite DNA. *Acta Entomol. Sin.* 59: 337-345.

Hua CJ and Shan LL (2004). Study on the biological characteristics and status of common mackerel (*Scomber japonicus Houttuyn*) fishery in the East China Sea region. *Mar. Fish.* 26: 73-78.

Liu YG, Bao BL, Liu LX, Wang L, et al. (2008). Isolation and characterization of polymorphic microsatellite loci from RAPD product in half-smooth tongue sole (*Cynoglossus semilaevis*) and a test of cross-species amplification. *Mol. Ecol. Resour.* 8: 202-204. https://doi.org/10.1111/j.1471-8286.2007.01923.x

Lunt DH (1999). An efficient method for PCR-based isolation of microsatellite arrays (PIMA). *Mol. Ecol.* 8: 891-893.

Ma HY, Ma CY, Ma LB and Cui HY (2010). Novel polymorphic microsatellite markers in *Scylla paramamosain* and cross-species amplification in related crab species. *J. Crustac. Biol.* 30: 441-444. https://doi.org/10.1651/09-3263.1

Marshall TC, Slate J, Kruuk LEB and Pemberton JM (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* 7: 639-655. https://doi.org/10.1046/j.1365-294x.1998.00374.x

Matsui T (1967). Review of the mackeral genera Scmber and Rastrelliger with description of a new species of Rastrelliger. Copeia 71-83.

Mohanty P, Sahoo L, Pillai BR, Jayasankar P, et al. (2016). Genetic divergence in Indian populations of *M. rosenbergii* using microsatellite markers. *Aquacult. Res.* 47: 472-481. https://doi.org/10.1111/are.12508

Oosterhout CV, Hutchinson WF, Wills DPM and Shipley P (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* 4: 535-538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

O'Reilly P and Wright JM (1995). The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. *J. Fish Biol.* 47: 29-55. https://doi.org/10.1111/j.1095-8649.1995.tb06042.x

Qiang L I and Wan J M (2005). SSRHunter: Development of a local Searching Software for SSR Sites. *Hereditas* 27: 808-810.

Raymond M and Rousset F (1995). GENEPOP (Version 1.2): Population Genetics Software for Exact Tests and Ecumenicism. *J. Hered.* 86: 248-249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Rice WR (1989). Analyzing tables of statistical of statistical tests. *Evolution* 43: 223-225. https://doi.org/10.1111/j.1558-5646.1989.tb04220.x

Sun Y, Sun H, Qian G, Zhao Y, et al. (2009). Isolation and characterization of microsatellite loci in the freshwater crab *Sinopotamon yangtsekiense* and cross-species amplification in related taxa (Decapoda: Brachyura). *J. Crustac. Biol.* 29: 413-418. https://doi.org/10.1651/08-3115.1

Tan MP, Jamsari AFJ and Siti Azizah MN (2016). Genotyping of microsatellite markers to study genetic structure of the wild striped snakehead *Channa striata* in Malaysia. *J. Fish Biol.* 88: 1932-1948. https://doi.org/10.1111/jfb.12956

Tang CY, Tzeng CH, Chen CS and Chiu TS (2009). Microsatellite DNA markers for population-genetic studies of blue mackerel (*Scomber australasicus*) and cross-species amplification in *S. japonicas*. *Mol. Ecol. Res*. 9: 824-827.

Utter FM (2006). Biochemical genetics and fishery management: an historical perspective. *J. Fish Biol.* 39: 1-20. https://doi.org/10.1111/j.1095-8649.1991.tb05063.x

Weber JL and May PE (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.* 44: 388-396.

Zeng L, Cheng Q and Chen X (2012). Microsatellite analysis reveals the population structure and migration patterns of *Scomber japonicus* (Scombridae) with continuous distribution in the East and South China Seas. *Biochem. Syst. Ecol.* 42: 83-93. https://doi.org/10.1016/j.bse.2012.02.014