Development and Characterization of 135 SNP Markers in Chiton *Acanthochitona Rubrolineatus*

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Abstract

Acanthochitona rubrolineatus (Lischke, 1873) (Polyplacophora, Neoloricata, Cryptoplacidae) is an important species widely distributed in the middle and low tide zone of coastal intertidal zone along the China Sea coast. In recent years, the serious pollution of seawater has caused a sharp decline in the number of wild populations of *A. rubrolineatus*. Lacking of effective molecular markers limits the effective protection and management of this species. Studies for the isolation and characterization of 135 *A. rubrolineatus* SNPs markers were carried out. The frequency of minor allele ranges from 0.0125 to 0.5000. The observed heterozygosity and expected heterozygosity are 0.0000-0.9750, 0.0731-0.6696, respectively. The inbreeding value varies from -0.2902 to 0.9966. Among them, there are 27 sites markedly differently from Hardy-Weinberg equilibrium ($P < 0.05$). The study of the polymorphic SNPs will provide a theoretical basis for further analysis of population genetic analysis on *A. rubrolineatus*.

Main Text

Acanthochitona rubrolineatus belongs to Mollusca, Polyplacophora, which is a special branch in the evolution tree of Mollusca. In the 500 million years, their shape and living habits have not changed markedly (Denny M W et al. 2008). Therefore, they have great value in studies of speciation and the evolution (Scherholz M et al. 2013). Now, studies on this species have been focused on its great economical values on new magnetic materials and biopharmaceuticals. According to ancient Chinese medical books, *A. rubrolineatus* can be used to treat leprosy and tuberculosis. Moreover, their extracts have immunological activity against tumor cells (Zhang L et al. 2005). In addition, the radula teeth of *A. rubrolineatus* is one of the few structures that can synthesize nano-single-domain magnetic materials in vivo (Qian X. 2018; Fleissner G et al. 2007; Liu C. 2001). However, due to the frequent human activities and ecological environment destruction, the wild resources of *A. rubrolineatus* are declining sharply in China (Zhuang S et al. 2001). To better protect wild resources, we performed an investigation in the population genetics of *A. rubrolineatus*.

Single nucleotide polymorphisms (SNPs), the third generation molecular marker, are mainly used to analyze DNA sequence polymorphism caused by genomic nucleotide level mutation (Kaikai et al. 2018). Genetic research based on SNP markers has several advantages, such as high frequency in genome and quickly genotype of many individuals (Schork N et al. 2000).

In this study, 40 *A. rubrolineatus* individuals were collected from Yantai coast, Shandong Province, China (37°27'N/121°30'E). Genomic DNA was extracted from blood samples using the DNeasy Blood & Tissue kit (QIAGEN, Germany) according to the manufacturer's instructions. Then RAD library construction, sample indexing and pooling followed for the natural populations (Baird et al. 2008). 287581 putative SNPs were judged from *A. rubrolineatus* with Illumina HiSeq 4000 (Shanghai BIOZERON Co., Ltd) performing the paired end (150-bp) sequencing to obtain SNP markers resource.

Twenty-five primer pairs were designed by Primer v3.0. According to the instructions of manufacturer (GeneStar, Beijing, China), PCR in a 25 µl volume with GenStar PCR Mix. The PCR amplification cycle was as below: the initial denaturation at 94 °C was carried out 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), 72 °C for 30 s; Finally, extend at 72 °C for 7 min. Amplified samples were purified by gel extraction and sequenced on ABI 3730 DNA analyzer (Applied Biosystems). For confirmed loci, the minor allele frequency (MAF), observed heterozygosity (HO), expected heterozygosity (HE), polymorphic information content (PIC), inbreeding coefficient (FIS), and P-values representing the deviations from the Hardy-Weinberg equilibrium (HWE) were involved in the statistics (Kalinowski et al. 2007).

135 SNP sequences were confirmed by Sanger sequencing. The minor allele frequency ranged from 0.0125 to 0.5000 (Table 1). The observed heterozygosity and expected heterozygosity were 0.0000-0.9750 and 0.0731-0.6696, respectively. In addition, Polymorphism information content ranged from 0.0696 to 0.5870, and inbreeding coefficient ranged from -0.2902 to 0.9966. Only 27 loci showed significant deviations from HWE after Bonferroni correction ($P < 0.05$). These SNP markers are of great significance to population genetic analysis of species.

Declarations

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Author contributions: Corresponding author contributed to the study conception and design. Shukui Zhang, Ying Qu and Zilin Jiang helped collect samples. Donghui Sun and Jirui Zhang were in charge of extracting genomic DNA. Zhikai Xing and Sen Wu helped collect data. Yan Ni, Chenghong

Wang and Yunqi Song were in charge of compiling the data. The first draft of the manuscript was written by Jirui Zhang 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 Characterization of 135 SNP markers in chiton *Acanthochitona rubrolineatus*.

