Development and Characterization of 123 SNP Markers in *Neotrypaea Japonica* (Ortmann 1891)

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Abstract

Neotrypaea japonica (Ortmann 1891) is a burrowing shrimp that lives in tidal flats. In this study, 123 SNP markers were isolated and characterized from *N. japonica*. The frequency range of minor alleles varied from 0.2500 to 0.4833, the observed heterozygosity ranged from 0.4333 to 0.9000, and the expected heterozygosity ranged from 0.4944 to 0.6503. The polymorphic parameter and inbreeding value varied from 0.3680 to 0.5651 and -0.2768 to 0.1045, respectively. Significant departures from Hardy-Weinberg equilibrium after Bonferroni correction ($P < 0.05$) were observed for 16 loci. The study of polymorphic SNPs will provide an important theoretical basis for further population genetic diversity analysis and species conservation of *N. japonica*.

Introduction

The Japanese ghost shrimp *Neotrypaea japonica* (Ortmann 1891), belongs to the group of Crustaceas (Kim et al., 2013). *N. japonica* is a dominant species in the macrobenthos community, which occupies important position of burrowing thalassinideans in soft-sediment environments (Mukai and Koike 1984; Posey et al. 1991; Dworschak 2000). The species quantity is facing a wide range of major threats, including environmental destruction, pollution, hydrological change and so on (Felder 2001). In addition, the species is not well managed and protected due to the lack of molecular marker research which is essential for protecting biodiversity and maintaining ecological balance. Thus, conducting genetic investigation on the population of *N. japonica* is an urgent need to make better use of natural resources and to protect biodiversity.

Single nucleotide polymorphisms (SNPs), as a third-generation molecular marker, are mainly used to analyze DNA sequence polymorphisms caused by mutations at the genomic nucleotide level (Kaikai et al., 2018). Moreover, SNPs have been widely used in genetics, as they can act as biological markers, helping scientists locate genes associated with certain characteristics, conditions, or diseases (Vignal et al., 2002). Here, SNPs were developed and characterized in *N. japonica* with the restriction-site associated DNA tags sequencing (RAD-seq), which enriched genetic marker resources for conservation genetics study (Qu et al., 2019).

Samples used in this study were collected from Yantai coast, Shandong province (37°27'N/121°30'E). Genomic DNA was extracted from tissues of 30 randomly selected *N. japonica* according to the manufacturer's instructions. For Illumina sequencing, RAD libraries with the paired-end short-insert of 150 bp were generated, and sample indexing, and pooling were carried out as previously described (Baird, etc. 2008). To obtain SNP marker resources, the libraries were sequenced using Illumina HiSeq4000 sequencing platform (Shanghai BIOZERON Co., Ltd.) with the total flux of one lane. Totally, 719,660 putative SNPs of *N. japonica* were identified in this research.

To verify the SNPs recognized by Illumina sequencing, Sanger sequencing was conducted. Primers used were designed using Primer v3.0. The PCR was performed in a total volume of 25 μ L containing 12.5 μ L 2 $\times$ PCR Mix (GenStar, Beijing, China), 3.5 μ L cDNA template, 1 μ L forward primers, 1 μ L reverse primers and 7 μ L ddH₂O. The PCR amplification cycle was as follows: initial denaturation for 5 minutes at 94°C, followed by 40 cycles at 94°C for 30 seconds, annealing for 30 seconds (annealing temperature of each primer pair, see table 1), 72°C for 30 seconds, final extension of 7 minutes at 72°C. Amplified PCR products were purified by gel extraction and subjected to sequence on ABI 3730 DNA analyzer (Applied Biosystems).

Summary statistics were calculated, which include the minor allele frequency (MAF), observed heterozygosity (H_o), expected heterozygosity (H_e), polymorphic information content (PIC), inbreeding coefficient (F_{IS}), and P-values representing the deviations from the Hardy-Weinberg equilibrium (P_{HWE}) (Kalinowski et al., 2007). 123 SNP sequences were further confirmed by Sanger sequencing. The MAF ranged from 0.2500 to 0.4833. The H_o and H_e ranged from 0.4333 to 0.9000 and 0.4944 to 0.6503 (Table 1), respectively. PIC was between 0.3680 and 0.5651. F_{IS} values varied from -0.2768 to 0.1045. After Bonferroni correction, significant departures ($P < 0.05$) from Hardy-Weinberg equilibrium were observed for 16 loci. The newly developed SNP markers play a significant role in population genetic analysis, conservation of natural resources, and directional breeding.

