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Research Report

Development and characterization of 32 microsatellite loci in the giant grouper *Epinephelus lanceolatus* (Serranidae)

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ABSTRACT. An economically important marine fish species, the giant grouper *Epinephelus lanceolatus* (Serranidae) is widely cultured in Taiwan and costal areas of China. We isolated and characterized 32 polymorphic microsatellite loci from a CA-enriched genomic library of giant grouper. The number of alleles per locus ranged from 3 to 7, with a mean of 4.69. Observed and expected heterozygosities per locus varied from 0.387 to 1.000 and from 0.377 to 0.843, respectively. Six loci significantly deviated from Hardy-Weinberg equilibrium. After sequential Bonferroni's correction, only two loci showed deviation from Hardy-Weinberg

equilibrium, and no linkage disequilibrium was found between any pair of loci. These microsatellites can be useful tools for the study of population genetics in the giant grouper.

Key words: Giant grouper; Microsatellite; Population genetics; Isolation

Giant grouper, *Epinephelus lanceolatus*, a large reef-dwelling fish species belonging to the family Serranidae (Nelson, 1994), is distributed throughout most tropical and temperate marine areas in the Indo-Western Pacific and Indian Oceans (Zeng et al., 2008). Attributing to its high economic and medicinal value, giant grouper has been widely cultured in Taiwan, and there has also been an increase in aquaculture practices in mainland China. The life history of giant grouper is typical of many other groupers, encompassing late reproduction, sex reversal, high degree of territoriality and cannibalism, which make it exceedingly susceptible to overharvest and habitat destruction (Heemstra and Randall, 1993; Morris et al., 2000). As a consequence, this species has been listed as Vulnerable on the Red List of the IUCN since the mid-1990s (www.iucnredlist.org).

To provide effective conservation and sustainable utilization of giant grouper, it is particularly important to study the population genetic diversity and population structure of this species. Previous studies on giant grouper mainly focused on the molecular mechanism of growth factors function (Dong et al., 2010) and intracohort cannibalism (Hseu et al., 2004), while little information is available on population genetics, due to the lack of enough molecular markers. At present, microsatellites have been proven to be a favorable molecular marker in the field of fisheries and aquaculture, owing to their traits of co-dominance, high polymorphism and relatively small size (Chistiakov et al., 2006). In this study, 32 new microsatellite loci in giant grouper were isolated and characterized. We believe that these microsatellites can be used for the population genetic study of giant grouper.

Genomic DNA was extracted from the fin tissues of two unrelated individuals, using the method of standard proteinase K/phenol extraction (Sambrook and Russell, 2001). Construction of microsatellite library was performed according to the protocol of Zane et al. (2002) with some modifications. A total of 500 ng genomic DNA were digested with *Mse*I restriction enzyme (New England Biolabs, USA) in a 25-μL volume. Fragments with a length of 300-800 bp were isolated from an agarose gel and then ligated to *Mse*I adaptors: oligo A (5'-TACTCAGGACTCAG-3') and oligo B (5'-GACGATGAGTCCTGAG-3'), using T4 DNA ligase (New England Biolabs). The product was subsequently amplified with adaptor-specific primers (5'-GATGAGTCCTGAGTAAN-3', *Mse*I-N) in a total volume of 20 μL containing: 10 μL Ex-Taq premix buffer (TaKaRa, Dalian, China), 1 μM *Mse*I-N, and 5 μL diluted digestion-ligation DNA. PCR amplification was performed as follows: 94°C for 5 min followed by 21 cycles of 94°C for 30 s, 53°C for 1 min and 72°C for 45 s, with a final extension at 72°C for 5 min. The PCR product was purified with the Wizard PCR clean-up system (Promega, USA) and

hybridized with 100 nM biotin-labeled $(CA)_{15}$ probe at 60°C for 1 h after 5 min of denaturation. Streptavidin-coated magnetic beads (Promega) were used to selectively capture sequences containing TG repeats, and later, specific DNA was eluted from the beads by denaturation at 95°C. The eluted DNA was amplified again using the same cycling program as before. After purification using the Wizard PCR clean-up system (Promega), the DNA products were cloned into pMD18-T vector (TaKaRa) and transformed into *Escherichia coli* DH5α competent cells. Transformed cells were plated on LB agar containing ampicillin, IPTG and X-gal used for blue/white selection and incubated at 37°C for 12 h. Positive clones were randomly selected and sequenced using an ABI PRISM 3730 automated DNA sequencer (Applied Biosystems).

