Development and Characterization of 177 SNP Markers in The Mud Shrimp *Upogebia Major*

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Abstract

The mud shrimp *Upogebia major* (De Haan, 1841) (Crustacea, Decapoda, Gebiidea) is an important species widely distributed in coastal shallow waters. As an active initiation species, it has contributed significantly to the maintenance of coastal benthic biodiversity. However, there is still a lack of effective molecular markers to effectively protect and manage this species. In this study, we used the DNeasy Blood & Tissue kit to extract DNA and Illumina HiSeq 4000 sequencing technology to obtain SNP marker resources. 177 single nucleotide polymorphism (SNP) markers were developed and characterized in *U. major*. The minor allele frequency ranged from 0.0500 to 0.5000. The observed heterozygosity and expected heterozygosity ranged from 0.0333 to 0.9000 and from 0.0966 to 0.5085, respectively. Polymorphic information content ranged from 0.0905 to 0.3750. The inbreeding coefficient values varied from -0.2902 to 0.5968. Seventeen loci showed significant deviations from the Hardy-Weinberg equilibrium ($P < 0.05$). The polymorphic SNPs will be helpful for the further population genetic analysis and natural resource conservation of *U. major*.

Introduction

The mud shrimp *Upogebia major* (De Haan, 1841) is one of the largest upogebiids, which forms massive populations in the upper sublittoral zone at depths of 0.5-3 m in slightly silted sand of the Japanese Islands, the Korean Peninsula, and the Shandong Peninsula (Selin, 2017). Like other hole-dwelling animals, it is an active edicator and defines the qualitative composition and abundance of the bottom fauna (Kinoshita et al., 2007) (Ubaldo et al., 2013). Besides the important role in the structure of benthic community, the pharmaceutical value used by lactagogue was recorded in *Chinese Medicinal Animals*. This arouses a strong interest in the study of this species. However, there are few studies on the population genetic investigation of *U. major*. At present, only a few studies have been conducted on the complete mitochondrial genome of *U. major* (Sun and He, 2021). In this study, we mainly studied the single nucleotide polymorphism (SNPs) of the mud shrimp *U. major*. SNPs are the most convenient and fast DNA marker in genetic studies because of their wide genomic distribution and high level of polymorphism (Liu et al., 2019) (Qu et al., 2019). Therefore, SNPs were developed and characterized in *U. major* with the restriction-site associated DNA tags sequencing (RAD-seq), and will provide important genetic markers for the studies of population genetic diversity and conservation genetics.

Muscle tissue was collected from 30 *U. major* individuals from Yantai in Shandong province (37°27'N/121°30'E). Genomic DNA was extracted using the DNeasy Blood & Tissue kit (QIAGEN, Germany) according to the manufacturer's instructions. RAD library construction, sample indexing and pooling followed for the natural populations (Baird et al., 2008). To obtain SNP marker resources, pair-end (150-bp) sequencing was performed using Illumina HiSeq 4000 (Shanghai BIOZERON Co., Ltd), and a total of 719,728 putative SNPs in *U. major* was identified.

Using primer V3.0 to design primers, 175 primer pairs were successfully created. PCR reactions were performed in a 25 µl volume with GenStar PCR Mix according to the manufacturer's instructions (GeneStar, Beijing, China). The PCR amplification procedure was as follows: an initial denaturation at 94°C for 5 min; then 40 cycles of 94°C for 30 s, annealing for 30 s (for annealing temperatures of each primer pair, see Table 1), and 72°C for 30 s; and a final extension at 72°C for 7 min. Amplified samples were purified by gel extraction and sequenced on ABI 3730 DNA Analyzer (Applied Biosystems). For validated loci, statistics including the minor allele frequency (MAF), observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphism information content (PIC), inbreeding coefficient (F_{IS}) and P value representing the deviations from Hardy-Weinberg equilibrium (HWE) were calculated using Cervus 3.0 (Kalinowski et al., 2010).

One hundred and seventy-five primer pairs could be amplified, and 177 SNPs located within these sequences were confirmed by Sanger sequencing. The minor allele frequency ranged from 0.0500 to 0.5000 (Table 1). The observed heterozygosity and expected heterozygosity ranged from 0.0333 to 0.9000 and from 0.0966 to 0.5085, respectively. Polymorphic information content ranged from 0.0905 to 0.3750. The F_{IS} values varied from -0.2902 to 0.5968. Seventeen loci showed significant deviations from the HWE after Bonferroni correction ($P < 0.05$). These polymorphic SNP markers will be useful for further population genetic analysis, natural resource conservation and selective breeding of *U. major*.

Declarations

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Code availability: Not applicable.

Ethics approval: Not applicable.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

Author contributions: Corresponding author contributed to the study conception and design. Ying Qu, Yan Ni and Zilin Jiang helped collect samples. Donghui Sun and Jirui Zhang were in charge of extracting genomic DNA. Shukui Zhang, Zhikai Xing and Sen Wu helped collect the data. Chenghong Wang and Yunqi Song were in charge of compiling the data. The first draft of the manuscript was written by Chenghong Wang and all authors commented on previous versions of the manuscript. Corresponding author read and approved the final manuscript.