Declarations

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Declaration of interests

The authors declare that they have no competing interests.

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Tables

Table 1 Characterization of 123 SNP markers in *N. japonica*.

Primer ID	Primer sequence (5'–3')	Size (bp)	T _m (°C)	SNP type	SNP position				P_{HWE}	PIC	MAF
12369	F: ATTCTGCAGTCTTGGTCGGC R: CGGACACACATGACGAGACT	303	61	C/T	31	0.7667	0.5034	-0.2153	NS	0.3725	0.4500
56598	F: TTCTTCGTACGCTGTCGCAT R: GAGAGTCTGACGGTCAGCAC	318	60	G/T	60	0.8667	0.5944	-0.2354	*	0.4945	0.4333
73093	F: TGCATATTTCAATCATACTTGTCCA R: GGCTTGAGAGTACTGCTTTTGG	280	60	G/C	110	0.6667	0.5898	-0.1066	NS	0.4918	0.4000
77424	F: TTCCTTGTAAGCGCCCATGG R: CGTGGCTGGGATCAAAGTGA	350	61	T/C	87	0.5667	0.5418	-0.0575	NS	0.4453	0.3500
82960	F: TCTCCTCCTAGCTATAAGTTATACC R: ACTTAAGGCCGAGTCCACAC	321	60	G/C	51	0.6667	0.5266	-0.1421	NS	0.4140	0.4000
105510	F: TGAAAGAAGGTCAGCAGTTGC R: GGTTGCATCAGTATTACCGCC	300	59	T/A	33	0.6667	0.5672	-0.1175	NS	0.4600	0.4333
106496	F: GTTGCTGTACAGCTCCTCT R: TGTGGTCTGCACAACGTAA	331	60	G/A	59	0.7667	0.5203	-0.2166	NS	0.4106	0.3833
187710	F: AGAGGCGGCTTGGATGAAAG R: TCCTGGCTTCCATTTACCCC	342	60	T/A	67	0.8333	0.5689	-0.2273	NS	0.4610	0.4500
196901	F: AGCATCTTCACCCATTGGAGG R: GGGGGCCTGTAGTCATTGTC	290	60	T/G	63	0.6333	0.6503	-0.0392	NS	0.5628	0.3833
237016	F: TCATCAAAGTGCCTCAGCGT R: TGGTGCCGCTATATGTTGGT	355	60	A/T	45	0.8333	0.5316	-0.2464	NS	0.4167	0.4167
252910	F: CCATGGCTGTCGTATCCCAA R: GACGGGAGGAAGATGTGGAC	275	60	G/C	108	0.9000	0.5384	-0.2768	**	0.4203	0.4500
257138	F: CTTCCGCGTCCTCGATCTC R: CGAAATGGAGATGCGCTTCG	276	60	T/C	96	0.7667	0.4944	-0.2239	NS	0.3680	0.4167

Table 1 (continued)