A total of 186 positive clones were screened and sequenced, in which 65 with enough flanking sequences were suitable for primer design. Primer pairs were designed using online software PRIMER 3 (Rozen and Skaletsky, 2000). Characterization of these microsatellites was assessed in a sample of 31 individuals collected from Hainan Province, China. Genomic DNA of each individual was isolated using the Wizard Genomic DNA Purification kit (Promega). PCR amplification was performed in a 20-µL volume containing the following components: 10 µL Ex-Taq premix buffer (TaKaRa), 1 µM of each primer set and 50 ng template DNA. The PCR conditions were 5 min at 94°C followed by 28 cycles of 30 s at 94°C, 40 s at the annealing temperature for each locus (Table 1) and 30 s at 72°C, with a final extension of 5 min at 72°C. Amplified products were separated on an 8% polyacrylamide gel and visualized by silver staining. The size of alleles was identified according to a pBR322/*Msp*I marker (Tiangen, Beijing, China). After screening all loci in the tested population, genotypes of polymorphic loci were scored. Genotyping errors due to null alleles, stuttering and allele dropout were analyzed using MICRO-CHECKER (Van Oosterhout et al., 2004). Number of alleles at each locus (N_A), observed heterozygosity (H_O) and expected heterozygosity (H_E) were calculated using CERVUS 3.0 (Kalinowski et al., 2007). Deviation from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) at each locus were tested using GENEPOP 4.0 (Rousset, 2008).

Of the 65 microsatellite loci tested, 32 were shown to be polymorphic, while the other 33 were either monomorphic or failed to amplify target products. Sequences of the polymorphic microsatellite loci have been deposited in GenBank (Accession numbers: JN185622-JN185653). The number of alleles per locus varied from 3 to 7, with an average of 4.69. The observed and expected heterozygosities varied from 0.387 to 1.000 and from 0.377 to 0.843, respectively. Six loci (An 4, An 12, An 14, An 16, An 29 and An 31) significantly deviated from HWE ($P < 0.05$; Table 1), as MICRO-CHECKER analysis showed no evidence of null alleles, stuttering or allele dropout for all the polymorphic loci, probably due to insufficient sample size or existence of a subpopulation. After sequential Bonferroni correction (Rice, 1989), only two loci (An13 and An29) still showed significant deviation from HWE ($P < 0.0016$). No significant LD was detected between any pair of the 32 polymorphic loci. These microsatellite markers will be useful for the study of population genetics and conservation assessment of giant grouper and other related species.

Table 1. Characterization of 32 microsatellite loci in giant grouper *Epinephelus lanceolatus* (Bloch).