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Tables

Table 1

Primer ID	Primer sequence (5'–3')	Size(bp)	T _m (°C)	SNP type	SNP position	H ₀	H _E	F _{IS}	HWE P	PIC	MAF
2342_2	F: AGTCAGGCTGAAGTGATGAAACT R: CGATGCTGCACAGAGATCCT	309	60	A/C	73	0.4000	0.3254	-0.1097	NS	0.2688	0.2000
5675_2	F: ACAGGGAGACAAGCGGTTTC R: CGTGGCGAATGAAAGTAGCG	259	60	T/C	95	0.8667	0.4994	-0.2765	*	0.3705	0.4300
6217_1	F: ACATTTGTCGTCAGCTGGTG R: TGCCTTCATTACTTTGCCCA	294	59	C/A	91	0.8667	0.4994	-0.2765	*	0.3705	0.4300
7952_1	F: TTTCACAGATAAGCCTGGCT R: GCCAGCAATTTCCGCATCAT	338	60	G/T	68	0.7333	0.4723	-0.2244	NS	0.3566	0.3600
8984_2	F: GACTGTGTCCTGCTGATGTT R: CTGGGGATGTTCAGCCATGT	256	60	A/G	84	0.5000	0.3814	-0.1423	NS	0.3047	0.2500
9719_1	F: ACAGATGTCGATTCGCAGTGA R: AGCATAGTCGCTTTAGCCCC	282	60	A/G	67	0.8667	0.4994	-0.2765	*	0.3705	0.4300
9719_2	F: ACAGATGTCGATTCGCAGTGA R: TGTTCACCCACGAGCATAG	265	60	T/G	84	0.8667	0.4994	-0.2765	*	0.3705	0.4300
10117_2	F: ACGTGTTGTGTCGTCTGTGT R: AGTAGCAGACAGGAGGAGGG	292	60	T/A	71	0.3000	0.2593	-0.0778	NS	0.2225	0.1500
14865_1	F: TTTAATAGTATTGTGTAGAGGGAGG R: CGACGAGTATAGCGACCACC	277	60	G/A	56	0.2000	0.2825	0.1628	NS	0.2392	0.1600
18507_1	F: ACAGATGTCGATTCGCAGAGA R: ACGGGCTCTAAATGAGTGGA	319	59	G/T	109	0.7000	0.4627	-0.2120	NS	0.3515	0.3500
20936_1	F: ACGGATCAGTCAAGTCGTGT R: TTTGTGCCATCATGGGTGGA	312	60	G/A	64	0.1333	0.1266	-0.0258	NS	0.1167	0.0600
26102_1	F: CGGCTGCCGTATCAATAGC R: ACGCCTTGCTACGTAGTACC	305	60	C/A	104	0.8000	0.4881	-0.2499	NS	0.3648	0.4000
28472_1	F: ACTCAATAGACTACCCTCTCAAGG R: ACTTCCTTGTCGTGAAGCGT	316	60	C/T	63	0.9000	0.5034	-0.2902	**	0.3725	0.4500
35998_1	F: ACAAATGTCGATTCGCAGTGA R: TCGCGCAACCAACAGAAAAAG	307	60	C/A	88	0.9000	0.5034	-0.2902	**	0.3725	0.4500
37685_2	F: AGGTAAAGCGCAGACAAACG R: TTCCTCACATCAAGAGCCAA	245	59	C/A	78	0.6333	0.4401	-0.1879	NS	0.3391	0.3100
43946_1	F: GTCTGTCAGACCCACGGTG R: AGCCTGTAAAGTCAGTGACAGA	282	60	C/A	40	0.1333	0.1266	-0.0258	NS	0.1167	0.0600
63249_1	F: TGTGCCCAATATAACAGTGACAG R: GGAGGCTGTGTAACGTGGG	282	60	A/C	71	0.9000	0.5034	-0.2902	**	0.3725	0.4500

T_m annealing temperature, H₀ observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient, HWEP results for Hardy–Weinberg Equilibrium test, PIC polymorphism information content, MAF minor allele frequency, NS non-significant

*P < 0.05; **P < 0.01

Primer ID	Primer sequence (5'–3')	Size(bp)	T _m (°C)	SNP type	SNP position	H ₀	H _E	F _{IS}	HWE P	PIC	MAF
96754_1	F: TCCATCTGTGGTTGTGTAGCT R: GTTTCCTCGCGCTATGCATG	356	60	C/G	72	0.9000	0.5034	-0.2902	**	0.3725	0.450
109215_1	F: ACAGATGTCGATTCGCGAGTGA R: GGACCCAACCCGGTATGAAA	283	60	G/A	82	0.8667	0.5062	-0.2703	*	0.3739	0.466
109744_1	F: AATTCTGTGGTATTAGCTGT R: TCAACCACCCTTGGATGAGC	273	60	C/A	71	0.1667	0.1554	-0.0358	NS	0.1411	0.083
112887_2	F: AGAACACTAACCTCAACAATTGGT R: TCCAAGCTTCCATTCTGTTGGG	333	60	A/G	87	0.9000	0.5034	-0.2902	**	0.3725	0.450
124474_2	F: TCTTTTGCCTTATTTACATTGT R: ATCATGCCTTAGCTTGCACT	293	58	C/T	47	0.8667	0.4994	-0.2765	*	0.3705	0.433
126155_1	F: GGACAGCAGTCTCTTTAGTACT R: TGCTTGTCTGTGAGTAGCCTG	287	60	A/G	100	0.0667	0.1266	0.2931	NS	0.1167	0.066
133592_1	F: GCAATGAAACGCACCCAGAA R: CCAGGAATGCGGTGGTTTTTC	244	60	A/C	49	0.0667	0.1831	0.4489	NS	0.1638	0.100
134738_1	F: TGAACATATGTCGATTCGCGAGTG R: TAACCCCTTTCCAACCTGGCG	283	60	A/G	86	0.1000	0.2096	0.3445	NS	0.1849	0.116
135002_1	F: ACTGTACAAGCTAAGAAAAACACCG R: TCTGATGTACTTTTGTGTGCTGT	330	60	G/A	65	0.1000	0.0966	-0.0164	NS	0.0905	0.050
135764_1	F: ACGCAAAGTTGGCCAAATC R: TTGCGCACTGTTTTGTGCGAG	313	60	C/T	67	0.8000	0.4881	-0.2499	NS	0.3648	0.400
135891_1	F: TTCAAATGAGCTACAGGTGAGA R: GCCATGAATAGTCCCGCCAT	294	60	C/T	88	0.1333	0.1266	-0.0258	NS	0.1167	0.066
135903_1	F: ATGACGAGACACAAACCCCC R: GGTGCTAGGTAGTGGATTGGG	331	60	T/C	106	0.1667	0.1554	-0.0358	NS	0.1411	0.083
136266_1	F: TTACCTGATTCAATTTAAAGAATGGT R: TGGAAACAGCCTGGCAATTC	295	59	A/T	81	0.8667	0.5062	-0.2703	*	0.3739	0.466
136602_1	F: ATTCTGCTGGCTCGGGTAAC R: ATCCCGCTATTTGCCCAACA	332	60	T/A	69	0.1000	0.1554	0.2076	NS	0.1411	0.083
137241_1	F: CCCATGAAAGAGGGCGAACT R: TGTCTCTACTGCTCTGACCT	340	60	A/T	43	0.1667	0.2096	0.1058	NS	0.1849	0.116
137471_1	F: TTCCTCACTTCTGGGTCCGA R: TTGGTAATTGCACGTGTTGT	316	60	G/A	106	0.1000	0.1554	0.2076	NS	0.1411	0.083
137488_1	F: CGTTGTTTTATTCCGCTTTTCG R: CTCCTTCTCTGCCTCCTCCT	352	60	T/C	50	0.1667	0.1554	-0.0358	NS	0.1411	0.083
137562_1	F: TGTCGATTGGCAGTGAAAACT R: GTTGCTCCTCCTCCACCATC	316	60	T/C	107	0.1000	0.0966	-0.0164	NS	0.0905	0.050

