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Development and characterization of 108 SNP markers in the Iwagaki oyster, *Crassostrea nippona*

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Abstract
A total of 108 single nucleotide polymorphism (SNP) markers from the Iwagaki oyster, *Crassostrea nippona* were isolated and characterized based on high-throughput sequencing in this study. The minor allele frequency per locus ranged from 0.0167 to 0.4844. The observed heterozygosity and expected heterozygosity varied from 0 to 0.9688 and 0.0333 to 0.5079, respectively. Eighteen loci showed significant deviation from Hardy–Weinberg equilibrium. These SNP markers described in this study will provide a valuable tool for population genetic analysis and natural resource conservation of *C. nippona*.

Keywords *Crassostrea nippona* · SNP · High-throughput sequencing · High resolution melting

The Iwagaki oyster *Crassostrea nippona* is a commercially important bivalve, which is distributed along the coasts of Japan, Korea and China (Boudry et al. 2003; Itoh et al. 2004; Yoon et al. 2008). The commercial price of *C. nippona* is nearly five times as high as that of the Pacific oyster *C. gigas* in Japan, as it is edible during the summer when the other oyster species are unavailable (Itoh et al. 2004). Since the production of *C. nippona* is based almost exclusively on natural stocks, over-exploitation has led to continuing decline of the natural populations over the decades (Fujiwara 1995; Li 2007). Therefore, it is urgent to perform population genetic investigation on *C. nippona* to conserve the wild resources. Single nucleotide polymorphisms (SNPs) are now the most popular DNA markers in genetic studies because of their high level of polymorphism, favorable reproducibility and wide genomic distribution (Vignal et al. 2002). With the rapid development on next generation sequencing technologies, discovery of large numbers of SNPs in any non-model organisms of interest is becoming easier and faster. In this study, we developed and characterized SNP markers in *C. nippona* for the first time based on the restriction-site associated DNA (RAD) sequencing. These markers will facilitate the researches on conservation genetics and genetic evaluation in *C. nippona*.

To obtain SNP marker resources, the RAD libraries were constructed and sequenced on the Illumina HiSeq 2500 platform using 150-bp paired-end reads. A total of 538,594 putative SNPs in *C. nippona* was identified, of which C/T and G/A were the most common substitution and the ratio of transition to transversion was 1.22. Four hundred and twenty putative SNPs were randomly chosen for primer design using Primer Premier 5.0 and evaluated for polymorphism by high resolution melting (HRM) analysis in a cultured population of *C. nippona* from Yantai, China (n = 32). Genomic DNA was extracted from adductor muscle by standard proteinase K digestion and phenol–chloroform extraction. PCR was performed in a total volume of 10 µL on a Light Cycler® 480 real-time PCR instrument (Roche Diagnostics Burgess Hill, UK). The reaction mixture contained 0.25 U Taq DNA polymerase (Takara, Dalian, China), 10 × PCR buffer, 0.2 mM dNTP mix, 0.4 µM of each primer set, 1.5 mM $MgCl_2$, 5 µM SYTO9 (Invitrogen Foster City, CA, USA), and about 10 ng template DNA. The PCR amplification procedure was as follows: first denaturation at 95 °C for 5 min, then 45–55 cycles of denaturation at 95 °C for 40 s, annealing and extension for 40 s at 62 °C for the first cycle and thereafter at 0.5 °C decrease each for 10 cycles, and a final extension at 72 °C for 40 s. Following amplification, the products were denatured at 95 °C for 1 min, and then annealed at 40 °C for 1 min to randomly form DNA duplexes. Melting curves were

Table 1 Characterization of 108 SNP markers in the Iwagaki oyster *Crassostrea nippona*