Locus	Accession No.	Primer sequences (5'-3')	Repeat motif	Ta (°C)	Size range (bp)	N_A	H_O	H_E	P value
An1	JN185622	F: ACTACAGAGCGTGGCAGGTT R: TGCAGTCAGTGCAAATGAGTC	$(CA)_{13}$	61	236-262	4	0.839	0.701	0.073
An2	JN185623	F: TGCCCCTCCGACAACTAATA R: AACGGGACTTGTGGTTTTTG	$(TG)_{10}AG(TG)_{14}$	61	226-250	5	0.871	0.717	0.116
An3	JN185624	F: CCACACTGATGGATGACATGA R: GAAACAGCGCCACCCTCT	$(TG)_{22}$	61	142-170	4	0.871	0.741	0.072
An4	JN185625	F: GATGCACACAAGCACAAACA R: GCAGGCTTATCCAAAACAGC	$(CA)_{22}$	60	192-220	5	0.807	0.711	0.015
An5	JN185626	F: GCTGGGGAATGCATTATGTT R: TCAGGTTGATGCTGAGTGGA	$(TG)_{15}$	60	208-238	4	0.903	0.713	0.261
An6	JN185627	F: GCTCGAAGATGAGCTGGAAG R: AAGGTGCTGCTCCTGCTTT	$(CT)_{10}(CA)_{12}$	60	192-210	7	0.710	0.805	0.054
An7	JN185628	F: TGAAGTGCAAACATCCTTGG R: CCAAAGGCTGAGTTCTCTGTG	$(CATA)_9$	60	142-154	3	0.400	0.386	0.290
An8	JN185629	F: ACCATGCATAAATGCCCACT R: GCTCTCTGTCTCGCAAGGAT	$(CA)_{23}$	60	148-162	6	1.000	0.843	0.156
An9	JN185630	F: ACAGGCACACAGAAATGCAG R: TGAGGCTTGTTGATTGCTTG	$(CA)_4TA(CA)_{25}$	61	186-216	5	0.839	0.774	0.233
An10	JN185631	F: GAGAGATAAATACCAGCTTCACTGC R: TTCACAGTTTCCTCGGTTCC	$(CA)_{26}$	60	168-216	5	0.967	0.762	0.063
An11	JN185632	F: GCTCTGTGGATGGCCTTTAT R: TTCATCCTCTGGGGACTACG	$(TTGG)_{12}$	60	144-170	5	0.733	0.694	0.591
An12	JN185633	F: AGGAAATGCCACAAAGATGG R: AGCTCTGAAAGCTCCCTGCT	$(AC)_{21}$	61	196-222	5	0.807	0.772	0.021
An13	JN185634	F: CCACTGAGGTTGCCTGTTTT R: TAGTCTGCGTGATCGTCTGG	$(AG)_5(TG)_{12}$	60	234-260	5	1.000	0.786	0.001
An14	JN185635	F: CAAGGAATAGTTCAGCTTTCATCTT R: TAAATGTGTCTCCCCCAAGG	$(CT)_2(TG)_{14}$	60	210-238	4	0.839	0.618	0.087
An15	JN185636	F: CCTGTGTGTGAGCTGGAGAA R: GGTGGAGGAGTACGAAACCA	$(GT)_{26}$	58	186-242	5	0.968	0.729	0.070
An16	JN185637	F: CTGGGATTGCTGGTTTTGTC R: CTGTGCTAGCTTTTCTCTCTGG	$(TG)_{22}$	61	244-254	3	0.710	0.527	0.032
An17	JN185638	F: AAGCTCTAAGCAGCGCTCAC R: CACACTCAGTTGTGCCTTGAA	$(TG)_8T(TG)_3$	60	250-262	4	0.800	0.737	0.138
An18	JN185639	F: ACACGGTATGGGCAAGAAAG R: ACTTGATGTGACGACGATGC	$(CA)_4…(CA)_{17}$	60	176-190	4	0.827	0.716	0.327
An19	JN185640	F: ATGTCTGTGTGCGCTCATGT R: AAGTCACGAAGCCATGAGGT	$(TG)_{13}$	60	202-214	3	0.710	0.607	0.126
An20	JN185641	F: AAAGATCAAAGACGCTCCTGA R: AGTCGTTGGCAACCCATAAA	$(CA)_{18}$	61	146-166	3	0.677	0.596	0.577

Continued on next page

Table 1. Continued.