Primer ID	Primer sequence (5'–3')	Size (bp)	Tm (°C)	SNP type	SNP position				P_{HWE}	PIC	MAF
262878	F: ATTCCTGATCTTGCCGAGGC R: TTTGCCCATTCGTTACCA	326	60	C/A	43	0.7667	0.5689	-0.1863	NS	0.4610	0.4833
263231	F: GATGAAGCACGTTGCCACAG R: ACTCTGTCCTCTCTCCGACC	309	60	C/T	107	0.9000	0.5689	-0.2645	**	0.4610	0.4500
272390	F: TCAGGTAATGGACGTTAACACA R: TGGTTCGTAACGTCTTTAACTT	354	57	C/T	85	0.7333	0.5944	-0.1517	NS	0.4945	0.4333
283060	F: TCGGAAATGACATGGAAAACA R: TCCATTCTGCAGTAGCCCTG	318	60	G/A	25	0.7000	0.5418	-0.1648	NS	0.4453	0.3500
292356	F: CCCAAATCCACAAAGCTGGG R: TTTGAGCAGGTCATCAGGGG	297	60	G/A	45	0.8333	0.5316	-0.2464	NS	0.4167	0.4167
299165	F: TCCAAAAAGGAAAGAGCACC R: AGGTGCTTTGGAACTCACA	305	58	G/A	87	0.7000	0.5689	-0.1407	NS	0.4610	0.4500
305477	F: ATCGATCTGAACACGGCGTT R: AAGAACATCCGCCTCACCAG	322	60	G/A	40	0.7667	0.5316	-0.2062	NS	0.4167	0.4167
307716	F: AGGACTGGGCAATGACACA R: CTTTGCAGCCCACTACCGTA	288	60	C/T	51	0.8333	0.4944	-0.2631	NS	0.3680	0.4167
315140	F: ACCGTATGAATACCCTTAGCTCT R: AGTGTCCCGATAATGCGCAA	263	60	G/A	74	0.6667	0.5266	-0.1421	NS	0.4140	0.4000
329675	F: CCAATGTAGGACTCGGTGCA R: TCTCTGCGACTCTTAAGGGT	288	60	T/C	59	0.9000	0.5672	-0.2748	**	0.4215	0.4833
350870	F: TCATGCATCAGAAAACGCGT R: TTGGTGTGTTTTGTGCATGT	331	59	A/G	109	0.8333	0.4944	-0.2631	NS	0.3680	0.4167
359170	F: GCACCAGGATGAATCAAACGC R: GGCCCTCTTAATGCCCTTT	317	60	G/A	66	0.6333	0.6164	-0.0633	NS	0.5224	0.4167

Table 1 (continued)

Primer ID	Primer sequence (5'–3')	Size (bp)	Tm (°C)	SNP type	SNP position				P_{HWE}	PIC	MAF
360212	F: CGGTATTGGCCGAGAAATG R: GAAATAGTGGCTGCACTCGC	304	60	T/C	77	0.8333	0.5316	-0.2464	NS	0.4167	0.4167
361253	F: TGTATCTTTAGAGCCGCCACC R: TACCTCTTCCCTCCTCCTGC	293	60	T/C	55	0.7667	0.5554	-0.1980	NS	0.4532	0.3833
369763	F: TGGGACAGGACTTTTGTGGC R: AGTCCTGTCCCATCCCTTGA	249	60	C/G	81	0.7333	0.5492	-0.1814	NS	0.4496	0.3667
371676	F: AGGAGCCCGGATAGGAAGAG R: CGTCCTCTGCCCCCTTTATT	281	60	A/C	106	0.8333	0.5689	-0.2273	NS	0.4610	0.4500
399752	F: GCGGAGGCTGTAAAGAGACT R: ACAGTGTAATGAATAAAGGTGGT	256	59	C/T	85	0.9000	0.5384	-0.2768	**	0.4203	0.4500
404645	F: ACTTTTGTTTTTCATTCCCCCT R: ACCTGTGACGTCACTCACAC	323	60	T/C	109	0.7000	0.5927	-0.1293	NS	0.4935	0.4167
417099	F: GTCTGCCTTCACTCTCGTAGG R: AGGTGTAAAAGATGTCGCTGGT	301	60	T/C	76	0.6667	0.5266	-0.1421	NS	0.4140	0.4000
419104	F: AGCTTGGTAAAAATGGTTACACCT R: ATTTCCACTCCCTCCCAGGA	282	60	G/A	90	0.6667	0.5356	-0.1337	NS	0.4188	0.4333
419110	F: TGCATATTTCAATCATATTTTCCATT R: TGCAAGAATTGGCCAACTAGTG	305	59	G/A	91	0.8333	0.5927	-0.2174	NS	0.4935	0.4167
423291	F: AAAATGTGGGATATATTGAGGTTTT R: TCAACGTAACTTTTAGACAGGGA	322	58	C/T	63	0.8333	0.5316	-0.2464	NS	0.4167	0.4167
435217	F: TCGAACCAATCAGCATCGGA R: AGTGTGCCCAAACCTTCATGAA	260	59	G/A	100	0.7333	0.5062	-0.1913	NS	0.3739	0.4667
439596	F: GGTGGTCTTGAGGGTGTGTGT R: TTGTGGATAATGCCCCCAGC	332	60	G/A	72	0.9000	0.5689	-0.2645	**	0.4610	0.4500