T_m annealing temperature, H₀ observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient, HWE P results for Hardy–Weinberg Equilibrium test, PIC polymorphism information content, MAF minor allele frequency, NS non-significant

*P < 0.05; **P < 0.01

Primer ID	Primer sequence (5'–3')	Size(bp)	T _m (°C)	SNP type	SNP position	H ₀	H _E	F _{IS}	HWE P	PIC	MAF
137566_1	F: TTCTCTGGCTTTGATGGTTT R: CTCTCGTATCCGACTGCACC	342	60	G/A	90	0.1667	0.1554	-0.0358	NS	0.1411	0.083
137731_1	F: TCGAATCCTACCTGTCCAGA R: GCTCATCAGGTAGGCCAAGG	340	60	T/C	61	0.1000	0.0966	-0.0164	NS	0.0905	0.050
137790_1	F: TGA CTGAGATGACCCAACTCA R: CACGAAGGGGCGTCATTTTC	274	60	T/C	86	0.1000	0.1554	0.2076	NS	0.1411	0.083
137803_1	F: GTGTCTGTGCTAGTGGGAGG R: CACACACACACACACACA	274	60	T/A	99	0.1000	0.0966	-0.0164	NS	0.0905	0.050
137820_1	F: AATCACATCTGGCCTCTGAT R: ACGGCTCTGCATAACAAACG	303	59	T/G	89	0.1000	0.0966	-0.0164	NS	0.0905	0.050
138017_1	F: ACCTTCACAATTTACGTTAATCGACA R: CCCGTATCAGTAACCCTGGC	326	60	A/T	92	0.1000	0.0966	-0.0164	NS	0.0905	0.050
138166_1	F: ACGGAAGTGTATCGTTGTTGT R: AGGTGAGGGATGAAGACTTGG	260	59	A/C	59	0.1333	0.1266	-0.0258	NS	0.1167	0.066
138172_1	F: TGAACAGATGTCGATTCACAGTG R: CAAAAATCCCTCAGCCCGC	343	60	T/A	66	0.1333	0.1266	-0.0258	NS	0.1167	0.066
138186_1	F: TGAACAGATGTAGATTCGAGTGA R: GCAAGGTCTGTGTGTCCTGA	328	60	A/T	54	0.9000	0.5034	-0.2902	**	0.3725	0.450
138329_1	F: TTGTGCCCTCTGGTCACTC R: GCTGCAAGCAGTAAACACCA	281	60	T/C	43	0.1333	0.1266	-0.0258	NS	0.1167	0.066
138496_2	F: GGTGGCGTAAGTACTTCAAGC R: TGGGTAATGTTACCTCGGC	310	60	C/A	81	0.1333	0.1266	-0.0258	NS	0.1167	0.066
138539_1	F: TGAACATATGTCGATTGCAAGTGA R: CCTGTACCAATTTTGCGCC	336	60	G/A	98	0.1333	0.1266	-0.0258	NS	0.1167	0.066
138849_1	F: CCCGCTGCTTTTCCCTTTT R: ACACTTCGTTGTCAGCCTGT	273	60	T/C	94	0.1333	0.1831	0.1488	NS	0.1638	0.100
139132_2	F: CATGAGCGAACAATGCCTCG R: TGACTGGCAACCTCTCGTTC	326	60	A/G	79	0.3333	0.2825	-0.0884	NS	0.2392	0.166
139146_1	F: TGACAAGTTGGAATTTTGTGAGT R: AGCATGAGACAGATTGCGGG	335	60	A/G	60	0.2333	0.2096	-0.0567	NS	0.1849	0.116
139166_1	F: TCGCAATTTGCTTATGTATCGGA R: TGGTGGTAAGTAGTCTTCATAATCAGG	254	60	A/C	83	0.1667	0.1554	-0.0358	NS	0.1411	0.083
139234_1	F: TCTCCAATTTATGTTTTACTCGCT R: CCACTGAACCAAGGGACGAT	321	60	T/C	102	0.1667	0.1554	-0.0358	NS	0.1411	0.083
139453_1	F: GCTTGGTTTTACGTTGCAAATGG R: AGTCTATGGTGGATCACTCTGC	333	60	T/C	86	0.1667	0.2096	0.1058	NS	0.1849	0.116
139485_1	F: TCGTAGCAAGATTTATAATACGGTGA R: TCTCTGAGGGCTGTAAGGCT	333	60	G/A	77	0.1667	0.1554	-0.0358	NS	0.1411	0.083
139529_1	F: TGGCTTATGCAGAAAGGCCA R: TGCACTGCATCGTTTCTTGC	320	60	T/G	105	0.1667	0.1554	-0.0358	NS	0.1411	0.083

