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Short Communication

Development and Characterization of Twenty-Three Novel Polymorphic Microsatellite Markers for Mussel, *Mytilus coruscus*

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Authors' Contributions
YY conceived and designed the experiments; ZF performed the experiments; YT and YY analyzed the data; PQ and CW contributed with reagents/materials/analysis tools; ZF wrote the paper; Zf and Yt collected and processed the samples.

Key words
***Mytilus coruscus*, Mussel, Microsatellite markers, Genetic diversity.**

ABSTRACT

Mytilus coruscus is one of the main mussel species in China, with a widely range of aquaculture markets and importantly economic value. In this study, we used one hundred and twenty primers pairs to screen all microsatellite loci and twenty-three polymorphic loci were identified. The number of alleles per loci ranged from six to twenty-one with an average of 12.913 and the allele size varied between 161 and 470 bp. The observed and expected heterozygosity varied from 0.143 to 1.000 and from 0.351 to 0.926, with average values of 0.614 and 0.771, respectively. Eighteen loci accorded with the Hardy-Weinberg Equilibrium (*HWE*)($P > 0.05$). The polymorphism information content (*PIC*) value ranged from 0.340 to 0.921 with an average of 0.751. These markers will help the further studies, provide more useful tools to choose for researching genetic diversity and population structure and provide more in-depth understanding of *Mytilus coruscus*.

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The thick shell mussel, *Mytilus coruscus* (Gould, 1861), belongs to the *Mytilidae* or true mussels (*Mollusca*; *Bivalvia*; *Lamellibranchia*; *Mytiloida*; *Mytilus*). It is an economically important mussel and widely distributed in the coastal waters of the Bohai Sea, the Yellow Sea, the East China Sea, and the South China Sea (Liao *et al*., 2013). With the high nutritional values, it is extensively cultured as an important shellfish species in Zhoushan archipelago of Zhejiang Province in China (Chang and Wu, 2007). With the advanced breeding technologies, aquaculture of *M. coruscus* in China has been rapidly expanding, and its cultural production has reached about 50,000 tons in Zhejiang per year (Ye *et al*., 2012). However, due to the over-exploitation and damage to natural habitats, natural resource of *M. coruscus* has been significantly decreasing. In 2014, only 19,600 tons of *M. coruscus* were harvested in Zhejiang province, China (Zhejiang Fisheries Technical Extension Center http://www.zjfishery.com/html/main/bzxxView/2015-01/82624.html). Thus, it is required to investigate the genetic background and make an effective conservation strategy for *M. coruscus*.

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Microsatellites, otherwise known as "Simple Sequence Repeats" (SSR), are tandem arrays of short nucleotide repeats (1-6 base pairs) and are interspersed throughout the genome (Tautz and Renz, 1984; Zhu *et al*., 2017). They are highly abundant in various eukaryotic genomes including all aquaculture species studied to date. Their high polymorphism and Polymerase Chain Reaction (PCR) based analysis have made them to be one of the most popular genetic markers (Boris *et al*., 2011; Duran *et al*., 2009). Microsatellite tracking provides the ability to study the population genetic structure (An *et al*., 2012), due to their relatively high abundance in the genome, a high mutation rate, polymorphism, codominant Mendelian inheritance and good reproducibility (Matthewa *et al*., 2009). Microsatellite genotypes has been proved as a powerful tool for accurate genetic assessment of population differentiation and pedigree tracing of hatchery populations from various fishery animals (Li *et al*., 2006). However, microsatellites are not useful for inter-specific assessment and sequence information needed. Although the use of SSR markers in genetic variation studies of mussel species is published (Ong *et al*., 2009; Ye *et al*., 2014), there is less microsatellite marker available for *M. coruscus*.

The purpose of this study was to develop and

characterize microsatellite loci for *M. coruscus* using high-throughput sequencing technology. These new markers will serve as valuable tools to conduct the genetic diversity and structure of *M. coruscus* and aid the design of spatial fishery management and conservation strategies in artificial propagation programs for *M. coruscus*.

Materials and methods

In this study, 35 specimens of *M. coruscus* were collected in Taishan Island, Fujian Province, China. The adductor muscle was taken out and preserved in 100% alcohol until DNA extraction using improved salting out method (Aljanabi and Martinez, 1997). The DNA was diluted to a final concentration of 50 ng/ul and stored at -20°C for further analysis.

The transcription data were used for microsatellite searching and primer designing with Illumina sequencing technology. A total of 21,723,913 paired-end clean reads (NCBI SRA database SRX 1411195) were generated from HiSeq 2000 sequencer (Xu *et al*., 2016). Online WebSat (http://wsmartins.net/websat/) was utilized to design primers based on the reads containing putative microsatellite regions.