Table 1 (continued)

Primer ID	Primer sequence (5'–3')	Size (bp)	Tm (°C)	SNP type	SNP position				P_{HWE}	PIC	MAF
439765	F: CGAGTGAAGGAAAATACTCCA R: AAGGTCTGTTTGGCCCTGTC	271	60	A/G	37	0.7333	0.5605	-0.1715	NS	0.4561	0.4000
441280	F: ACCTTAAAGAAAGTCTAATTACCT R: TTGGAATGGTGGAGTGTCCG	328	60	G/A	92	0.8333	0.5644	-0.2311	NS	0.4584	0.4167
443015	F: AAGCTGTTTTGCATGAGGTG R: AAAGTGAACACGACCGACGA	309	60	C/T	107	0.7000	0.5927	-0.1293	NS	0.4935	0.4167
443558	F: GACACGGAATCACAGGTGTC R: AATAGTAGTCCCGCCAGGT	258	60	C/A	71	0.8333	0.5316	-0.2464	NS	0.4167	0.4167
445726	F: TCATGTGGCGTGACAAGTTCT R: GAGGGTCAGGTGTGGATGTG	353	353	A/T	80	0.9000	0.5407	-0.2748	**	0.4215	0.4833
450138	F: ACCCAAAAATGCCAACATCG R: GCACAAATCCGCCATGTGAG	326	60	C/A	66	0.7333	0.5492	-0.1814	NS	0.4496	0.3667
453937	F: TCATCGACAAGACCATGATCA R: AATCCTTTTGGCCTCGTGGG	280	60	C/A	85	0.7000	0.6503	-0.0924	NS	0.5628	0.3833
480414	F: AGGACTAGGTGTTCCGAGGG R: ATCCAGAGTGAGAGTGGCAA	277	60	T/G	71	0.7000	0.6119	-0.1192	NS	0.5195	0.3833
489466	F: ATATACCCCTCCCAGGACC R: CCTTTTTCCAGCCCCGAGAT	333	60	T/A	101	0.8333	0.5316	-0.2464	NS	0.4167	0.4167
493010	F: ATTCAACTCAACGTCGTGAC R: AGACGACGAACAGAAACGCT	323	60	G/A	53	0.8000	0.5944	-0.1956	NS	0.4945	0.4333
499089	F: AACACCTTCTCCACACGTCT R: TCCTGCGTGTTATAGTGTGGT	302	60	A/G	75	0.8333	0.5644	-0.2311	NS	0.4584	0.4167
506736	F: TGGACTGCATATCTGAAGCACA R: ATGCCACCTCATCCGAACAG	328	60	C/T	68	0.8000	0.5266	-0.2314	NS	0.4140	0.4000

Table 1 (continued)

