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Short Communication

Polymorphic microsatellite loci for Japanese Spanish mackerel (*Scomberomorus niphonius*)

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ABSTRACT. We isolated and characterized 21 polymorphic microsatellite loci in Japanese Spanish mackerel (*Scomberomorus niphonius*) using a $(GT)_{13}$-enriched genomic library. Forty individuals were collected from Qingdao, China. We found 3 to 24 alleles per locus, with a mean of 8.8. The observed and expected heterozygosities ranged from 0.263 to 0.975 and from 0.385 to 0.946, with means of 0.655 and 0.685, respectively. Deviation from Hardy-Weinberg proportions was detected at three loci. Two loci showed evidence for null alleles. These microsatellite markers will be useful for population genetic analysis of Japanese Spanish mackerel.

Key words: *Scomberomorus niphonius*; Japanese Spanish mackerel; Microsatellite loci; Population structure

INTRODUCTION

Japanese Spanish mackerel (*Scomberomorus niphonius*), a pelagic fish widely distributed in subtropical and temperate waters of the northwest Pacific (Shui et al., 2008), is one of the important catch targets in China's fishery industry. Recent two-decade cruise data indicate that biological characteristics related to the population structure of the *S. niphonius*, such as the mean age, length and the age at sexual maturity, had obviously changed (Jin et al., 2006). The changes were mostly due to overexploitation and environmental changes (Jin et al., 2006). The protection and sustainable utilization of Japanese Spanish mackerel resources in China's coastal waters have drawn the attention of relevant authorities. Analysis of the population structure of *S. niphonius* may provide new perspectives on population assessment and efficient management in the *S. niphonius* resources.

Characterized by hypervariability, abundance, neutrality, codominance, and unambiguous scoring of alleles, microsatellite markers are referred as to the finest identification of population structure in marine fishes, among molecular markers (Tautz, 1989; Zhan et al., 2009). In *S. niphonius*, 18 polymorphic microsatellite loci were isolated (Yokoyama et al., 2006; Xing et al., 2009). Here, we describe the development of an additional 21 loci that will increase the power available for detecting fine-scale population genetic structure and gene flow of *S. niphonius*.

MATERIAL AND METHODS

Forty individuals were collected from Qingdao, China. Samples were preserved at -20°C until DNA extraction. A dinucleotide-enriched genomic library was constructed following the method of Ma and Chen (2009). In brief, genomic DNA was extracted from muscle tissue and digested with *Mse*I restriction enzyme (New England Biolabs, USA). The DNA fragments were ligated to the adapters (5'-TAC TCA GGA CTC AT-3'/5'-GAC GAT GAG TCC TGA G-3'). The ligated products were then pre-amplified in a 25-μL reaction system using the adapter specific primer 5'-GAT GAG TCC TGA GTA A-3' to verify successful ligation and increase DNA concentration. The biotin-labeled probe $(GT)_{13}$ was applied to hybridize with the products from pre-amplification. Subsequently, the hybrids were captured by the streptavidin-coated magnetic beads (Promega, USA), and the DNA fragments obtained and eluted from the magnetic beads were amplified by the adapter specific primer. The final amplification products were ligated into the pMD18-T vector (TaKaRa, Japan) and transformed into *Escherichia coli* DH5α competent cells. The positive clones were randomly sequenced with an ABI Prism 3730 automated DNA sequencer (Applied Biosystems, USA). Microsatellite repeats were found in 92 of the sequenced clones. PCR primer pairs were designed to amplify 72 microsatellite loci with suitable flanking regions using the PRIMER PREMIER 5 software (Premier Biosoft International, USA).

The designed primers were evaluated using 40 individuals of *S. niphonius*. PCR was performed on a Veriti Thermal Cycler (Applied Biosystems) in a total volume of 25 μL containing 0.4 μM of each primer, 0.2 mM of each dNTP, 1X PCR buffer, 2 mM $MgCl_2$, 1 U *Taq* polymerase (Fermentas, USA) and 10-100 ng DNA. The PCR cycling profile consisted of one cycle at 94°C for 5 min, 35 cycles of 45 s at 94°C, 1 min at the locus-specific annealing temperature (Table 1), and 45 s at 72°C, and a final cycle of 10 min at 72°C. The PCR products were

Table 1. Characteristics of microsatellite loci in *Scomberomorus niphonius*.