Locus ID	Primer sequence (5′–3′)	Size (bp)	Tm ( )	Types	*Ho*	*He*	MAF	F_{IS}	P_{HWE}
CNP1	F: TGTTGACGACTGAGAGCTGG R: CGATCACATACTGACGTGTCC	90	60	G/A	0.3548	0.2967	0.1774	− 0.2157	0.2553
CNP3	F: AGTGGTTTGAGGCACTCGTC R: TAGTTCTGGCCAAGCACCC	116	60	C/A	0.3667	0.3045	0.1833	− 0.2245	0.2442
CNP8	F: ATGCTGTTTCATGTTTGGGA R: TCTGTTTGGGTTTCGGTTGT	113	60	G/T	0.3333	0.4520	0.3333	0.2500	0.1419
CNP10	F: ATTGCATAGCAAAGATCGCC R: ACATTCGGGATGAAGACTCG	124	60	C/A	0.1562	0.1463	0.0781	− 0.0847	0.6704
CNP11	F: AATTCGCCTTATTTACTTAGAGGA R: ATCTCTGAAACACCATCCGC	113	60	G/C	0.0645	0.4823	0.3871	0.8640	0.0000*
CNP14	F: ATAAGTCACATGCGCGGTTC R: GAAAAAGCACACCAAAGCGA	85	60	A/C	0.0000	0.4528	0.3333	1.0000	0.0000*
CNP16	F: CGCAAGATTTGACAATTTCTGT R: CCACTTCGATTTCAATTTGTG	111	60	G/A	0.3548	0.2967	0.1774	− 0.2157	0.2553
CNP19	F: CTCCGCCCCTTTAAAAGTCT R: AAAAGTCTGAAATTGCTTGCG	124	60	C/T	0.1250	0.1190	0.0625	− 0.0667	0.7458
CNP24	F: TGGCCATTGGTAAGTAAGGC R: AAGCGAGCATCTGTCGATCT	115	60	C/T	0.0000	0.3913	0.2593	1.0000	0.0000*
CNP26	F: CGGCCAAGAATACCTTGACT R: CTACTTGGATGCTTCCGGTG	83	60	G/A	0.2500	0.2222	0.1250	− 0.1429	0.4528
CNP27	F: CCCATTGCACAACGTTACAA R: CCGGGGTTACGTGGTATTTA	104	60	T/G	0.3571	0.2987	0.1786	− 0.2174	0.2786
CNP38	F: GGAAGAAAACCAGTGCATCAA R: ATGAACTTTTTGGCGACCTG	86	60	C/G	0.0312	0.0908	0.0469	0.6503	0.0000*
CNP41	F: TCGTGTCTTTCTTCGCCTTT R: AAGGCACTCTGAAACAAGCC	102	60	T/G	0.1250	0.1190	0.0625	− 0.0667	0.7458
CNP45	F: TCTAGGCCATTTATCCGTGG R: CAGAAAATGCACTCCCTTGG	87	60	C/T	0.0938	0.0908	0.0469	− 0.0492	0.8216
CNP55	F: TGCAGGAAGAGGATGATCTG R: ATAAGCAACAAACCGCAACC	113	60	A/G	0.7097	0.4654	0.3548	− 0.5500	0.0029
CNP67	F: TTTGAGAGAATTGCAGCCG R: CAGTCTGGATTTCTCTGCTGG	82	60	T/C	0.0645	0.0635	0.0323	− 0.0333	0.8964
CNP68	F: CCACTTTCACTTTTTGGGACA R: CCCCCAACTTCCTAAAGTCC	91	60	C/T	0.3871	0.3173	0.1935	− 0.2400	0.2036
CNP79	F: TGCTTTGCACCTTTCAATATACA R: TGCTCTAACGGCCTTTTGTT	99	60	C/G	0.0625	0.0615	0.0312	− 0.0323	0.8981
CNP91	F: TGTCCGCCCTTCTTACAAAC R: CAAATAAACGCACCCCACTT	103	60	G/A	0.5938	0.4241	0.2969	− 0.4222	0.0207
CNP95	F: AGTTCATCCCGTATTGCGTC R: ACAATGCTTCAAAACTCGCC	117	60	G/A	0.2333	0.2593	0.1500	0.0850	0.5632
CNP105	F: GCAGTCTCTGAAGAGCAGGAA R: CACGTTTTTGCCATGCTGTA	98	60	C/T	0.5938	0.4955	0.4219	− 0.2172	0.2543
CNP113	F: AGGACATTTCGTGGTTCGAT R: CCATCTTTTGGGATACAGGC	99	60	G/A	0.0645	0.0635	0.0323	− 0.0333	0.8964
CNP124	F: AAGGCGCTGTAAAAACGAAG R: GATGAAGAGAGGCGTTTTGC	104	60	T/C	0.3333	0.2825	0.1667	− 0.2000	0.3020
CNP133	F: CGTAGTCTTTGGTTTGCAATCTT R: CAGGGAAGGAACTGCAAGAG	89	60	G/A	0.2903	0.2522	0.1452	− 0.1698	0.3020
CNP137	F: GCAGCTGAAATGTTCCTCCT R: GGAAATAGAGACCAAATACGCA	117	60	T/C	0.5938	0.4241	0.2969	− 0.4222	0.0207
CNP145	F: CCTCTTTCGAGAAAATAACCCA R: TCAAAGCAGAGCAACAAGGA	115	60	G/T	0.2069	0.1887	0.1034	− 0.1154	0.5736
CNP150	F: AAGAAGCACACATACCGCCT R: AACTGTTCGGTGAGAGCCAG	118	60	T/G	0.0000	0.4950	0.4194	1.0000	0.0000*

