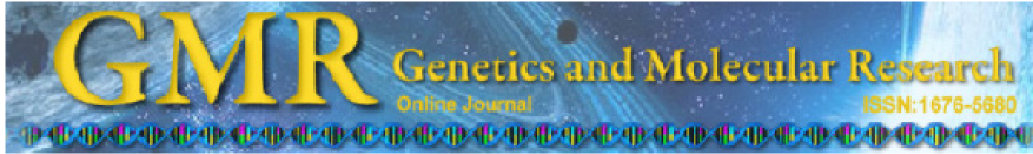

Polymorphic microsatellite loci isolated from the yellowbelly threadfin bream, *Nemipterus bathybius*

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ABSTRACT. Twenty-two polymorphic microsatellite loci were isolated and characterized from a $(GT)_{13}$-enriched *Nemipterus bathybius* genomic library. The number of alleles per locus ranged from 4 to 13, with an average of 7.86. The observed and expected heterozygosity was 0.167-0.889 and 0.278-0.904, respectively, with averages of 0.590 and 0.690. Three loci deviated from Hardy-Weinberg proportions, and 2 loci showed evidence of null alleles. No significant linkage disequilibrium was detected in the pairwise comparisons among the 22 loci. These markers are expected to be useful for the population genetic analysis of *N. bathybius*.

Key words: *Nemipterus bathybiu*s; Yellowbelly threadfin bream; Population genetic structure

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INTRODUCTION

The yellowbelly threadfin bream, *Nemipterus bathybius* is a small-size demersal fish that mainly inhabits the tropical and subtropical oceanic waters of the western Pacific from southern Japan to Indonesia and northwestern Australia. *N. bathybius* is an important fisheries resource in China. However, data collected over the last 2 decades indicate dramatic declines in the stock density and yield of *N. bathybius*. Furthermore, the proportions of juveniles in *N. bathybius* catches continue to increase in China (Huang and Chen, 2005; Chen et al., 2012). The recovery and sustainable utilization of *N. bathybius* resources in the coastal waters of China have drawn the attention of relevant authorities. It is therefore critical to evaluate the genetic population structure to appropriately inform conservation and management decisions for this species. Microsatellite markers represent an important tool for examining genetic diversity and genetic structure of wildlife populations. However, microsatellite markers specific to this species are not available at present. Therefore, here, we isolated and characterized 22 microsatellite markers in *N. bathybius*.

MATERIAL AND METHODS

Total genomic DNA was extracted from the muscle tissue of *N. bathybius* using the standard phenol-chloroform method. A dinucleotide-enriched genomic library was constructed following the protocol described by Ma and Chen (2009). Briefly, genomic DNA were digested with *Mse*I (New England Biolabs, Beverly, MA, USA), and ligated to the adapters (5'-TAC TCA GGA CTC AT-3'/5'-GAC GAT GAG TCC TGA G-3'). Then, the products were amplified using the adapter specific primer MA (5'-GAT GAG TCC TGA GTA A-3'). The PCR amplicons were then hybridized to biotin-labeled probes $(GT)_{13}$. Subsequently, the hybrids were captured by streptavidin-coated dynabeads (Promega, Madison, WI, USA). The obtained DNA fragments eluted from the magnetic beads were amplified using the adapter specific primer. The PCR amplicons in the range of 500-1000 bp were excised from 1% agarose, and purified using a gel extraction kit (TaKaRa, Kyoto, Japan). These amplicons were then ligated into the pMD 18-T vector (TaKaRa) and transformed into *Escherichia coli* DH5α competent cells. One hundred and fifteen positive clones were isolated and sequenced. Seventy-nine PCR primer pairs were designed to amplify the microsatellite-containing clones using the PRIMER PREMIER 5 software (Premier Biosoft International, USA).

The designed primers were evaluated using 40 individuals of *N. bathybius* collected from the waters near the Spratly Islands, China. PCR was performed with each 25 µL reaction containing 2.5 µL 10X PCR buffer, 0.4 µM of each primer, 0.2 mM of each dNTP, 2 mM $MgCl_2$, 1 U *Taq* polymerase (TaKaRa), and 10-100 ng DNA. The PCR cycling profile was run at 95°C for 5 min, 35 cycles of 45 s at 95°C, 1 min at the locus-specific annealing temperature (Table 1), and 45 s at 72°C, and a final extension for 10 min at 72°C. The PCR amplicons were separated on 6% denaturing polyacrylamide gel, and then visualized by silver staining. Allele size was estimated according to the pBR322/*Msp*I marker (TianGen, Shanghai, China). GENEPOP 4.0 was used to calculate the number of alleles, observed and expected heterozygosities, deviations from Hardy-Weinberg proportions and linkage disequilibrium (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

Twenty-two of 79 loci were cleanly amplified and shown to be polymorphic. The number of alleles per locus ranged from 4 to 13, with an average of 7.86 (Table 1). The observed and expected heterozygosities were 0.167-0.889 and 0.278-0.904, respectively, with averages of 0.590 and 0.690 (Table 1). Three loci (Nbv32, Nb121, and Nb139) deviated from Hardy-Weinberg proportions, and 2 loci (Nb121, Nb160) showed evidence of null alleles. No significant linkage disequilibrium was detected in the pairwise comparisons among the 22 loci.