Primer ID	Primer sequence (5'-3')	Size (bp)	Tm (°C)	SNP type	SNP position				<i>HWE</i> P	<i>PIC</i>	<i>MAF</i>
110	F: TCTGAGACACTGTGTGATTAAGAGA R: TGATCATACGAGGAGGAAACGT	313	59	A/G	67	0.9000	0.5013	-0.2902	***	0.3725	0.4500
115	F: GAGAGAATGGGGGCGTCTTC R: CCTGTTACTGCAGTGC GTTG	317	60	G/A	54	0.1750	0.2066	0.0680	NS	0.1910	0.0875
152	F: GCAATGCCTGACTGTTGCTC R: CGGGGAGAGGACGAAATCAG	338	60	T/A	52	0.3000	0.4418	0.1641	NS	0.3955	0.1500
153	F: ATGCTGACTGTTGCTCACA R: TGTGGAGTATGTGTCTGGGC	262	60	T/G	87	0.3000	0.4418	0.1641	NS	0.3955	0.1500
255	F: TGC GCACTTAAATGACGAGC R: ACGGGCCGTAAATGCTAAC	338	60	T/A	75	0.9000	0.5051	-0.2868	***	0.3744	0.4750
843	F: CGACCGCCATTTAGCTGTA R: AAGTCAAAATTTGCGCCGGC	296	61	A/C	85	0.8750	0.4984	-0.2799	***	0.3711	0.4375
1091	F: AAGATGGCTGTAATTAACAAAAGA R: ACTCCAAAAGTACTGGGCCG	269	60	A/G	108	0.7000	0.4608	-0.2120	NS	0.3515	0.3500
1092	F: AAGATGGCTGTAATTAACAAAAGA R: ACTCCAAAAGTACTGGGCCG	293	60	A/T	109	0.7000	0.4608	-0.2120	NS	0.3515	0.3500
1145	F: CAAGACGTGGCCTGAATCCT R: GCTGCCGAATATACAACCGC	273	60	A/G	95	0.5750	0.4149	-0.1676	NS	0.3258	0.2875
14760	F: TCGCCAGTTGAGTATGACCC R: ATGGCTGCCGTACTCAAGAG	311	60	C/T	53	0.9500	0.5051	-0.3144	***	0.3744	0.4750
14955	F: TGA CTGGTCATTGTCCAAGGA R: ATTGTTGTGGAGGCGTTTGC	319	60	C/A	93	0.7250	0.4680	-0.2213	NS	0.3554	0.3625
15195	F: TTCTACCCCTACGTGGACCA R: ACCTCCGTATACGAGTCCTGA	311	60	G/C	68	0.9500	0.5051	-0.3144	***	0.3744	0.4750
15433	F: ATCTTACACTATTTATACACTGGGA R: AGAAGACAGATTCTGGAGCTGT	337	59	C/G	95	0.2750	0.2826	-0.0030	NS	0.2516	0.1375
15481	F: TCATTGGTTAGCTGCTCTGGT R: TCATCGGTTTGCCTCAGTCA	336	60	T/C	66	0.8750	0.4984	-0.2799	***	0.3711	0.4375
16111	F: TG TCACTCAATCACACATTGTCT R: TGGGGACAGAATAGTCTAGCCA	310	60	T/G	96	0.9750	0.5060	-0.3221	***	0.3748	0.4875
16287	F: C TACTGAGCTTCGGCCACAA R: GCGGCATTGTCAGAATAGGC	334	60	A/G	41	0.2000	0.1823	-0.0462	NS	0.1638	0.1000
16582	F: TCAGAACTGCTTGACCCTGG R: TAATGAAACGTGTGTCGCGC	273	60	A/G	97	0.8000	0.4861	-0.2499	*	0.3648	0.4000
16714	F: TGCTTGCGTTTTAAACCACCG R: TGCTTGCGTTTTAAACCACCG	335	60	C/T	73	0.9750	0.5060	-0.3221	***	0.3748	0.4875
47003	F: ACAAGAGGCTCCAAATTATCCTT R: CCGTGGTTTGGCGGTAATTC	278	60	T/C	77	0.8500	0.4949	-0.2698	**	0.3693	0.4250
63291	F: GGGGCCCATAGCGTGAATT R: TTCGACTGCTACACGGATCC	326	60	T/C	40	0.0750	0.3522	0.6463	NS	0.3080	0.0375

