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Short Communication

Isolation and characterization of microsatellite loci in the purpleback flying squid (*Sthenoteuthis oualaniensis*)

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ABSTRACT. The purpleback flying squid (*Sthenoteuthis oualaniensis*) is a pelagic squid with tremendous potential for commercial exploitation. We isolated and characterized 21 polymorphic microsatellite loci for *S. oualaniensis* using a $(GT)_{13}$-enriched genomic library. The number of alleles per locus varied from 6 to 32. The observed and expected heterozygosities ranged from 0.188 to 0.890, and 0.537 to 0.968, respectively. No significant linkage disequilibrium was detected at these loci. Five loci significantly deviated from the Hardy-Weinberg equilibrium, and four loci may have exhibited null alleles. These microsatellite markers will facilitate

further studies in population genetics and the sustainable utilization of *S. oualaniensis*.

Key words: Purpleback flying squid; *Sthenoteuthis oualaniensis*; Population genetics; Microsatellite

INTRODUCTION

The purpleback flying squid (*Sthenoteuthis oualaniensis*) is a pelagic squid that is widely distributed in the tropical and subtropical areas of the Pacific and the Indian Oceans, particularly in the South China Sea and the northwest areas of the Indian Ocean (Nesis, 1977; Mohamed et al., 2006). In recent years, because of the drastic decline in traditional fishery resources in the South China Sea, the focus of marine exploitation has shifted to *S. oualaniensis* owing to its high biomass, short life cycle, high growth rate, and high fecundity (Chen et al., 2007; Zhang et al., 2010). Analysis of the genetic diversity and population structure of *S. oualaniensis* can provide essential information for the better management and sustainable utilization of this species.

Microsatellites are one of the best markers for studying population genetics in pelagic marine organisms, because they are reproducible, multi-allelic, co-dominant, and relatively abundant (Zhan et al., 2009). No microsatellite marker is currently available for *S. oualaniensis*; therefore, we developed and characterized 21 microsatellite markers for this species. These markers will facilitate further studies in population genetics and the sustainable utilization of *S. oualaniensis*.

MATERIAL AND METHODS

A $(GT)_n$-enriched genomic library of *S. oualaniensis* was constructed following a previously published method (Ma and Chen, 2009). In brief, genomic DNA was extracted from muscle tissue and digested with the *Mse*I restriction enzyme (New England Biolabs, USA) at 37°C for 3 h. The digested fragments were ligated to specific adapters (5'-TACTCAGGAACTCAT-3'/5'-GACGATGAGTCCTGAG-3') using T4 DNA ligase (Fermentas, USA) at 16°C for more than 8 h. The ligated fragments were amplified in a 25-μL reaction system, using an adapter-specific primer (5'-GATGAGTCTGAGTAA-3'), to verify the ligation and increase the DNA concentration. The polymerase chain reaction (PCR) products were hybridized to biotin-labeled $(GT)_{13}$ probes, and the hybridized complexes were captured by streptavidin-coated magnetic beads (Promega, USA). Subsequently, nonspecific binding and unbound DNA was removed by several non-stringent and stringent washes. The microsatellite-enriched DNA fragments eluted from the magnetic beads were amplified using the adapter-specific primer, and the amplified products were purified on 1.5% agarose gel. The DNA fragments (ranging in size from 500 to 1000 bp) were extracted using a DNA gel extraction kit (Axygen, USA) and ligated into pMD18-T vectors (Takara, Japan), which were then transformed into *Escherichia coli* DH5α-competent cells. The positive clones were sequenced on an ABI 3730 automated DNA sequencer (Applied Biosystems, USA).