Table 1. Characteristics of 22 microsatellite loci in *Nemipterus bathybius*.

Locus	Primer sequence (5'-3')	Repeat motif	Ta (°C)	Allele size range (bp)	N_A	H_O	H_E	P_{HW}	GenBank accession No.
Nbv1	F: ACCAGATTATAGTTACAAGCG R: TGTCCCTCACAGGTAGATAG	$(AC)_5(CA)_{14}$	55	272-318	11	0.833	0.826	0.378	KC346933
Nbv9A	F: CATTGTTTTGGATTGTTGA R: TGGCTGCTGGTGTCGTTC	$(TG)_5(GT)_5$	55	272-290	5	0.611	0.753	0.123	KC346938
Nbv11	F: GTCGGTCGTCACAAAACT R: CTTCATCGCACGCTCTAA	$(AC)_5GAC(GT)_{12}$	55	290-324	13	0.750	0.904	0.013	KC346940
Nbv32*	F: TTCTAATGGTGCAGGACA R: AACTGGTCAGCGGTAATC	$(CA)_{11}A(AC)_{11}$	55	234-272	10	0.823	0.571	0.000	KC346952
Nbv33	F: ACATCTCCGTCTGTCTGG R: ATGTGGCATCATTTGTAA	$(TG)_9$	55	278-296	6	0.657	0.764	0.212	KC346953
Nb25	F: CACCTGAGTCTCCTCCACC R: TGTTTTACCGCCTACTTGC	$(AC)_8$	55	274-300	8	0.417	0.485	0.081	KC192680
Nb52	F: TGGAGGAGGAGAAGAACCG R: ATCGCCTTGTCCAGCATG	$(TG)_9$	55	274-308	13	0.750	0.874	0.258	KC192697
Nb54	F: CATTGTATTTGTGGTGGCA R: GTCTGGTAACGGCGAGGAG	$(AC)_8C(CA)_6$	55	254-278	8	0.667	0.615	0.814	KC192698
Nb59	F: AAGTGTGACGACGAGTGC R: TTATCATCTATCCTACAAGTT	$(TG)_5(TC)_8T(CT)_5$	55	266-290	6	0.639	0.786	0.363	KC192701
Nb62	F: AAAGAATAGCGAAATGGC R: CGTCGTCGTAGGGAGTAA	$(CA)_{10}$	55	270-308	10	0.515	0.797	0.003	KC192704
Nb75B	F: TTTGGTGACAGAAGGATTTA R: CTTTATGATATGCTGAAGTGA	$(CA)_9$	55	212-244	11	0.889	0.826	0.665	KC192714
Nb77A	F: CTCCGCTTCTTCCTTTTC R: TATTTGGGACTGGGGCTC	$(CA)_5(CT)_7ATA(AC)_9$	55	214-230	7	0.514	0.582	0.358	KC192716
Nb77B	F: ACTCTGCTGTATCCATGC R: TACGGGCTATTATCTGTT	$(TC)_6(AC)_7$	55	202-218	6	0.600	0.594	0.097	KC192716
Nb85	F: ATTGCTCTGTTGTGGCTCC R: CCAGTTAGGGCGATACGG	$(AC)_6GCC(AC)_5$	55	208-220	4	0.167	0.278	0.164	KC192722
Nb121*+	F: GGATGCTCAGAATGTCAT R: GAGATCATAGTAGCCAGG	$(CT)_9$	55	248-286	10	0.235	0.854	0.000	KC346902
Nb125B	F: GGGAGGTAAAGGGCTGAA R: GCACCGTGGATGATAAACTA	$(CA)_6(AC)_8$	55	242-270	7	0.735	0.776	0.502	KC346904
Nb132	F: GCTGGTGTATGAATGTGGA R: ACTAAAACTCGGGGTAAAT	$(TG)_7$	55	210-238	7	0.722	0.735	0.075	KC346910
Nb135A	F: GACAGAGGACGGGTGATG R: CAGAAATTATGGGAGGAAAA	$(AG)_9$	55	200-218	5	0.611	0.696	0.106	KC346913
Nb139*	F: TCTTCCCCTTCTTACTCCTC R: CTGAATACCGCTGTCTGCTC	$(AC)_{21}$	55	198-220	7	0.412	0.788	0.000	KC346915
Nb160+	F: ATGTTCCCTTCCACTTAT R: GGATGGAATGATGTTTAGT	$(GT)_9$	55	214-234	6	0.343	0.489	0.018	KC346930
Nb163	F: GCTGTGGTGGCGTTTCTG R: GGAGGTCTCGGTGACTCATG	$(GT)_7$	55	290-312	5	0.500	0.502	0.958	KC346931

Ta = optimized annealing temperature; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; P_{HW} = Hardy-Weinberg probability test. *Indicates locus deviated from Hardy-Weinberg proportions (adjusted P < 0.0023). +Indicates that locus may harbor null alleles (null allele frequency >5%).

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