63292	F: GGGGCCCATAGCGTGAATT R: GCCAGTCCGCCAGTTTAAGA	315	60	T/C	83	0.0750	0.3522	0.6463	NS	0.3080	0.0375
64525	F: GCTCCTGCTGGTTTTCGAAC R: TTCTGGTTCCCCTGCGTTTT	318	60	G/C	50	0.4750	0.4497	-0.0453	NS	0.3652	0.2875
65017	F: GTAGGTTGCCAGCTCACTGT R: GGAGCTAAGCACGCGTAGTA	272	60	A/G	84	0.1250	0.3946	0.5068	NS	0.3557	0.1375
67289	F: CGAACGATTAGAGCGGGACA R: GTAGGACCTAAACTGCCCGG	260	60	C/T	103	0.8500	0.4949	-0.2698	**	0.3693	0.4250
67481	F: TGTGTTTCCGTTGTTGTTGGT R: CTGGTGTGTGTATCTGCCCA	325	60	T/C	88	0.8500	0.4949	-0.2698	**	0.3693	0.4250
67913	F: GGGCAGGGGTGCAAAGTATA R: TCGCATCAACAACGTACACG	292	60	A/T	75	0.1750	0.5934	0.5352	*	0.5197	0.1875
68037	F: TCCAGTTCTGCATGAAATTATCCA R: CGGGAACCTCGGAAGAACCAA	310	60	G/A	97	0.2750	0.2402	-0.0699	NS	0.2091	0.1375
68108	F: AGTTTGCAAGCAATTTCAAGA R: ACGGAGGATTGTTGCCATT	341	60	G/A	41	0.9500	0.5051	-0.3114	***	0.3744	0.4750
97286	F: GAATACCCTTCCCGTGCACT R: GACTTTGCCGCCAAGCATAC	324	60	G/A	69	0.3250	0.3168	-0.0300	NS	0.2771	0.1625
97287	F: GGAATACCCTTCCCGTGCACT R: CCAGGGGCTTGATATTGGCA	281	60	G/A	77	0.3250	0.3168	-0.0300	NS	0.2771	0.1625
97451	F: TCCTCGGAACAATAATCCT R: GTCACCGCTGACTATCGACC	291	60	C/T	35	0.9000	0.5013	-0.2902	***	0.3725	0.4500
97605	F: CGAACGATTAGAGCGGGACA R: ATTTGACGGGACTCTGGTCG	324	60	G/A	95	0.3500	0.4722	0.1258	NS	0.3947	0.2750
98716	F: AGCGGTGAAACAGGTAAGGA R: CTCCCTCTGACTGGCTCCAT	338	61	T/C	43	0.9750	0.5060	-0.3221	***	0.3748	0.4875
101130	F: TCATGCCGAGTAGATATTCCA R: AGAATCTGTACCGTTGCCTCT	306	59	G/A	61	0.6250	0.5275	-0.1133	NS	0.4278	0.3625
102257	F: TGA CTGGTCAGGGACTTTGT R: ATCCCTGGAGCTGATGTCCT	292	60	G/A	70	0.2750	0.2402	-0.0699	NS	0.2091	0.1375
103281	F: TG TAGTTCTTGATATATTGCACA R: GGCCAACGAATGTTGGGAC	336	60	G/C	83	0.3000	0.3241	0.0323	NS	0.2688	0.2000
105060	F: GGCTTCGGCGATTTTCACG R: CCGTTCAGCGTTGACAGTTC	306	60	G/A	106	0.4000	0.3532	-0.0684	NS	0.2879	0.2250
115993	F: CGTTTCCGTGTTGTGTGGAA R: AACGGCGTAAAACCCAATCA	330	59	C/G	57	0.2250	0.3484	0.2012	NS	0.2996	0.1875
117340	F: CCAGCCAATCAAATCTTGCGG R: CTGGAAAACGCGCCGAATT	371	60	G/A	96	0.1500	0.1405	-0.0307	NS	0.1291	0.0750
117387	F: TCCTGTGGAGCCATTGAAGT R: GCCCATGGACATCAATGATGG	332	59	C/T	31	0.8250	0.4908	-0.2598	**	0.3672	0.4125
133437	F: TTTGCTATCCGAGACGCACC R: CCTGAGGTTGAGGCAAGAGG	294	60	C/A	48	0.0750	0.0731	-0.0102	NS	0.0696	0.0375
133496	F: TTTTCTGTAGAGAGAGATTCCA	326	56	T/C	89	0.2500	0.3911	0.2060	NS	0.3285	0.2250