Primer ID	Primer sequence (5'–3')	Size (bp)	T _m (°C)	SNP type	SNP position				P_{HWE}	PIC	MAF
514249	F: AATTCGACTCTCCGGTCAGG R: TGAAGCAGATGGAACCGTCC	305	60	G/A	106	0.9000	0.5384	-0.2768	**	0.4203	0.4500
514512	F: ATTCAAGATGGCCGATGGGG R: GCTGTTCCCTACTCCGCTAC	350	60	A/T	60	0.8333	0.5644	-0.2311	NS	0.4584	0.4167
516617	F: TTCATCAACCTCAACAATGGTGT R: CCGGCGGCCTCAAAATAATT	268	59	T/A	35	0.7667	0.5859	-0.1812	NS	0.4894	0.3833
516704	F: TCTGCCTCCATTTAGATAGTGA R: GTCGGCTATCTGCAAACGGA	347	60	T/A	83	0.7667	0.5689	-0.1863	NS	0.4610	0.4500
517080	F: TGCATATTTCAATCATATTTGTCCA R: TGGGCTATCAACAATGGGGA	269	59	T/C	52	0.8333	0.5644	-0.2311	NS	0.4584	0.4167
517538	F: TCTCAGCCAACAGTTTCTGGA R: TTGTCAGTGGTCGGACTACG	342	59	A/C	87	0.8333	0.4944	-0.2631	NS	0.3680	0.4167
517813	F: TCGGATTGCTTCATGCTTTCC R: AGAGAGTGTGTGTGTTTGCTCA	320	60	A/G	50	0.8333	0.5316	-0.2464	NS	0.4167	0.4167
518823	F: TCTTGGGGTTACTCGTGTGAC R: AGAAGAGCAGCAAGACAAGACA	281	60	G/A	57	0.8333	0.5927	-0.2174	NS	0.4935	0.4167
519134	F: TGCAGTCGCAGAATAACAGT R: TCGCTCATGAACAATATGTGC	338	58	G/A	48	0.8000	0.5266	-0.2314	NS	0.4140	0.4000
519735	F: AGTGTGTGTTTGGAGTGCCA R: CTGTAGCAGGCTGTGGGAAA	298	60	T/C	86	0.8000	0.5672	-0.2088	NS	0.4600	0.4333
526287	F: ACATTATATATTTATTTTGCCCGTGGA R: GTCGTGGGTTATGGTGTCTCT	272	59	T/G	110	0.8000	0.4994	-0.2392	NS	0.3705	0.4333
531502	F: GACCACACAACCTCCGCCTA R: AGAAATCGGGCTAACTCGGG	327	60	T/A	110	0.7333	0.5356	-0.1809	NS	0.4188	0.4333

Table 1 (continued)

Primer ID	Primer sequence (5'–3')	Size (bp)	Tm (°C)	SNP type	SNP position				P_{HWE}	PIC	MAF
532574	F: AATCCAACAGGTCGTCGTCC R: CCCTTTCCCGTCGTA CTGTT	284	60	C/T	97	0.7000	0.5554	-0.1526	NS	0.4532	0.3833
542479	F: CAAGGCTTGGTCATCGATTGT R: ACCAAGCCTAAATTTCCATGAA	268	59	C/T	66	0.9000	0.5384	-0.2768	**	0.4203	0.4500
545314	F: TCGAGCATTCTAATGGGGAACA R: CCCCTACCTTAACCCCTCA	354	60	T/G	55	0.7333	0.5605	-0.1715	NS	0.4561	0.4000
546066	F: TCGTAAGGTCATTCCAGTCATGG R: TCACGTCCAGTATGTCTACACTC	355	60	C/G	42	0.6333	0.5588	-0.1071	NS	0.4724	0.3167
546218	F: TGGATCGACCACAAATCTACTGT R: AACTGCGACTGAGTAATCGA	351	59	C/T	86	0.9000	0.5407	-0.2748	**	0.4215	0.4833
549829	F: TGTCTCTGCTGTCTTTCTGT R: CCCCTGTGGTATCAGTTCC	252	60	G/A	79	0.8000	0.5944	-0.1956	NS	0.4945	0.4333
553717	F: ACAGAAGTTAGGAAACCAACCGA R: TGTTGAGCAACCTCCCTGAC	325	60	A/G	102	0.8333	0.5316	-0.2464	NS	0.4167	0.4167
554432	F: CATGGACGCTTACCACGTCT R: ACAAACCCAGAATCTTCAAATCA	324	60	G/T	64	0.6667	0.6305	-0.0815	NS	0.5419	0.3667
555044	F: GCATGAGCCTCCAGACAT R: TTACGGTCCCTTTTCCACGG	291	60	C/A	89	0.7000	0.5644	-0.1446	NS	0.4584	0.4167
555786	F: TCTTTACTTGGTTGAATAGCGCT R: AGTAAAAGCCATGGTGGTCCC	322	60	C/T	67	0.8333	0.5689	-0.2273	NS	0.4610	0.4500
556060	F: TGGGAGCAAGGAATCCAATGA R: ACTACCACAACCACCACCAC	265	60	A/T	97	0.7000	0.5418	-0.1648	NS	0.4453	0.3500
556526	F: AACGACCTTCTCCACACGTC R: CGTGTTGCAGTGTGGTGAAG	266	60	C/T	103	0.9000	0.5407	-0.2748	**	0.4215	0.4833