T_m annealing temperature, H₀ observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient, HWE P results for Hardy–Weinberg Equilibrium test, PIC polymorphism information content, MAF minor allele frequency, NS non-significant

*P < 0.05; **P < 0.01

Primer ID	Primer sequence (5'–3')	Size(bp)	T _m (°C)	SNP type	SNP position	H ₀	H _E	F _{IS}	HWE P	PIC	MAF
139593_1	F: TGGATTGAAATTTTTCATTATCGAGG R: CTGCGTCAAGTGGGGGTTAT	294	60	G/T	69	0.2667	0.2350	-0.0672	NS	0.2044	0.133
139796_1	F: ACAGATGTCGATTCGCAGTGA R: ATGCATACAGACAGACAAACA	270	60	G/C	98	0.2000	0.2825	0.1628	NS	0.2392	0.166
139863_1	F: TCAACGCAAAGATGGTCACA R: AACGGTGCCACCTATGAAAA	313	58	G/T	37	0.2000	0.2350	0.0722	NS	0.2044	0.133
139883_1	F: AACCACGCAGGCACCTTTAT R: TTTCTGCTTCCCCTCCTCCT	256	60	A/G	92	0.2000	0.2350	0.0722	NS	0.2044	0.133
139892_1	F: TGTTAGCGAATTTGGGGCCT R: GCAGTTCGGAAGAGAGGGAG	299	60	G/C	99	0.2667	0.2350	-0.0672	NS	0.2044	0.133
139977_1	F: GACAGGCAGGAAGGAAGGAC R: CCCGAACCTTTTCTGCCTCT	288	60	T/C	66	0.2667	0.3254	0.0909	NS	0.2688	0.200
140068_1	F: ACAGGCATTTCCCACTATCCA R: ACCACTGCTCTACGTAGACCA	329	60	T/C	55	0.2667	0.3254	0.0909	NS	0.2688	0.200
140071_1	F: TCTTCCTTCTCTTCTTCTTCA R: CTCCTAATTCCGCCTCCACC	329	60	C/T	29	0.4667	0.5062	0.0323	NS	0.3739	0.466
140071_2	F: TCTTCCTTCTCTTCTTCTTCA R: CTCCTAATTCCGCCTCCACC	329	60	G/A	64	0.4667	0.5062	0.0323	NS	0.3739	0.466
140180_1	F: TTCGTGATACAGTCTTCTGAAA R: ACCAACAGTCTTTGCAACGC	308	60	C/T	77	0.2000	0.1831	-0.0462	NS	0.1638	0.100
140308_1	F: TCCTATGTATTCCCTGCATGTCG R: TGCTCCCATCCACAGTGAAC	337	60	C/T	92	0.2667	0.2350	-0.0672	NS	0.2044	0.133
140586_2	F: CTCTCTAAGCGGCGTTAGGG R: CGGAAAGAGTGTTGGTGGGA	314	60	C/T	65	0.2333	0.2593	0.0444	NS	0.2225	0.150
140778_1	F: TTCTTCCTGTTTTGACGCGC R: GTGACTGTTAGTGAGTTGCCG	337	60	G/A	99	0.2333	0.3045	0.1241	NS	0.2546	0.183
140821_1	F: AAATTCAAAGTAGAATAACATGGTCT R: TCGATCACTTCCTCCAGGGT	319	60	G/A	89	0.3000	0.4130	0.1503	NS	0.3236	0.283
140914_1	F: GAGGAGCAGCAGATTCACAC R: GGGACGTTTTGACAGTGGGA	334	60	G/T	79	0.7333	0.4723	-0.2244	NS	0.3566	0.366
140956_1	F: ATTCGCCACAACAAACGGTG R: TGTCCCATGGCTGATTGTCC	306	60	G/A	33	0.2667	0.3638	0.1459	NS	0.2938	0.233
140959_1	F: AGGGAGAGAATAAGTCTGTCA R: CGTTACAAGGAGAGTCCGCA	343	60	A/G	62	0.4000	0.4271	0.0244	NS	0.3318	0.300
140963_1	F: ACAGATGTCGATTCGCAGTGA R: TCTACTCCACAAAGCCTCCC	321	60	G/A	67	0.2667	0.2350	-0.0672	NS	0.2044	0.133
140990_1	F: AACAGTTTCGGCAGCTTCCT R: CATCTGTGTCAGGGCTCGAG	343	60	G/A	52	0.4000	0.3254	-0.1097	NS	0.2688	0.200
141091_1	F: CTGTGAACAGCAGTCGACCT R: AACTGGATGGTGACTGCTGG	333	60	G/A	95	0.2667	0.3977	0.1892	NS	0.3146	0.266

T_m annealing temperature, H₀ observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient, HWE P results for Hardy–Weinberg Equilibrium test, PIC polymorphism information content, MAF minor allele frequency, NS non-significant