Table 1 (continued)

Locus ID	Primer sequence (5′–3′)	Size (bp)	Tm ( )	Types	*Ho*	*He*	MAF	F_{IS}	P_{HWE}
CNP151	F: GGGTGTCACCTTTTCCACAA R: GTGCCTTGGTCCTCTACCAG	82	60	T/C	0.4138	0.3339	0.2069	− 0.2609	0.1815
CNP155	F: CAGTTTGAACCTTTACGCCC R: TTAGCCGGAGGAAATAAAAGG	107	60	G/T	0.5484	0.4045	0.2742	− 0.3778	0.0423
CNP165	F: CTTCTGGGGAACAGCAACAT R: GGTGTAAGCAGGTTTAGCCG	90	60	A/G	0.2581	0.2285	0.1290	− 0.1481	0.4436
CNP166	F: TTCCAAGTCAACGCAGTTTG R: TGAACGGTTTGTCTTGCATC	87	60	C/A	0.2857	0.2494	0.1429	− 0.1667	0.4130
CNP170	F: GGCTGTTTTCTCGAAGCATT R: CTATGACGCTCCACAGCCAC	85	60	G/A	0.9688	0.5074	0.4844	− 0.9394	0.0000*
CNP171	F: AATCAGATTCTGGGCTTTGC R: AACCTTCGACCTTTCGACTG	111	60	G/A	0.8750	0.5000	0.4375	− 0.7778	0.0000*
CNP176	F: ATGGAGAAGCAATTTGGGC R: CCCAGTTCCGAGAAATACCA	98	60	A/G	0.0625	0.0615	0.0312	− 0.0323	0.8981
CNP178	F: GCCTCCTAAACTCCAAATTTCC R: GCTGATAGAGAACCAAACTGTTGA	93	60	T/C	0.7742	0.4823	0.3871	− 0.6316	0.0006
CNP180	F: CGAACAATGTTTTAGCCCGA R: TGGTACTGCTGGTGTCGAAG	101	60	T/C	0.0000	0.4881	0.4000	1.0000	0.0000*
CNP182	F: ATAACCAGCTGCATTTTGCC R: CGAAGAACAAGACGACCTGG	101	60	T/C	0.0000	0.4791	0.3793	1.0000	0.0000*
CNP189	F: TGCTTCAGATTAATGCCGTTC R: ATCGGAAAACGCGTTACAAA	106	60	T/C	0.0667	0.0655	0.0333	− 0.0345	0.8946
CNP192	F: TGCTACCATTTTCCTCAGCC R: TCATCAAGCGTGCCTTAGTG	90	60	C/T	0.9688	0.5074	0.4844	− 0.9394	0.0000*
CNP196	F: ACCCTGAACATCAAAATGCC R: GCGTAAAGAAGAAACCAGCG	120	60	G/T	0.8387	0.4950	0.4194	− 0.7222	0.0001*