Table 1 (continued)

Primer ID	Primer sequence (5'–3')	Size (bp)	T _m (°C)	SNP type	SNP position				P_{HWE}	PIC	MAF
556747	F: TCTTTGGTCGTGTGGGTAGA R: TGGCATGCCCACTAATTTGTG	352	59	T/A	78	0.6667	0.5492	-0.1336	NS	0.4496	0.3667
556794	F: CATGCGGAAGGAGCGAGAG R: TACGAGTCCATGGTGGGTCT	314	61	G/A	47	0.8333	0.5644	-0.2311	NS	0.4584	0.4167
557312	F: TCATCCGGTGGATTGATTCCTC R: CCCCCTTCCTCTTCCTCGTA	338	60	A/G	102	0.9000	0.5384	-0.2768	**	0.4203	0.4500
557410	F: TCCAAAGTCCCTTTCTCTGT R: CTCACAGAGGGCCTTGTGTT	335	60	T/A	105	0.5667	0.5554	-0.0450	NS	0.4532	0.3833
557474	F: TCGATTCCCGTTAGTATTCTTGCA R: CCCCTGTCCTCGCTTTCTTT	349	60	G/A	57	0.6333	0.6367	-0.0491	NS	0.5525	0.3167
557550	F: TGGCGTTGCAACTCATTTGG R: TCATCCCGACTCTCACCTC	320	60	T/A	62	0.7667	0.5554	-0.1980	NS	0.4532	0.3833
557714	F: ATCATACCATACCCCGCCAA R: TTCAGCGTGGGATATCGGTG	307	60	G/A	54	0.6667	0.5672	-0.1260	NS	0.4778	0.3333
557785	F: TCCTGTTCTATTTGACTCGGGC R: AGGGTGATAGGTGGAGGGAG	338	60	G/A	71	0.7333	0.6079	-0.1464	NS	0.5169	0.3667
557832	F: CGAGAGACAGAGACAGGCAC R: CTCTTCTTCTGCCGGTCGTT	312	60	G/C	107	0.7000	0.5689	-0.1407	NS	0.4610	0.4500
557964	F: GGGCTAAATCAAGTTCTTACGGC R: CCTTACACCGACCCAATGT	322	60	A/C	45	0.7333	0.6169	-0.1392	NS	0.5228	0.4333
558065	F: TGATTGTCGTCTGTCACTCCT R: CTGAGCATGATGGGAGGCAA	291	60	G/A	38	0.8333	0.5384	-0.2403	NS	0.4203	0.4500
558087	F: TCCTTCGGTAATTAGTGGGCA R: TGGTATGAGCTAGCGTTGGT	297	59	A/C	84	0.5333	0.6305	0.0366	NS	0.5419	0.4667

Table 1 (continued)