*P < 0.05; **P < 0.01

Primer ID	Primer sequence (5'–3')	Size(bp)	T _m (°C)	SNP type	SNP position	H ₀	H _E	F _{IS}	HWE P	PIC	MAF
141156_1	F: ACCCAAGACTGAAGACCGAAC R: TGTTTCGTCCTTTTGGGGTGT	260	60	C/T	78	0.5333	0.3977	-0.1534	NS	0.3146	0.266
141181_1	F: GAGGAAAAGAGCTGCAGTAGGA R: TCATAGCTCCACTGCCATGG	259	60	A/C	80	0.3667	0.4130	0.0510	NS	0.3236	0.283
141183_1	F: TCACACTTTCTTCCTCTTTTCA R: ACCCAACCCCATCTTGTGTTGT	330	60	C/T	56	0.3000	0.2593	-0.0778	NS	0.2225	0.150
141487_1	F: TCCGAAAGTCATTAAATTTCCATGA R: TCATCTCGACGTGAGCTTCG	346	60	G/A	105	0.4000	0.3254	-0.1097	NS	0.2688	0.200
141545_1	F: TCGGTATTTTGAAGCGAACT R: AATCGACCGGAACTGGTCT	297	59	C/T	32	0.4000	0.3977	-0.0112	NS	0.3146	0.266
141565_1	F: TCCTGTATTCTTCGTGTGCA R: ATGGAATAAAATACTAAGATTGCAT	338	59	A/G	90	0.4000	0.4881	0.0909	NS	0.3648	0.400
141652_1	F: TCGAGCGTTGCACAATATTGA R: GGGTAACTGTGGAAGGGCT	338	60	G/C	108	0.3667	0.4627	0.1075	NS	0.3515	0.350
141762_2	F: TTCGCACTGAAAAAGGTGT R: GGACAGTGGGTGACTTTGA	288	61	A/G	80	0.9000	0.5079	-0.2862	*	0.3747	0.483
141795_1	F: ACAGATGTCGATTCGCAGTGA R: CACCCGCCATTCAATGCTTC	337	60	T/G	93	0.9000	0.5034	-0.2902	**	0.3725	0.450
141832_1	F: GTTCTCTTGGGAGCGAGACG R: CCTTCTCGCCTCCTCTCAAC	317	60	C/T	72	0.6000	0.4271	-0.1762	NS	0.3318	0.300
141972_2	F: CAAGGACATACACGGGCACT R: TACTTGCCCTCGTTCCAGTG	332	60	G/A	106	0.7000	0.4627	-0.2120	NS	0.3515	0.350
141991_1	F: AACAGACGTCGATTTCGCAGT R: GGGTCACCAAAAACGTGAAGG	281	60	A/C	43	0.5000	0.4944	-0.0141	NS	0.3680	0.416
142068_1	F: GTTGAAACGGAGGATGTAATTCGA R: CCAACGATGAAGTGAGCAGC	340	60	C/T	69	0.5667	0.4627	-0.1093	NS	0.3515	0.350
142089_1	F: TTTTCCTAACTAGTAAACGCCT R: CGCATGGAAGCAAAGCTCTC	277	60	G/C	54	0.7667	0.4944	-0.2239	NS	0.3680	0.416
142234_1	F: ACAGATGTCGATTCGCAGTGA R: CACCACAAAACCGAGGATGG	293	60	C/T	108	0.8667	0.5085	-0.2683	*	0.3750	0.500
142735_1	F: TGTGGGATCCATGATGCAGG R: CCCACCTGTTTGTTCCCACT	339	60	C/T	80	0.0667	0.1266	0.2931	NS	0.1167	0.066
151278_1	F: TCAAAGGGTAAAAGTGTCATGT R: GGTCTTGCAAGGCTGTTTG	284	60	T/C	91	0.4000	0.3254	-0.1097	NS	0.2688	0.200
159979_2	F: TCAGCTGGTCCGTTGGTTTT R: ATGGTGCCACAAAACAGTGT	296	60	C/T	69	0.1000	0.0966	-0.0164	NS	0.0905	0.050
196855_1	F: TCATTTGGTGTGGATACTGATCT R: GGACACCATTTCAAAGTGAGTG	344	60	C/T	71	0.7667	0.4808	-0.2370	NS	0.3610	0.383
196932_2	F: CGGTGTCCTTCTCTGAGTGG R: TTTGGAGCAACACCCCGATT	287	60	C/T	45	0.2000	0.1831	-0.0462	NS	0.1638	0.100

T_m annealing temperature, H₀ observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient, HWE P results for Hardy–Weinberg Equilibrium test, PIC polymorphism information content, MAF minor allele frequency, NS non-significant