CNP197	F: AGACAGACTCGCTTTCAGCC R: CCTCCTCTGGTTCGTCTGTG	90	60	A/G	0.8966	0.5033	0.4483	− 0.8125	0.0000*
CNP199	F: ATAGGCTGACGCTGATTGGT R: GGTTTCTTCGTGTTGACGTG	115	60	G/A	0.6250	0.4365	0.3125	− 0.4545	0.0126
CNP200	F: TTCAGGAGGTCTGATACCCAA R: GACTTCGATTTGCACCTTCC	101	60	C/T	0.7500	0.4762	0.3750	− 0.6000	0.0009
CNP202	F: TGCCGTTCATGACTTACGTG R: ATCGGAAAACGCGTTACAAA	93	60	G/T	0.0625	0.0615	0.0312	− 0.0323	0.8981
CNP204	F: TGATAAACCTCTGCTCGCAA R: AAGTGGCATGGGTCTAGGAG	85	60	A/G	0.5667	0.5079	0.4833	− 0.1346	0.5193
CNP209	F: AGGGGCCTATCTGCATTTCT R: ATTTTCCGTGAAAGGGTGTC	98	60	G/A	0.0000	0.1190	0.0625	1.0000	0.0000*
CNP225	F: AATCAGCTTTGATTCGTGGC R: AAGCAACAACAACACAGAGGAA	91	60	G/A	0.3438	0.2892	0.1719	− 0.2075	0.2660
CNP233	F: CTGGCTTCAATCAGGTCACA R: GCAAGTGCAAGCTTTCCAAC	95	60	C/T	0.9688	0.5074	0.4844	− 0.9394	0.0000*
CNP242	F: CAAGTGCCAATGTAACCCCT R: TGTTGCAGAGATGTCAAAAGC	89	60	A/G	0.1250	0.1190	0.0625	− 0.0667	0.7458
CNP252	F: AAAGGGATGCAACTCTTGGA R: TGACTCAATACATGCCAGAACA	92	60	T/C	0.4516	0.3554	0.2258	− 0.2917	0.1198
CNP254	F: GATTGCTGACGGTGTTTGTG R: AAAACCTCAAGGTGGATTGAGA	123	60	A/G	0.0000	0.1831	0.1000	1.0000	0.0000*
CNP256	F: TTTCATGGTAGATGAGTAGCATCC R: GCGAGGAAATCCGAGTCTTA	86	60	C/T	0.7407	0.4752	0.3704	− 0.5882	0.0030
CNP259	F: AGTCAGCCCTGGAGCACTTA R: GTGAAGCCAGTCCTTGAAGC	116	60	T/C	0.2258	0.2036	0.1129	− 0.1273	0.5148
CNP269	F: TGGAATGTTTCATTTTCCGC R: TGTCAGCAAAGTGTACAAAAAGG	115	60	A/C	0.3448	0.4065	0.2759	0.1369	0.4011

Table 1 (continued)