Primer ID	Primer sequence (5'–3')	Size (bp)	T _m (°C)	SNP type	SNP position				P_{HWE}	PIC	MAF
558228	F: TCGATTACTCTCCGTCCTTCAC R: CGTCTCCCAGGCATCGTTAT	335	60	C/T	82	0.9000	0.5384	-0.2768	**	0.4203	0.4500
558275	F: ATTCTCGGTGTGGTCGTACC R: CGCATCACCTACAGCCGATT	351	61	A/G	75	0.6333	0.6266	-0.0574	NS	0.5391	0.3500
558335	F: TGCAACCGCTACTTTGAGTG R: ATTGGAGAGGTACGGTGCAC	274	60	C/T	43	0.5333	0.6486	0.0540	NS	0.5651	0.3000
558337	F: TCCTTCGCTAATTAGTGGTCCA R: AGTCCCCACAGACCTTTCT	309	60	A/G	66	0.7000	0.6028	-0.1264	NS	0.5135	0.3500
558356	F: AGAGCACACAGAGGAACAGC R: TGGTGTGGTTGATGGTTGG	326	60	G/A	40	0.7667	0.5316	-0.2062	NS	0.4167	0.4167
558377	F: TCTGACTTCGAGTGTACAAATCCT R: GCATCGTAGTACCACAGCGA	267	60	A/T	60	0.6667	0.5605	-0.1235	NS	0.4561	0.4000
558441	F: ATGTCACTGCAGGTGGTCTC R: GGAGTCCAGTATAGGCAGGC	340	60	C/T	45	0.5667	0.5712	-0.0426	NS	0.4921	0.2833
558457	F: TCACTCTCGGCTGCATTTCA R: TTCACAGAACAGGGCCACTT	301	60	A/G	81	0.7667	0.5316	-0.2062	NS	0.4167	0.4167
558470	F: GAGTTGATTGGGACATGCACC R: AGCAGATTGTCTCACGTCCC	322	60	A/G	88	0.7000	0.5644	-0.1446	NS	0.4584	0.4167
558474	F: TTCAAGGTAATCATTAGTAAATGGT R: AATGCTCAACCCCTCAACGT	342	60	C/G	32	0.6333	0.5203	-0.1224	NS	0.4106	0.3833
558480	F: ATTCATACCCGCTCACTCG R: ACTCAAGCACGCACTCTCTC	311	60	T/A	79	0.7333	0.5672	-0.1655	NS	0.4600	0.4333
558483	F: TCGGAAATGACAGGGAAAACAA R: GGTGGTCGCAGGAGTTTCTT	357	60	T/C	28	0.7000	0.5418	-0.1648	NS	0.4453	0.3500

Table 1 (continued)

Primer ID	Primer sequence (5'–3')	Size (bp)	Tm (°C)	SNP type	SNP position				P_{HWE}	PIC	MAF
558491	F: TTCCTCTGAAGAAGCCACTGT R: AGTGTGTGAAGTGGATAAGGCT	321	59	G/A	71	0.6333	0.5644	-0.0938	NS	0.4584	0.4167
558494	F: ATCAGCACATATCAGCCGCT R: GACTGTACAGGGCTTGCACA	251	60	A/C	66	0.6000	0.6350	-0.0223	NS	0.5450	0.4000
558500	F: TCAGAAAATTATGTTTCCCAGAAGGC R: TGCTGGTGCATAGACAGGTG	256	60	G/A	56	0.7000	0.5644	-0.1446	NS	0.4584	0.4167
558501	F: CACCACCACATTTCTGTATGGC R: GGTGGCGAAGAGAACAGGAA	273	60	G/C	57	0.9000	0.5384	-0.2768	**	0.4203	0.4500
558502	F: CGAGCATAGCTTACATTTGCCC R: TGTGGAGTCACGGAGTAAGC	318	60	T/C	71	0.7000	0.5418	-0.1648	NS	0.4453	0.3500
558504	F: TACTGGCATGGTGCTTGACC R: GCCATACTTCCAGCAATGGC	284	60	T/A	105	0.6333	0.5588	-0.1071	NS	0.4724	0.3167
558506	F: TTGGCCAGTGTAACAGCCA R: AAGCTATCCAGGGGTCTCGT	340	60	T/A	88	0.7667	0.5203	-0.2166	NS	0.4106	0.3833
558512	F: TGTCTGTGGAATCACCCAG R: ACAGATACAGCCACAGACGT	253	59	G/A	108	0.6333	0.5927	-0.0777	NS	0.4935	0.4167
558516	F: CGCACTCGTTTATTACTTCCGT R: CTCTGGGCGAAAACCTGCTA	280	60	G/A	81	0.8000	0.5401	-0.2192	NS	0.4212	0.4667
633885	F: CCTTGTAGTCTTGGAATACTACCC R: GGGTGGGGGTGTTTGGGG	323	63	C/T	86	0.7667	0.5644	-0.1901	NS	0.4584	0.4167
634516	F: TGATCGTCGTCTGTCACTCC R: ATTTTCTGGGGTGTGGAGCC	312	60	C/A	67	0.4667	0.6079	0.0891	NS	0.5258	0.3000
641305	F: AGCAACAATATGGGGAAGCCA R: CGTCTTACTTGGCTTCCCCA	295	60	A/T	59	0.7000	0.6028	-0.1264	NS	0.5135	0.3500