*P < 0.05; **P < 0.01

Primer ID	Primer sequence (5'–3')	Size(bp)	T _m (°C)	SNP type	SNP position	H ₀	H _E	F _{IS}	HWE P	PIC	MAF
197553_1	F: TGGGACTTTACATGTGCGTC R: GGACACTGTACGCTACCGG	265	60	C/T	63	0.1333	0.2825	0.3510	NS	0.2392	0.166
201056_2	F: GTACCCAGGTGCCAGTTC R: ACACCACACCACACAACACT	321	60	A/T	72	0.0333	0.1554	0.5968	NS	0.1411	0.083
205020_2	F: ATGCAGACATGGGCGAGAAA R: AGGAAGTATCAACAGCAACTCCA	286	60	C/T	53	0.1000	0.0966	-0.0164	NS	0.0905	0.050
205068_2	F: TCAGTGGTTGATTGTAGCCAGT R: GGCTACGAGTCGATGTCACT	286	60	C/T	52	0.1000	0.0966	-0.0164	NS	0.0905	0.050
205190_2	F: GTGCGTCAAAATCCTGCCAA R: CTCTCAGTCAGCCCCGTAGA	280	60	C/T	47	0.1333	0.1266	-0.0258	NS	0.1167	0.066
205281_2	F: GTAAGCTGGGGAGGTGTACG R: CTCTCCTCCCTCCTCTTGCT	329	60	C/A	84	0.1333	0.1266	-0.0258	NS	0.1167	0.066
205522_2	F: ATTCACAGCGACCTTCCTGG R: CACTGCCACAGTTGTGTTGG	317	60	A/G	82	0.1333	0.1831	0.1488	NS	0.1638	0.100
205570_2	F: TGTTCAGCGATTTCGTCTGCA R: TCCTGGAACACACAATTTTGTCA	257	60	C/T	64	0.1000	0.1554	0.2076	NS	0.1411	0.083
205723_2	F: GTACAAAGCCTCAACACACCG R: CCCACCATCTACACCAGTGG	289	60	A/G	81	0.0667	0.1266	0.2931	NS	0.1167	0.066
206029_1	F: AATGACCCGACTCAACGTCT R: CCTCACCTTGCAACACTGGT	330	60	C/T	51	0.1333	0.1266	-0.0258	NS	0.1167	0.066
206073_2	F: TCCATCGTCCAGGTAGACTGT R: CCGCCAATAACTGCAAAGGG	352	60	G/A	78	0.1000	0.0966	-0.0164	NS	0.0905	0.050
206126_2	F: ACATGGTTCAGAAAGTTGAAGA R: CAACTGTGCCACAAATGCGA	305	60	A/T	99	0.1333	0.1266	-0.0258	NS	0.1167	0.066
206423_2	F: AGCCACACACGATAAGACCC R: GCCCTTACTCACACTCCCAC	332	60	C/A	42	0.1333	0.1266	-0.0258	NS	0.1167	0.066
206630_1	F: TGTATAAAGTACTTTCTGCAGAGT R: GAGTATGTAACGCCCGCTCA	315	60	C/T	45	0.1667	0.1554	-0.0358	NS	0.1411	0.083
206677_2	F: TGAACAGATGTCGATTACAGTG R: TGCAGCAATTTATAAACACCATTC	341	59	G/A	90	0.2667	0.2350	-0.0672	NS	0.2044	0.133
207162_1	F: TCCCTGCGTGTTATAAGAGCT R: TCCCTCAACTAGGTATGATTTCCT	259	59	G/A	59	0.1000	0.0966	-0.0164	NS	0.0905	0.050
207365_2	F: AGATGCTCATTTGGCTTGGC R: AGCTGTTGGACGGTAGGATT	264	59	T/C	85	0.1000	0.0966	-0.0164	NS	0.0905	0.050
207425_2	F: TCACGGGAAATACATTGAGCA R: GTCTGTGCTTGCAACTCGTC	256	60	C/G	102	0.1000	0.0966	-0.0164	NS	0.0905	0.050
207490_2	F: TATCATGGTGCTTGGGTGGC R: TCTGCATCAAGTGTGTGCCA	267	60	A/G	110	0.1000	0.1554	0.2076	NS	0.1411	0.083
207539_2	F: GCTTCCTCCAGAGTCGCATC R: TCCGTGCGCAATCAGATAGC	291	61	A/G	94	0.1000	0.1554	0.2076	NS	0.1411	0.083

T_m annealing temperature, H₀ observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient, HWE P results for Hardy–Weinberg Equilibrium test, PIC polymorphism information content, MAF minor allele frequency, NS non-significant

*P < 0.05; **P < 0.01

Primer ID	Primer sequence (5'–3')	Size(bp)	T _m (°C)	SNP type	SNP position	H ₀	H _E	F _{IS}	HWE P	PIC	MAF
207554_1	F: GGAACGTGCAGATTTCCAGC R: GTGGTGCCACATCTCTTCCA	291	60	A/G	57	0.2000	0.1831	-0.0462	NS	0.1638	0.1000
207650_1	F: TCCCACATATCCACTGCGTG R: GGCAAGCAACTGTCCCATA	318	60	T/C	84	0.1333	0.1266	-0.0258	NS	0.1167	0.0667
207684_1	F: TCTTCACAATTTTACCTTCGCA R: AAAGCTGTGACAAATGGCCG	349	60	C/T	49	0.1000	0.1554	0.2076	NS	0.1411	0.0833
207728_2	F: TCATCGTGCAGTTCTTACAGT R: AAACGCCTATGTTCTGTGGGT	317	60	C/A	52	0.1333	0.1266	-0.0258	NS	0.1167	0.0667
207730_1	F: ACAGATGTCAATTCGCAAGTGA R: ACAAACGGCGGAAATGTGTG	334	60	C/A	63	0.1000	0.0966	-0.0164	NS	0.0905	0.0500
208318_2	F: AGGGCTAGAGTTATAGAGGGCT R: GAATGAAGGAGAGGCTGGGC	316	60	C/T	47	0.1000	0.1554	0.2076	NS	0.1411	0.0833
208453_2	F: ATTCATTACATGCGGGGGCA R: TTGAATGTCAACTTCGCCGC	289	60	C/A	54	0.1333	0.1266	-0.0258	NS	0.1167	0.0667
208568_2	F: ATTCTGCCTCTGTACACCG R: TCCATTATCATCACTTGTGAGCT	276	60	C/G	66	0.2333	0.2096	-0.0567	NS	0.1849	0.1167
208577_1	F: ACTATTCATAGCCATAAACACACA R: CCACAAATCAGTGTATGGTGGC	321	60	C/T	44	0.1333	0.1831	0.1488	NS	0.1638	0.1000
208717_2	F: ACTGATGAAGACGGGTATCGC R: CACTGAATGTTGTGGGTGCG	303	60	C/A	82	0.1333	0.1831	0.1488	NS	0.1638	0.1000
208822_2	F: ACGTAGACAGACACAAACGA R: CCCTCCAACCTTTCCCATCG	278	60	T/G	52	0.2000	0.1831	-0.0462	NS	0.1638	0.1000
208828_2	F: AATTCATCGAGGCGCTGGAT R: TGGCCGATTCCACATACGTT	340	60	C/T	108	0.1333	0.1266	-0.0258	NS	0.1167	0.0667
209004_2	F: TGAACAGATGTCGATTGGTAGTGA R: TCTGCTTACACCAGCTTGCA	291	60	A/C	102	0.1000	0.0966	-0.0164	NS	0.0905	0.0500
209120_2	F: ACACTACACTGACAGAGGTGTG R: TGTCAAACCTATGCACCGCT	328	60	A/G	82	0.2000	0.1831	-0.0462	NS	0.1638	0.1000
209159_2	F: AGATGTCGATTTCGAGTGAA R: CGCAGAGGACAGACGAACTC	268	60	T/C	56	0.1333	0.1266	-0.0258	NS	0.1167	0.0667
209249_1	F: ATGTCAGGTTTTCTCTCCCT R: CGAATCCTCATAGGCCTGCC	325	60	G/A	89	0.1667	0.2096	0.1058	NS	0.1849	0.1167
209286_2	F: ACACTCTTTTGAAATGTTGTGCA R: CCCCAGGTCTCCTTCTCTA	335	60	C/T	62	0.1333	0.1266	-0.0258	NS	0.1167	0.0667
210043_2	F: ACACCTTTTCGAGTCTTCACCA R: TGCTCCTCATAAACACTGTGT	306	60	T/A	72	0.1000	0.0966	-0.0164	NS	0.0905	0.0500
210089_2	F: GCCACTTTCATTTAGCCCC R: TTGCAGCAAAAGCTCACACA	326	60	C/T	65	0.1667	0.2096	0.1058	NS	0.1849	0.1167
210100_2	F: TCACAACTGCACTGTAATGTGT R: AGGAGATGGACCAGGAGAGA	329	59	A/G	92	0.2333	0.2593	0.0444	NS	0.2225	0.1500