DNA sequences containing microsatellite loci were selected, and primers were designed based on the flanking regions of the microsatellite loci using the Primer Premier 5 software (Premier Biosoft International, USA). The designed primer pairs were evaluated us-

ing 50 individuals of *S. oualaniensis* collected from the South China Sea. PCR amplifications were performed separately in a 25-μL reaction volume that contained 0.4 μM primer (each), 0.2 mM dNTP (each), 2 mM $MgCl_2$, 1X PCR buffer, 1 U *Taq* polymerase (Takara, Japan), and 50 to 100 ng template DNA. The PCR cycling profile was as follows: an initial denaturation at 94°C for 5 min; 35 cycles of 45 s at 94°C, 45 s at the optimal annealing temperature (Table 1), and 45 s at 72°C, followed by a final extension step at 72°C for 10 min. The PCR products were separated on 6% denaturing polyacrylamide gels, and visualized by silver staining. Allele size was estimated using a pBR322/*Msp*I marker (TianGen, China).

The observed and expected heterozygosities, the Hardy-Weinberg equilibrium, and linkage disequilibrium were calculated using Genepop 4.0 (Rousset, 2007). Null allele frequencies were estimated using Micro Checker 2.2.3 (Van Oosterhout et al., 2004). Bonferroni corrections (Rice, 1989) were used to correct the results of all of the multiple tests conducted.

Table 1. Characterization of 21 polymorphic microsatellite loci for *Sthenoteuthis oualaniensis*.

Locus	Repeat motif	Primer sequences (5'-3')	Size (bp)	Ta (°C)	N_A	H_O	H_E	GenBank accession No.
SO2	$(GT)_6$	F: CACAACTAATCAAGTGGACAA R: TATGCTATCAATAAACCGAAA	270-309	58	8	0.440	0.537	KF999033
SO6	$(TG)_{10}(GT)_6(GT)_{16}$	F: TTTAGTTTATACCCTCAATGT R: CACTCATACAGACATCCATAC	250-331	58	23	0.804	0.935	KF999034
SO8*†	$(CA)_5$	F: TCGTGGAAAATTGAAACA R: AAAATGCCCTCATAAACA	230-260	58	22	0.740	0.897	KF999035
SO14*†	$(AC)_5$	F: CACCAAAGTAACTGGGACAAC R: AGTTTCATTACATGAAGCGTG	195-258	60	6	0.283	0.707	KF999036
SO18	$(TG)_{16}(GT)_{17}$	F: TCAAGCACCAAACGAGAT R: AAATGAGCAAAACGAACA	255-337	60	18	0.890	0.900	KF999037
SO20	$(AC)_8$	F: CCCACCAGAAGTTTTAGG R: AGTCCCAATCTCGTCTTT	231-285	60	13	0.760	0.882	KF999038
SO23	$(GT)_{29}$	F: GCCTTTGTATGACCAGAT R: TGAGTCTACTTCAATGGGT	287-349	62	18	0.850	0.924	KF999039
SO24	$(CT)_6(CA)_{22}$	F: TGCTGAGTTTTCCAAAGG R: ATATTCGTAGATGACACCATA	315-388	58	14	0.780	0.896	KF999040
SO39	$(TG)_{10}(GT)_6(GT)_6$	F: TTTTAGTTTATACCCTCAATG R: CACTCATACAGACATCCATAC	264-333	58	16	0.826	0.933	KF999041
SO45	$(TCA)_{11}$	F : CATTCTACCTTTAGTCGC R: GTGTCAAACAATGGATTT	300-356	58	17	0.847	0.911	KF999042
SO47*†	$(TG)_{25}$	F: ATTGATAGCCTTTGGTGA R: TGAGCCTTATTTCGGTCT	238-289	60	20	0.256	0.940	KF999043
SO51	$(AC)_{15}(AC)_{11}(AG)_5$	F: ATCGCTGTAGGGTGAGGA R: CACTATGCCAAATGTTGTTT	286-355	63	13	0.790	0.878	KF999044
SO59	$(AG)_{16}(GT)_5(TG)_{10}(GA)_9$	F: CCAAATACGCATTCAAACAA R: GATCACAAAGCGAATCCTTC	262-346	63	23	0.871	0.935	KF999045
SO60	$(TG)_{20}$	F: TTTATCGGAAAATCGCTAC R: TACGCAGAATCATACGCTA	179-252	60	18	0.890	0.920	KF999046
SO74	$(GT)_9$	F: AACGATTGGTCCTCAGTG R: CCTTGCTCCTACAGTCTTA	220-258	63	11	0.776	0.781	KF999047
SO79*†	$(ATGT)_6$	F: TCACTATCGTTATAGACCCAG R: TTTGAGATTTTGAGCGTTG	268-309	63	13	0.188	0.871	KF999048
SO80	$(CA)_{17}$	F: GATTAGTGGACGTTTCCG R: TCCCGATTCAATTCTTGT	262-324	60	15	0.833	0.916	KF999049
SO83	$(GA)_8(GA)_{20}$	F: GTACCCACAACCTCACAT R: TTTTCCCAGCTATAAATT	190-277	50	32	0.812	0.968	KF999050
SO86	$(CA)_7$	F: TGGAAGGGGAGAAAAGTA R: GATGGGAAATATGATGCA	299-325	60	6	0.360	0.322	KF999051
SO96	$(TG)_{29}$	F: CTCCAGTGCCACCCCAGTAAA R: AGAGGACCATAACCCCGACAA	220-272	60	16	0.747	0.878	KF999053
SO101†	$(AC)_{11}(CA)_5$	F: ATGCCTGGAAGATGTGAATG R: TTGGCTTTGCTGAACGAATA	314-333	58	13	0.490	0.879	KF999055