Locus ID	Primer sequence (5′–3′)	Size (bp)	Tm ( )	Types	*Ho*	*He*	MAF	F_{IS}	P_{HWE}
CNP270	F: ATCCCATGGTGCATTCAAGT R: CATAGGAGGACTCGGCTGAC	81	60	G/T	0.3750	0.3095	0.1875	− 0.2308	0.2142
CNP273	F: TGGAGCGCATTTCTTCATAA R: TGCCAATTGTAATGGACGAT	91	60	C/T	0.2812	0.2455	0.1406	− 0.1636	0.3860
CNP274	F: ACCCATTAGAGGTCGAGGGT R: GAGCATTTTAACACCCGTGC	105	60	T/C	0.2812	0.2455	0.1406	− 0.1636	0.3860
CNP282	F: TGGCAAACTTGTCGGTATCC R: TCCTCATCGCTTACATTCCA	97	60	C/T	0.7143	0.4675	0.3571	− 0.5556	0.0043
CNP283	F: ACCGGTAATTTGAACACCGA R: GGGGGTTGTTTACTTAGGTGC	98	60	C/T	0.5556	0.4088	0.2778	− 0.3846	0.0549
CNP288	F: GGGGTTCCGTTGGAATTATC R: TGTGGGAGTACCTTTTTGGC	85	60	G/A	0.4194	0.3728	0.2419	− 0.1433	0.4744
CNP291	F: GGGGGAACACCTGTCACTAA R: CCCTCCAGAATCAGACATCC	112	60	T/C	0.6452	0.4442	0.3226	− 0.4762	0.0101
CNP292	F: GTTCAACGAGCACCCTTCTC R: TTTACCTGGAAAGACCCTGC	84	60	A/C	0.3125	0.2679	0.1562	− 0.1852	0.3235
CNP294	F: CTCATGCCTTTGGAATGGTT R: TCGGTGTTTACTTTTTGCATCTT	92	60	T/C	0.1935	0.1777	0.0968	− 0.1071	0.5888
CNP295	F: TCCTGTGTCAGATAAAGCTCCA R: GACATTCACAGATACACAGCCC	117	60	T/C	0.2812	0.2455	0.1406	− 0.1636	0.3860
CNP298	F: GCAAAATTCAGTGGTAGAGGAAA R: CAGATCCCTGTGTATAAGGACCA	81	60	G/A	0.4828	0.3727	0.2414	− 0.3182	0.1008
CNP299	F: TCAAGGCAAAATGGATTCTATG R: GGATGGTTTTGTATGCCGAC	86	60	C/T	0.2143	0.1948	0.1071	− 0.1200	0.5653
CNP303	F: TTCAAAGAATCGCCATAGCA R: CTTCAGATTTCGGGATGGAG	107	60	G/A	0.3548	0.2967	0.1774	− 0.2157	0.2553
CNP307	F: TTGCCCCCTAAATGAACATC R: AATTGTGGGCATTTGGATCA	110	60	A/G	0.1875	0.1726	0.0938	− 0.1034	0.5958
CNP308	F: CTATCCAGGAGCCTTTGTGC R: CAGGCAATGAAGGGGACTTA	116	60	C/T	0.6429	0.4442	0.3214	− 0.4737	0.0153
CNP312	F: CTCTCTCCAATGGAAAAACAGA R: GTTTCCGGAATCCTTTTGGT	80	60	T/C	0.7500	0.4762	0.3750	− 0.6000	0.0009
CNP315	F: CTGTAAGCGATTCGATCGTG R: GAAACCGATCGGAGTTCAAA	85	60	G/A	0.3125	0.2679	0.1562	− 0.1852	0.3235
CNP316	F: GAGGATGCATTATCAGGGGA R: TTGACAATGAAAGTGTGTGGC	83	60	A/T	0.1379	0.1307	0.0690	− 0.0741	0.7319
CNP319	F: AATTCCTGTCGGATACCCAG R: ACAAATCTAGCAGCGGAGGA	92	60	G/A	0.5161	0.3892	0.2581	− 0.3478	0.0622
CNP320	F: AATTCACTTGATTGGCATCC R: GCTTCGAGAAAAATGGTTGG	126	60	C/T	0.0625	0.0615	0.0312	− 0.0323	0.8981
CNP321	F: ATCTCGTCGTCGATGGAATC R: TTTTTGGGAATTGTGGGGTA	94	60	T/G	0.6875	0.4583	0.3438	− 0.5238	0.0039
CNP322	F: TCCAAAGTTTGTCAATGCTGA R: AGAAAACCTGTTCCAATGCG	97	60	G/C	0.3667	0.3045	0.1833	− 0.2245	0.2442
CNP324	F: TCCCAAGCCAAACTCCTAAA R: AATTGGACATGTGGGTCCTC	93	60	C/T	0.4062	0.3646	0.2344	− 0.1320	0.5057
CNP326	F: ATATTTTGAGGAAACGGGGC R: GTTGCCATTAACGGCTGTA	118	60	T/C	0.4375	0.3472	0.2188	− 0.2800	0.1291
CNP331	F: GAACTTGCCACCAACGAAAT R: TGTTCGTGGTCATTTTCCTG	82	60	T/C	0.5000	0.3814	0.2500	− 0.3333	0.0795
CNP333	F: GCCAATGTTACACACCAACG R: AGAGCAAACACACCTGAGGG	120	60	G/A	0.4688	0.3646	0.2344	− 0.3061	0.0962
CNP341	F: GATGACCCGGTAGTTGTGCT R: GTCGTAAGGGGGATGGGATA	86	60	A/C	0.4516	0.3554	0.2258	− 0.2917	0.1198