Locus	Accession No.	Primer sequences (5'-3')	Repeat motif	Ta (°C)	Size range (bp)	N_A	H_O	H_E	P value
An21	JN185642	F: CAAACGAACAAGACCAAGCA R: CACCAGTAACCTCTCTGTTGTGA	$(CA)_{21}$	61	230-272	6	0.767	0.682	0.464
An22	JN185643	F: TCAGGTTGATGCTGAGTGGA R: GCTGGGGAATGCATTATGTT	$(CA)_{13}$	61	214-242	4	0.600	0.593	0.226
An23	JN185644	F: GACAGCAACGTCAGACCAGA R: TGCACAAAGTGTTATTAGTCATCA	$(AC)_{21}$	61	202-236	4	0.774	0.673	0.496
An24	JN185645	F: GGTAAGGGGGCTAGGGAAT R: GCTTACGCAACCGATACCTC	$(TG)_{24}$	61	174-200	6	0.774	0.752	0.385
An25	JN185646	F: TCTGTGCTGATGCCGACTAC R: CCGTGTTTGCACACTCTCTG	$(TG)_{21}...(GT)_{11}$	58	146-200	6	1.000	0.810	0.124
An26	JN185647	F: GGAGCTGAAGCAGGATGTTC R: TTCTCCTCAGAGAGCCTTGG	$(TG)_{22}$	61	188-200	4	0.867	0.716	0.130
An27	JN185648	F: AGCACAAAGACCTGGAGGAA R: AGCAGGTCTTGGGAATTGTG	$(CA)_{25}$	58	235-267	6	1.000	0.813	0.136
An28	JN185649	F: CATTGTGGAGGACATTGCAG R: TTGGTAAACTCTACAGCCAACG	$(CA)_{28}$	61	146-186	4	0.710	0.732	0.168
An29	JN185650	F: CACCAGTAACCTCTCTGTTGTGA R: CATTGTGGAGGACATTGCAG	$(TG)_{24}CA(TG)_3$	61	214-250	5	0.968	0.747	0.001
An30	JN185651	F: GGCAGGTGTGTTTATTGCAG R: CTAGCCATGGTTTGGTGGAT	$(GT)_{18}$	58	200-250	7	1.000	0.817	0.119
An31	JN185652	F: TCATGTGTGCAAACGCTGTA R: CAACATGGCCGAAACCTAAT	$(GT)_{22}$	61	184-218	6	0.936	0.777	0.005
An32	JN185653	F: TTCTCCATCATTAGTCGCACA R: TCTTTGTCTCCCTGTTTTTGC	$(TG)_2GA(TG)_{11}$	61	196-218	4	0.700	0.545	0.367

Ta = annealing temperature; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; P value = probability values for exact tests of Hardy-Weinberg equilibrium.

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REFERENCES

Chistiakov DA, Hellemans B and Volckaert FAM (2006). Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. *Aquaculture* 255: 1-29.

Dong HY, Zeng LX, Duan D and Zhang HF (2010). Growth hormone and two forms of insulin-like growth factors I in the giant grouper (*Epinephelus lanceolatus*): molecular cloning and characterization of tissue distribution. *Fish Physiol. Biochem.* 36: 201-212.

Heemstra PC and Randall JE (1993). Groupers of the World (Family *Serranidae*, Subfamily *Epinephelidae*): An Annotated and Illustrated Catalogue of the Grouper, Rockcod, Hind, Coral Grouper and Lyretail Species Known to Date. FAO, Rome.

Hseu JR, Hwang PP and Ting YY (2004). Morphometric model and laboratory analysis of intracohort cannibalism in giant grouper *Epinephelus lanceolatus* fry. *Fish. Sci.* 70: 482-486.

Kalinowski ST, Taper ML and Marshall TC (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* 16: 1099-1106.

Morris AV, Roberts CM and Hawkins JP (2000). The threatened status of groupers (Epinephinae). *Biodivers. Conserv.* 9: 919-942.

Nelson J (1994). Fishes of the World. John Wiley and Sons, New York.

Rice WR (1989). Analyzing tables of statistical tests. *Evolution* 43: 223-225.

Rousset F (2008). Genepop 007: a complete reimplementation of the genepop software for Windows and Linux. *Mol. Ecol. Resour.* 8: 103-106.

Rozen S and Skaletsky HJ (2000). Primer 3 on the WWW for General Users and for Biologist Programmers. In: Bioinformatics Methods and Protocols: Methods in Molecular Biology (Krawets S and Misener S, eds.). Humana Press, Totowa, 365-386.

Van Oosterhout C, 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.

Zane L, Bargelloni L and Patarnello T (2002). Strategies for microsatellite isolation: a review. *Mol. Ecol.* 11: 1-16.

Zeng HS, Ding SX, Wang J and Su YQ (2008). Characterization of eight polymorphic microsatellite loci for the giant grouper (*Epinephelus lanceolatus* Bloch). *Mol. Ecol. Resour.* 8: 805-807.