Ta = optimized annealing temperature; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; *locus may harbor null alleles (estimated null allele frequency > 5%); †locus deviated from Hardy-Weinberg proportions (adjusted P < 0.0023).

RESULTS AND DISCUSSION

In total, 150 positive clones were identified and sequenced, and microsatellite repeats were found in 106 sequences. Fifty-three primer pairs were designed based on the microsatellite sequences. Twenty-one loci were amplified cleanly and found to be polymorphic, and the number of alleles per locus varied from 6 to 32. The observed and expected heterozygosities ranged from 0.188 to 0.890, and 0.537 to 0.968, respectively. No significant linkage disequilibrium was detected among these loci. Five loci deviated significantly from the Hardy-Weinberg equilibrium after a Bonferroni correction (adjusted $P < 0.0023$), which may have been due to the small sample size or the existence of null alleles. Null alleles may have been present at four loci (estimated null allele frequency > 5%). These markers will serve as useful tools for population genetics and sustainable utilization studies of *S. oualaniensis* (Table 1).

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REFERENCES

Chen XJ, Liu BL, Tian SQ, Qian WG, et al. (2007). Fishery biology of purpleback squid, *Sthenoteuthis oualaniensis*, in the northwest Indian Ocean. *Fish. Res*. 83: 98-104.

Ma HY and Chen SL (2009). Isolation and characterization of 31 polymorphic microsatellite markers in: barfin flounder (*Verasper moseri*) and the cross-species amplification in spotted halibut (*Verasper moseri*). *Conserv. Genet.* 10: 1591-1595.

Mohamed KS, Joseph M and Alloycious PS (2006). Population characteristics and some aspects of the biology of oceanic squid *Sthenoteuthis oualaniensis* (Lesson, 1830). *J. Mar. Biol. Ass*. 48: 256-259.

Nesis KN (1977). Population structure in the squid *Sthenoteuthis oualaniensis* (Lesson 1930) in the Western Tropical Pacific. *Oceanology* 107: 15-29.

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

Rousset F (2007). GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Mol. Ecol. Notes* 8: 103-106.

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.

Zhan A, Hu J, Hu X, Zhou Z, et al. (2009). Fine-scale population genetic structure of Zhikong scallop (*Chlamys farreri*): do local marine currents drive geographical differentiation? *Mar. Biotechnol*. 11: 223-235.

Zhang P, Yang L, Zhang XF and Tang YG (2010). The present status and prospect on exploitation of tuna and squid fishery resources in South China Sea. *South China Fish. Sci.* 6: 68-74.