For detection of microsatellite markers, each pair of primer was tested on 35 specimens of *M. coruscus*, PCR was carried out in volumes of 20µl, containing 15-40 ng of template DNA, 4 pmol of each forward and reverse primer, 10µl CW0716 2×Taq MasterMix (Cwbiotech., Peking, China). PCR amplification was performed using Applied Biosystems Veriti 96-Well Thermal Cycler (Applied Biosystems, Inc., Foster City, CA, USA). The reaction process was as follow, after initial denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30s, 55°C-61°C for 30s (each pair of primer was screened in gradient temperature), 72°C for 40s, and final elongation at 72°C for 10 min. In order to evaluate the amplifications, the PCR products were tested on an 8% non-denaturing polyacrylamide gel electrophoresis. Then, the genotypic of PCR products by capillary electrophoresis were performed using the BiopticQsep100 DNA Analyzer (Hangzhou Houze Biotech Company, Zhejiang, China).

For the successful primers, the number of alleles (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e) and *P*-value of Hardy-Weinberg Equilibrium (*HWE*) test were obtained by the program GenAlEx 6.501 (Peakall *et al*., 2006). Polymorphism information content (*PIC*) (Botstein *et al*., 1980) was calculated using the PIC_CALC 0.6. Micro-checker 2.2.3 (Van Oosterhout *et al*., 2004) was used to analyze the null alleles.

Results and discussion

Among 120 polynucleotide-repeat microsatellites in *M. coruscus*, 23 polymorphic loci were successfully amplified from the specimens of *M. coruscus*. The characteristics of these loci were presented in Supplementary Table I.

The number of alleles per locus ranging from 6 (MC18) to 21 (MC54, MC57 and MC99) with an average of 12.913, and the alleles size (bp) varied between 161 and 470 bp. The observed and expected heterozygosity varied from 0.143 (MC43) to 1.000 (MC31, MC63 and MC76) and from 0.351 (MC44) to 0.926 (MC101), with average values of 0.614 and 0.771, respectively. Five loci (MC22, MC27, MC43, MC49 and MC101) were deviated from Hardy-Weinberg equilibrium significantly ($P<0.01$). The polymorphism information content (*PIC*) value ranged from 0.340 (MC44) to 0.921 (MC101) and the average was 0.751.

In this study, we successfully found 18 different microsatellite markers which conformed to the *HWE* ($P > 0.05$). However, some loci tested could not attain the predicted effect (*i.e.,* MC22, MC27, MC43, MC49 and MC101). The Wahlund effect, genotypic errors, groupings of relatives and the presences of null alleles could be the reasons for these loci deviations from *HWE*. It could be because these loci did not exisit in the subset of 35 individuals, or the PCR was influenced by some unknown causes or it may be because of the small sample size. So, MICRO-CHECKER 2.2.3 software was used to test these loci, and null alleles were detected from these five loci. The null alleles perhaps appear variations in SSR flanking sequence, loss of large fragments alleles or differences in the quality requirements of DNA with special loci. The presence of null alleles is part of the main reason due to the five loci deviated from *HWE*.

Eventually, 23 out of 120 primers pairs were selected as novel polymorphic microsatellite markers. These loci sequences were submitted to the GenBank (GenBank accession numbers: KX423595-KX423617, Supplementary Table I). These new 23 microsatellite loci showed a medium genetic diversity (based on the data of alleles number per locus (N_a), observed and expected heterozygosity (H_o and H_e), *HWE* and *PIC*), and suitability for studies of potential value. In addition, these markers will help the further studies, serve as more useful tools for researching genetic diversity and population structure and more in-depth understanding of *M. coruscus*.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2018.50.4.sc3

Statement of conflict of interest

Authors have declared no conflict of interest.

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Supplementary Material

Short Communication: Development and Characterization of Twenty-Three Novel Polymorphic Microsatellite Markers for Mussel *Mytilus coruscus*

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Table I.- Characterization of 23 polymorphic microsatellite loci isolated from *Mytilus coruscus*. The table shows the locus name code, primer sequences (5'-3'), repeat motif, annealing temperature (Ta), allele size range, test population sample size, number of alleles (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e), *P*-value of Hardy-Weinberg Equilibrium (HWE) test, polymorphism information content (PIC).