Table 1 (continued)

Locus ID	Primer sequence (5′–3′)	Size (bp)	Tm ( )	Types	*Ho*	*He*	MAF	F_{IS}	P_{HWE}
CNP344	F: CGTCAACATATTGCTGGCTG R: TGCACAGGATTTTAAAGACGG	84	60	A/G	0.8387	0.4950	0.4194	− 0.7222	0.0001*
CNP347	F: TCCCTCTTGCTCCAGCTCTA R: CACATTCATGGTCAAGGCAC	124	60	A/G	0.7097	0.4654	0.3548	− 0.5500	0.0029
CNP352	F: GGCATTGTTTGAAACTCGTG R: GTGCGATCTCCGGCTAAATA	119	60	C/A	0.0333	0.0333	0.0167	− 0.0169	1.0000
CNP353	F: AGCATTGTGTTTCCTCCTCC R: TGGCAGTGACCAGTATGTGTG	104	60	G/A	0.0333	0.4944	0.4167	0.9314	0.0000*
CNP356	F: CTTTCCCGTTTGTCACCACT R: TGTAGGAAGCTGGCAGTGAA	91	60	T/C	0.1290	0.3173	0.1935	0.5867	0.0006
CNP357	F: AGGAAACGTCGCAACTCAAC R: GATTTGATAGGGCCCTTGGT	96	60	G/C	0.1875	0.1726	0.0938	− 0.1034	0.5958
CNP365	F: CAATAGCAAGCTGTTGGTGC R: CCAATGCCTGATTGTCATTCT	92	60	T/C	0.5172	0.3902	0.2586	− 0.3488	0.0712
CNP368	F: ACAGTTTGTGTCTTCATCACGG R: CCCACAATTCTGTGCTGCTA	80	60	A/G	0.4815	0.3725	0.2407	− 0.3171	0.1158
CNP372	F: CATACATGGCTAAGACCCCG R: TCAAAATGACTTAATCCTGTCCA	91	60	A/G	0.4828	0.3727	0.2414	− 0.3182	0.1008
CNP375	F: ACGCGTCATCTGCAATCATA R: TGGTTTTGCCTTTTAAGTACGA	81	60	G/A	0.1290	0.1227	0.0645	− 0.0690	0.7414
CNP377	F: GCTTCGGTCTAAGTTCTCCG R: ATGGCTTTGTGGTAACCGAG	81	60	A/C	0.6897	0.4598	0.3448	− 0.5263	0.0059
CNP379	F: GCAAAGTTGAAGAAAAGAACTCC R: GATCTGGTCTTGGTTGGGAA	98	60	C/T	0.1562	0.2892	0.1719	0.4511	0.0067
CNP381	F: TTCGTTGTACAGACAAGCAACA R: CCCTGAACAGGTGTGTCAAA	91	60	T/C	0.4000	0.3254	0.2000	− 0.2500	0.1927
CNP382	F: TGCTAGCTGTTGTCAGTCGG R: TGGTGTACCTGACAGTTCCCT	85	60	T/C	0.1379	0.1307	0.0690	− 0.0741	0.7319
CNP384	F: CGCATAATGATGGCGATTCT R: GTTCTCTCCCTGTCACCCAA	109	60	A/G	0.0625	0.0615	0.0312	− 0.0323	0.8981
CNP385	F: ACCCAAACACTACGAGGACG R: TCCTTCATAGCTCGTTACTGACC	85	60	T/C	0.4062	0.3289	0.2031	− 0.2549	0.1685
CNP386	F: CCTCGCAAGAAACTACGCTT R: AGCAGCCAGGTTGAAGTGTT	117	60	A/G	0.5806	0.4188	0.2903	− 0.4091	0.0276
CNP387	F: TCATCTTGGAGCCTCAGTTG R: CTGCCATTCATCAACTGCTC	80	60	A/G	0.3125	0.2679	0.1562	− 0.1852	0.3235
CNP389	F: CGTGCATGATAGCATACATTCC R: GCGGGCAGATCGATTAGTAT	110	60	G/T	0.6786	0.4565	0.3393	− 0.5135	0.0085
CNP390	F: CAGTCGAAGACAAATGGCAA R: GAAAATTGTGTACCTTCCGCA	102	60	T/C	0.1562	0.1463	0.0781	− 0.0847	0.6704
CNP391	F: AAGTGCATCAATTTCTGTGGA R: CAGAGCCAGGCTTGTGATTT	106	60	G/A	0.1667	0.1554	0.0833	− 0.0909	0.6586
CNP400	F: ATACTCCGACGCCAAAGATG R: TAAGGGACTGTTCTCGGCAT	96	60	C/G	0.0938	0.0908	0.0469	− 0.0492	0.8216
CNP402	F: ATTCTGTCCGCGTTTTTGAC R: CGATCAACATTGCCTCTTCA	114	60	G/T	0.0625	0.0615	0.0312	− 0.0323	0.8981
CNP405	F: TCTCTACCCATCTCCCATAGAAA R: AGGCAACATTTGCTAAAGCC	91	60	A/G	0.2500	0.2222	0.1250	− 0.1429	0.4528
CNP409	F: CCTTTGGGTGGAGAAAACAA R: GCCTTGTCCATGTGGAATCT	116	60	G/A	0.8387	0.4950	0.4194	− 0.7222	0.0001*
CNP417	F: TCTTTAAAAGCCCTCCCCCT R: GCGGATACTAATTCCTTGCG	101	60	T/G	0.0938	0.0908	0.0469	− 0.0492	0.8216
CNP420	F: GATGCCTGCCTTCAATCAAT R: AAATTCTTTTCCCTCTCCAGC	81	60	C/T	0.4815	0.3725	0.2407	− 0.3171	0.1158