T_m annealing temperature, H₀ observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient, HWE P results for Hardy–Weinberg Equilibrium test, PIC polymorphism information content, MAF minor allele frequency, NS non-significant

*P < 0.05; **P < 0.01

Primer ID	Primer sequence (5'–3')	Size(bp)	T _m (°C)	SNP type	SNP position	H ₀	H _E	F _{IS}	HWE P	PIC	MAF
210197_1	F: TTCAAGATTTTGTAAAGCAATTTAGAAT R: ACATGTGGAAGTGAAGCGG	349	59	C/A	97	0.1667	0.1554	-0.0358	NS	0.1411	0.083
210217_2	F: GTGTAGCGAATTTGGGCC R: ACAGATGTCGATTCGCAGTGA	341	60	T/A	78	0.1667	0.1554	-0.0358	NS	0.1411	0.083
210222_1	F: ACCCCAAAGAGCAAAACCA R: GCCACAAGCAACAGTGGTTT	336	60	A/G	97	0.1333	0.1831	0.1488	NS	0.1638	0.100
210380_1	F: TGGTCACTCTAGGTCTGCCA R: TGGCAATGTGAATACCGTGT	274	60	A/G	99	0.1667	0.2593	0.2094	NS	0.2225	0.150
210447_1	F: ACCTTGC GTTCCATACCGAG R: CTGTCTGGCTGGACTGTCTG	335	60	C/T	71	0.1667	0.1554	-0.0358	NS	0.1411	0.083
210590_2	F: ACTTTAACCTTTACGCCTGA R: AGCAGGCGTTTGACTTCTGG	247	61	G/C	105	0.1667	0.1554	-0.0358	NS	0.1411	0.083
210908_1	F: AACGCAAATAACATAGTTAAGCACA R: ACAACAGAATACCCGAGTCAGT	266	59	G/A	64	0.1667	0.4130	0.4180	NS	0.3236	0.283
210949_2	F: TCCACATAAAGAAAATCGACGT R: AAGTCTGGGTGTCTCGGTCA	334	60	G/A	55	0.1333	0.1266	-0.0258	NS	0.1167	0.066
210957_2	F: ACCACTGATGTGTAGGTGT R: GAGCGGGTGATATTCGCCT	348	60	C/T	47	0.1667	0.1554	-0.0358	NS	0.1411	0.083
211154_1	F: ACAGATGTCGATTGGCAGTGA R: ACACCTGCTGATCCTTGCAA	304	60	G/T	49	0.2000	0.2825	0.1628	NS	0.2392	0.166
211179_2	F: ATTGGGGGTTGCAGTTCTTT R: AGCAAAATTGTTCTTTGATGCCA	292	58	G/A	73	0.1667	0.2593	0.2094	NS	0.2225	0.150
211219_2	F: TGAACAGATATCGATTGCGAGTG R: GGAAGACGGACAATGGAGCA	281	60	T/C	59	0.2333	0.3045	0.1241	NS	0.2546	0.183
211287_2	F: TGCCAGAAAACAATTCCTGCA R: AAGAGCTGTGTTCCGTCTGT	283	59	T/C	103	0.2000	0.1831	-0.0462	NS	0.1638	0.100
211328_2	F: ATGAACCACGCAGACACCTT R: AAACCATACGTCCTGCAGGG	315	60	C/G	62	0.1333	0.1266	-0.0258	NS	0.1167	0.066
212467_2	F: TCGTCAACTGACTGATAATGCT R: TCGAACTCCCTCCAGACAGA	337	60	A/G	97	0.3667	0.5079	0.1533	NS	0.3747	0.483
212480_1	F: AGAGCCAGAACTAACAATAAGCA R: TTCCCCGTTATCCAACCACC	324	60	A/T	71	0.2667	0.2350	-0.0672	NS	0.2044	0.133
212580_2	F: ACGAGAAAAACAAGAACTGGG R: GGGTGAAGGTCAACAATGAGG	347	60	G/C	82	0.2333	0.2593	0.0444	NS	0.2225	0.150
212695_1	F: ACAGATGTCGATTCGCAGTGA R: AGCTAGCCATGACTTCCTCA	281	60	A/T	55	0.2333	0.3045	0.1241	NS	0.2546	0.183
212715_1	F: AGGAAACAAGTCAATGCCAAGT R: TGTGATTCAACAAGTTTACCGTGT	271	59	C/T	58	0.2333	0.3045	0.1241	NS	0.2546	0.183
212784_1	F: ACAGATGTCGAATCGCAGTGA R: ACAACTCGGGTCACCAACAG	343	60	A/G	34	0.3667	0.3045	-0.0990	NS	0.2546	0.183