Table 1 (continued)

Primer ID	Primer sequence (5'–3')	Size (bp)	T _m (°C)	SNP type	SNP position				P_{HWE}	PIC	MAF
668058	F: AACAAATTTACGTATTGTAAGGAA R: TGCAAAGGACTTCAGGGTGT	264	59	A/C	70	0.8333	0.5689	-0.2273	NS	0.4610	0.4500
692392	F: TCACTCGCAAATTAAGGTGTT R: CAACCACCGAGAAGGGAAGT	282	60	A/G	60	0.9000	0.5384	-0.2768	**	0.4203	0.4500
699932	F: AAATGACCTCAGAGGCAGGG R: AGCGCAGATAGAGGCCAATC	271	60	C/A	74	0.4333	0.5791	0.1045	NS	0.5045	0.2500
707253	F: ACGAGGCCCTTCAATCTGAGG R: TGTGGCCCACTTTTCTCTGA	338	59	G/A	77	0.6333	0.6119	-0.0669	NS	0.5195	0.3833
715816	F: TCTCAAGAATATCTCGTTTTATGCCA R: TCACACAATTGACTGGAGTACT	260	59	T/G	98	0.8000	0.5266	-0.2314	NS	0.4140	0.4000
718190	F: AAGGCTCCAAACACAGGTCC R: TTCCTGCCAACACTCTTCCC	276	60	A/G	93	0.7000	0.5949	-0.1274	NS	0.4949	0.4500
718824	F: AAATCGCCCAGAGCTAACCA R: AAAAGGGCCGAGAGAGATGC	251	60	T/A	84	0.6333	0.5418	-0.1143	NS	0.4453	0.3500
719225	F: TCAGCCAAAGAAGTGAAATGA R: ACCGTTGTCAATTAAGCACT	314	59	G/C	54	0.6333	0.5927	-0.0777	NS	0.4935	0.4167
719503	F: CCACACATTCATAGGAGGACCA R: TGTCTTCTCTCCCTGGTCCA	311	60	G/A	61	0.8333	0.5644	-0.2311	NS	0.4584	0.4167
719545	F: AGTCTGAAGTCTACTGCTTGGC R: CAGGCCTAGTGTAAGTAGCCA	267	60	A/G	78	0.6667	0.5356	-0.1337	NS	0.4188	0.4333
719652	F: AATTCATATTTTTCCATGCACCT R: GGGAGTTTGTTTTGACCCCC	277	60	C/T	99	0.9000	0.5384	-0.2768	**	0.4203	0.4500
719660	F: AGCTGTGAGAAGAACCACCC R: AGACGTGATTCTTTGGGTGGT	245	60	C/A	80	0.8000	0.5401	-0.2192	NS	0.4212	0.4667

Table 1 (continued)

Primer ID	Primer sequence (5'–3')	Size (bp)	T _m (°C)	SNP type	SNP position				P_{HWE}	PIC	MAF
719705	F: AACCCAACTTTTCAGCGGGA R: AACGAAATGGCCCGAGATGG	332	61	C/A	37	0.6667	0.6350	-0.0780	NS	0.5450	0.4000
719727	F: CCACACACATCTTGCCTGC R: AGAAGATTGCTGGATGGGGTG	262	60	T/C	101	0.7000	0.6333	-0.1050	NS	0.5439	0.3833
719728	F: TCTAACACCAGCCCATGTCT R: AGCACCGAGTTGTCGTTCTG	272	61	A/G	50	0.6333	0.5927	-0.0777	NS	0.4935	0.4167

T_m annealing temperature, H_o observed heterozygosity, H_e expected heterozygosity, F_{IS} inbreeding coefficient, P_{HWE} the P value of the departures from Hardy-Weinberg equilibrium, PIC polymorphism information content, MAF minor allele frequency, NS non-significant

* $P < 0.05$, ** $P < 0.01$