Locus/ Accession No.	Primer sequences (5'-3')	Repeat motif	Ta (°C)	Size (bp)	Samples	N_a	H_o	H_e	HWE	PIC
MC08/ KX423595	F:TCACATGACTCTATACAACCCCTTT R:TATCCAGCCACAACGGACTTA	(ATTT)3	60	204-270	35	13	0.686	0.840	0.065	0.822
MC18/ KX423596	F:CACGGATCTCGGGTATTCTCTG R:CAGTCGGATGTTGTTTATTGATGTG	(TA)7	60	177-187	34	6	0.971	0.774	0.052	0.740
MC22*/ KX423597	F:CTTTGGCAGCAGAATATCACAG R:ACAGGATCGGAAGTGAATGAGT	(TA)7	60	335-389	33	19	0.485	0.909	0.000	0.902
MC27*/ KX423598	F:GCAACATTTACCGATGAAGACA R:CGGACATTCCAGAAGAAACATA	(AT)6	60	319-337	35	8	0.371	0.807	0.000	0.780
MC31/ KX423599	F:TGTACTGCACTTCTTTTCTTGGC R:TGCAAGCTGACTCTTACAATAACC	(AT)9	60	342-424	35	15	1.000	0.895	0.146	0.885
MC34/ KX423600	F:GGCAGAGGGTCATAGTTTTCAC R:CCCTTTCTGTGATTTCCTCAAC	(TA)7	59	240-252	35	7	0.400	0.617	0.152	0.563
MC36/ KX423601	F:GCTTTTGTGATTACTGTGAAGACG R:CATGATATGGCAACATTGGACT	(TA)7	60	161-187	35	8	0.971	0.688	0.307	0.661
MC43*/ KX423602	F:TACTGTCCTTTCCAGAGCAGAC R:CCAAAACGCACCATACATTAAC	(TGA)6	58	386-442	35	11	0.143	0.794	0.000	0.771
MC44/ KX423603	F:TCCCTTTGTCTCTTGTTTCCAT R:TGAATGGCGTTTACCCTCTTAT	(CA)6	59	182-208	35	8	0.257	0.351	0.061	0.340
MC47/ KX423604	F:GATAGAGTTGGGAAAAGCAACG R:CATACAAATCAAACATGACGGG	(AT)7	60	249-329	34	19	0.676	0.824	0.507	0.812
MC49*/ KX423605	F:GCGCAGAAGGTATTTGATTGTT R:GAAGAACTTGCCAGAAAACAGG	(GCT)6	60	253-333	32	16	0.625	0.908	0.000	0.902
MC54/ KX423606	F:TATTTTGACGAAGTGCTACCCC R:AAAGTAAGTGGACGAAGGTGGA	(AC)7	60	228-306	35	21	0.514	0.847	0.190	0.832

Locus/ Accession No.	Primer sequences (5'-3')	Repeat motif	Ta (°C)	Size (bp)	Samples	N_a	H_o	H_e	HWE	PIC
MC57/ KX423607	F:TGTCAGAGGCCAATAAAACAAC R:TTCGGGTTCCTAAACATTAACC	(ACAA)5	59	239-333	31	21	0.742	0.888	0.125	0.879
MC62/ KX423608	F:AATTCTCTTTCCATCAGGTCCA R:GTGTTTCTAGGGGTGATCTTGC	(TTC)6	59	253-315	35	13	0.571	0.809	0.417	0.784
MC63/ KX423609	F:CTGAGACGGTAATAGAAATGAATGG R:GGCCTCATCAAGGAACATAAAA	(TG)10	60	253-299	35	8	1.000	0.757	0.053	0.724
MC66/ KX423610	F:TGTTTACCCATTGCTGGTGA R:GACCATTGTGCTGACATTTCTT	(TA)7	59	242-264	35	7	0.314	0.518	0.166	0.489
MC68/ KX423611	F:GCTGAAGTCTGCGATAAAACCT R:ATGAAAGACACACTGGTTGTGG	(GA)8	59	195-299	35	11	0.857	0.691	0.244	0.666
MC74/ KX423612	F:CATGTCAGCCAATGTTAAAAGC R:AGCTGGGTCTATGTTATCGCTC	(AT)8	59	287-305	35	10	0.486	0.726	0.147	0.696
MC76/ KX423613	F:GGAAATCGCTGAATTGGTTATC R:GCATTTGAGAAAATAAGGGACG	(AT)7	60	260-318	35	15	1.000	0.894	0.518	0.885
MC90/ KX423614	F:GCACACACATCATACTTGCACA R:CAGAGGGTCCAAACCAAAATAC	(AC)6	60	257-303	35	11	0.514	0.804	0.119	0.779
MC99/ KX423615	F:ACAACTTAGAAAACAGGGGCAA R:GCTGTAGGTAGTATTTGGCATGAA	(AC)9	60	388-470	35	21	0.743	0.903	0.633	0.896
MC101*/ KX423616	F:CAAAGAATGGGAAGCAGTTTG R:ATTTGGTCAGTTGCGTTCATC	(TA)8	59	217-351	31	19	0.323	0.926	0.000	0.921
MC104/ KX423617	F:ATTACGCGCATGTGTCTATCAC R:TTACCGAAACTGCATGTCAACT	(TTAT)5	60	237-301	34	10	0.471	0.558	0.796	0.538

*Significant deviating from HWE (P<0.001).

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