T_m annealing temperature, H₀ observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient, HWE P results for Hardy–Weinberg Equilibrium test, PIC polymorphism information content, MAF minor allele frequency, NS non-significant

*P < 0.05; **P < 0.01

Primer ID	Primer sequence (5'–3')	Size(bp)	T _m (°C)	SNP type	SNP position	H ₀	H _E	F _{IS}	HWE P	PIC	MAF
213359_1	F: TCCCTTGCCCAACTGTCTA R: TTACCGTATGTGGGGCTGTG	279	60	G/A	44	0.3667	0.3814	0.0112	NS	0.3047	0.250
213359_2	F: TCCCTTGCCCAACTGTCTA R: ACAGGTGCTTGTCTCAGGTACC	312	60	A/G	79	0.4000	0.4994	0.1022	NS	0.3705	0.433
213445_1	F: TCACCCAGACAATTTCTAACAGTCA R: GCGATCCAGTAGTCCTCAGC	316	60	A/C	28	0.3667	0.4401	0.0827	NS	0.3391	0.316
213445_2	F: TCACCCAGACAATTTCTAACAGTCA R: GCGATCCAGTAGTCCTCAGC	316	60	A/T	91	0.3333	0.3638	0.0354	NS	0.2938	0.233
213458_2	F: ACAGATGTCGATTCGCGAGTGA R: TCTCATTTGTTGTGCTGCGC	301	60	C/G	88	0.3667	0.4401	0.0827	NS	0.3391	0.316
213469_1	F: CGGTGATGTTCAGAAATTGGCA R: ACATCATAACTGAGGCGCCC	317	60	A/G	73	0.9000	0.5034	-0.2902	**	0.3725	0.450
213475_2	F: ACGACTCCATTCTGTTACCT R: CTCAGGAGTGAAAACAATCCGG	310	59	T/G	99	0.3000	0.2593	-0.0778	NS	0.2225	0.150
213600_1	F: ACAGATGTCGATTCGCGAGTGA R: AGGTCTCATTGCGTGTGAAAG	268	60	A/T	97	0.5333	0.3977	-0.1534	NS	0.3146	0.266
214019_1	F: TAAGAGTTGCGCCATGTCCA R: AGATCACGTCAAGGGGAACA	259	60	G/A	57	0.4000	0.3254	-0.1097	NS	0.2688	0.200
214038_2	F: TAACCCCTTTCCAACCTGGCG R: ACCTGTAACAAAATCCAGAGTT	301	60	T/G	91	0.2000	0.1831	-0.0462	NS	0.1638	0.100
214045_1	F: ACAAAGACTTTCATGTTAGAGGT R: TCGTCTTCTCAAATGGGGGC	270	60	T/C	43	0.2000	0.2350	0.0722	NS	0.2044	0.133
214045_2	F: ACAAAGACTTTCATGTTAGAGGT R: TCTCAAATGGGGGCCGTAAC	302	60	C/T	52	0.4667	0.4881	0.0141	NS	0.3648	0.400
214185_1	F: TCGTCTTGGGAGGAATTGGG R: GCAGTCTTCTTTCGCCTCCT	303	60	A/G	90	0.4333	0.5079	0.0709	NS	0.3747	0.483
214226_2	F: CACCGTAGTGACAGCCTGC R: ACTAACTGCCAACCTCTGC	315	61	C/G	72	0.7333	0.4881	-0.2088	NS	0.3648	0.400
214260_2	F: AAGTCCGCTACTGAACCCCT R: TCGTGTCCAAGGGAGAAGGA	319	61	A/G	76	0.2000	0.1831	-0.0462	NS	0.1638	0.100
214302_2	F: TCAACAACCTTCACTACATCTGT R: GGCTAGACGTGAAGGTGACC	280	60	A/T	86	0.4667	0.4994	0.0255	NS	0.3705	0.433
214321_1	F: CCAGGAGAGTGAAAGGCGTA R: CTTGGGCAACAACCTCACCT	298	60	A/G	45	0.5000	0.4808	-0.0280	NS	0.3610	0.383
214327_2	F: TGAGTTTGTGGTTTTCGGTG R: ATCATGCTGAGAGGCAGTGG	328	60	T/C	57	0.7667	0.5079	-0.2111	NS	0.3747	0.483
214342_2	F: AGTCGCATGAGATGGTCTGC R: CGCAAGGTCTGTCTCTGAT	272	60	C/T	72	0.1000	0.0966	-0.0164	NS	0.0905	0.050
214354_2	F: TTCAAAATAGGGAAAAAGTAAAACAC R: GCGCGCTACCAACTGTACTA	282	60	A/G	74	0.5333	0.4723	-0.0690	NS	0.3566	0.366

T_m annealing temperature, H₀ observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient, HWE P results for Hardy–Weinberg Equilibrium test, PIC polymorphism information content, MAF minor allele frequency, NS non-significant

*P < 0.05; **P < 0.01

Primer ID	Primer sequence (5'–3')	Size(bp)	T _m (°C)	SNP type	SNP position	H _O	H _E	F _{IS}	HWEP	PIC	MAF
215686_1	F: ACAGATGTCGATTCGCAGTGA R: CCCGTCCCAAGAGTCTGTTG	331	60	A/C	55	0.3667	0.3814	0.0112	NS	0.3047	0.250
215719_2	F: TGAACAGATGTCAATTCGCAGTG R: TACAAACAGTTTCCCCGCA	344	60	G/C	79	0.2333	0.2441	0.0518	NS	0.2266	0.066
T _m annealing temperature, H _O observed heterozygosity, H _E expected heterozygosity, F _{IS} inbreeding coefcient, HWEP results for Hardy–Weinberg Equilibrium test, PIC polymorphism information content, MAF minor allele frequency, NS non-signifcant											
*P < 0.05; **P < 0.01											