Locus	Primer sequence (5'-3')	Repeat motif	Ta (°C)	Allele size range (bp)	N_A	H_O	H_E	P_{HWE}	GenBank accession No.
Sn2	F: CTTATTGGTAGAAGAGGAGGAT R: GATAGATTTGACAGCGAGGA	$(GT)_9$	55	218-252	6	0.604	0.593	0.134	HQ317488
Sn12	F: ACAAACACCGATGCCCATACTG R: AAACACTGGTCTGATGGCTCCC	$(AC)_{22}$	62	167-205	13	0.750	0.885	0.010	HQ317489
Sn21	F: TGTGGCTTTGGGAGATTCAGGA R: ACGGCAACAGAGCAGAGGGTAA	$(TG)_{17}$	62	198-242	12	0.949	0.824	0.880	HQ317490
Sn39*	F: ACCCAACACTTGATTGATTT R: GTTTAGGTTTACACGCCACT	$(TG)_{17}$	60	170-262	24	0.975	0.946	0.227	HQ317491
Sn60	F: CTCTAATATCCCTGTTCAT R: ACACTGCTGTAAAGTTCTC	$(CA)_6AAC(CA)_{11}$	55	272-322	8	0.743	0.731	0.234	HQ317492
Sn90	F: ACACTCGCACTACTCTAAACA R: AGAAGGCAAGACAAGGGA	$(AC)_7$	55	220-240	3	0.516	0.427	0.427	HQ317493
Sn92	F: TCATTATCATAGCCAGGAAG R: CAAGCACTGTCAGCGTCT	$(CA)_{17}AA(AC)_{10}$	48	228-360	7	0.532	0.713	0.077	HQ317494
Sn94	F: AGGTTTGAGCATTACCGACAT R: TCTACTGACCCAGGCTTTCAC	$(AG)_{18}$	50	242-280	5	0.641	0.544	0.014	HQ317495
Sn111	F: CACTTATTAGTTGGAGGACAT R: TAGGCAAGTAGTGATTATGGT	$(CA)_8CCA(AC)_7AA(CA)_8$	63	228-252	6	0.471	0.440	0.848	HQ317496
Sn140	F: GAGATTGGATCTGCATCGT R: TGGTTTGCTTGCTTTAGTG	$(AC)_{21}$	63	160-224	14	0.917	0.884	0.012	HQ317497
Sn141	F: TCCATCTTCACATCACGTCCAC R: CTCCCCTCCCTCGCTTCA	$(AC)_{11}$	55	208-238	7	0.590	0.717	0.172	HQ317498
Sn170	F: GCCAGCGAAGCACAAACAT R: GCAAGGCAGAGTGACAGAG	$(AC)_6CCCA(AC)_7$	63	284-312	9	0.816	0.795	0.204	HQ317499
Sn180*+	F: AACCTTACAAGTTAGGGACG R: CTCAGGGATTGGAAACAG	$(AC)_{20}$	60	206-238	11	0.500	0.786	0.000	HQ317500
Sn199	F: TCTCAGCAAAATCCTCT R: AAGCAATAGAAAAGAACAG	$(AC)_{10}$	55	190-224	5	0.579	0.511	0.809	HQ317501
Sn200	F: ACACTCACCAGCTCCACCG R: CCAAAACCTGATGCCGATG	$(AC)_7ATTC(TC)_{14}$	55	148-200	8	0.895	0.786	0.392	HQ317502
Sn218+	F: ACAGTAGGTGGAGGTTTCTT R: TGTTTTACTCTTCAGGCTTC	$(GT)_8$	55	268-288	5	0.300	0.531	0.000	HQ317503
Sn224	F: TGGCAGGTGAACAGACAA R: CACTCACAACCCAGTCAATA	$(TG)_8$	55	226-262	11	0.750	0.768	0.577	HQ317504
Sn277	F: CAGGAGATCAGGCTACATC R: GCAAACATATTTTCCAACT	$(TG)_5AGTA(GA)_8$	55	242-294	6	0.700	0.668	0.138	HQ317505
Sn299	F: TAAAGAAGATGATGTAAGCACA R: CAGCCATTATCCAGCAGT	$(AC)_5(AC)_9$	50	206-214	5	0.263	0.385	0.029	HQ317506
Sn305	F: ATTACACCAATGTGCCAACT R: ACCAAAGCGCAGATCAAA	$(AC)_{12}$	55	204-232	7	0.575	0.588	0.338	HQ317507
Sn330+	F: TTGGAGCAAAGACAGAGC R: ATGATTAGAAATGGGAGC	$(CA)_{15}$	55	282-320	12	0.686	0.866	0.000	HQ317508

Ta = optimized annealing temperature; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; P_{HWE} = Hardy-Weinberg probability test. *Locus may harbor null alleles (null allele frequency >5%). +Locus deviated from Hardy-Weinberg proportions (adjusted $P < 0.0024$).

separated on a 6% denaturing polyacrylamide gel, and visualized by silver staining. Allele size was estimated according to the pBR322/*Msp*I marker (TianGen, China). The observed and expected heterozygosities together with tests for Hardy-Weinberg equilibrium and linkage disequilibrium were determined by GENEPOP 4.0 (Rousset, 2007). Null allele frequencies (Brookfield, 1996) were calculated by MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004). All results for multiple tests were corrected using Bonferroni's correction (Rice, 1989).

RESULTS AND DISCUSSION

A total of 21 of 72 loci were cleanly amplified and shown to be polymorphic. The number of alleles per locus ranged from 3 to 24 with an average of 8.8 (Table 1). The observed and expected heterozygosities ranged from 0.263 to 0.975 and from 0.385 to 0.946, with averages of 0.655 and 0.685, respectively (Table 1). No loci showed significant deviation from Hardy-Weinberg proportions except for loci Sn180, Sn218 and Sn330. Two loci (Sn39 and Sn180) showed evidence of null alleles (null allele frequency >5%). No significant gametic disequilibrium was detected between locus pairs.

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