Ho observed heterozygosity, *He* expected heterozygosity, *MAF* minor allele frequency, F_{IS} inbreeding coefficient, P_{HWE}, the *P* values for Hardy–Weinberg equilibrium test (*$P < 0.05/108 = 0.0005$)

generated by heating samples from 60 to 90 °C with 25 data acquisitions per degree. At the end of each PCR reaction, the Light Cycler® 480 Gene Scanning Software was used to determine genotypes by analyzing the peaks in the melting curve. The minor allele frequency (MAF), expected heterozygosity (*He*), observed heterozygosity (*Ho*), inbreeding coefficient (F_{IS}; Weir and Cockerham 1984) and χ^2 tests of deviations from Hardy–Weinberg equilibrium (HWE) were calculated using Popgene 1.32 (Yeh et al. 2000).

Of the 420 primer pairs, 108 SNP loci (25%) were polymorphic and produced distinct melting curves that can be genotyped by HRM (Table 1). The minor allele frequency was detected to be from 0.0167 to 0.4844. The observed heterozygosity (*Ho*) and expected heterozygosity (*He*) varied from 0 to 0.9688 and 0.0333 to 0.5079, respectively. The values of F_{IS} were estimated from −0.9394 to 1.0000. Eighteen SNPs showed significant deviation from HWE after the Bonferroni correction ($P < 0.0005$). These polymorphic SNP markers will be useful for further population genetic analysis, natural resource conservation and selective breeding of *C. nippona*.

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