R: AGACAAAAGCTTCACAAAGGA

153242	F: AGCAAGAGAAACACAGCAGC R: CGCTGTTGTGTTTCTCTGGC	328	60	G/A	97	0.9750	0.5060	-0.3221	***	0.3748	0.4875
159368	F: CACCGGTCAAGGACCTGTC R: AGGACGGAGGGACCACTAAA	342	60	G/T	97	0.9750	0.5060	-0.3221	***	0.3748	0.4875
159555	F: ATTGAATGAAGACTGGCGGT R: AACAGCAACTAAGACCCCGC	304	61	G/A	71	0.8750	0.5060	-0.2730	**	0.3748	0.4875
167472	F: AATTTCGAGTTGCCGGACTGT R: GGTTCATCAGGTACCCCG	302	60	T/C	58	0.2750	0.3168	0.0545	NS	0.2771	0.1625
167473	F: AATTTCGAGTTGCCGGACTGT R: ATACAGGAGTCGACGTCGGA	358	60	C/T	63	0.2750	0.3168	0.0545	NS	0.2771	0.1625
170581	F: ACCTTCTCTGTGAACGATCCA R: ACCATCAGATCTGACACGGC	302	60	A/G	45	0.2250	0.2022	-0.0545	NS	0.1797	0.1125
186929	F: CTCGACTACTGAGCTTCGGC R: GCCAGATCTGCGTCTTTCCT	342	60	T/C	60	0.4250	0.5389	0.0838	NS	0.4610	0.2875
186930	F: GACTACTGAGCTTCGGCCAA R: CTCACGTGACGTCTGCAGAA	341	60	G/A	105	0.4250	0.5389	0.0838	NS	0.4610	0.2875
188818	F: TTCCGACACAGGTATGGACA R: AGCCAAAACAGCATCCGTA	340	60	A/G	57	0.9750	0.5060	-0.3221	***	0.3748	0.4875
188819	F: TTCCGACACAGGTATGGACA R: TGTCAAATGGGTTGGTGCCT	345	60	C/T	69	0.9750	0.5060	-0.3221	***	0.3748	0.4875
189292	F: TTCAAGGCTGTGATTTAGGGT R: ACGCTCAAAGTACGAGTCCA	353	60	A/T	92	0.7250	0.4908	-0.1987	NS	0.3672	0.4125
189308	F: ATTCCGTACTAGCCACATGT R: AAAGACCAGCGAGTCATGGG	355	60	C/G	89	0.9500	0.5051	-0.3114	***	0.3744	0.4750
190334	F: ATGCACTGATCCTGCTGGTT R: GCTGTTGGTTTCCGCACAAT	336	60	C/A	64	0.1250	0.2085	0.2356	NS	0.1958	0.0625
191667	F: ATGGAAAGTGTCGCTGGTA R: AGTAGGCCACTGTGAAACGG	269	60	T/C	52	0.3250	0.5282	0.2087	NS	0.4613	0.2375
191668	F: ATGGAAAGTGTCGCTGGTA R: AGTAGGCCACTGTGAAACGG	269	60	G/C	102	0.3250	0.5282	0.2087	NS	0.4613	0.2375
192722	F: AAAACGACCGGCACATGTTT R: ATCAATATGTCCGGCCGTT	349	60	C/A	34	0.7000	0.6696	-0.0693	NS	0.5870	0.3500
192723	F: AAAACGACCGGCACATGTTT R: ACACCTAGGCCCATCAGAT	341	60	T/A	35	0.7000	0.6696	-0.0693	NS	0.5870	0.3500
193182	F: AGTTCCACGTACAATGCTAGAG R: ACGCTGTTTCCGGCACATAA	311	61	C/T	84	0.4750	0.5275	0.0265	NS	0.4278	0.3625
202668	F: ATGATTCCACCTCGCTCACT R: CGGCCATAAAACACCATGGT	274	59	T/C	86	0.0750	0.4839	0.7268	NS	0.4298	0.1375
216926	F: GAACGATTAGAGCGGGGCAT R: TGCAGCTCTTCCGATCTTCC	322	60	A/T	78	0.1250	0.3225	0.4284	NS	0.2901	0.1375
227919	F: TCAGCACATTTTGCGTTGG R: AAGTGGCCGCTTTAAGCAAC	338	60	C/T	96	0.1000	0.2924	0.4842	NS	0.2471	0.1750

228342	F: ATTGCACAATATAGTGACATCTATG R: AAGCGCACATCAATGAGACT	315	58	G/A	66	0.2750	0.3085	0.0512	NS	0.2583	0.1875
228862	F: TTCGGCAGTACAATCCCCAC R: TGA AAAACAATGGCGGACGTG	336	60	C/A	108	0.1750	0.3934	0.3720	NS	0.3530	0.0875
229977	F: ACCTCACTGTTTCCTTCCGG R: TAACGCAACTCAACTTCGCG	268	60	T/C	108	0.4250	0.4617	0.0159	NS	0.3881	0.2625
236835	F: TCTGAGACACTGTGTGATTAAGAGA R: ACGGTTGTAAGATGTTTGTGCC	299	60	C/A	75	0.5000	0.3797	-0.1423	NS	0.3047	0.2500
237216	F: AGCATTGCCTGAGAACAGTCA R: AGTGGAAGGCAAGCATGTTG	323	60	G/C	89	0.6000	0.4861	-0.1111	NS	0.3648	0.4000
257954	F: TCAAACCTCGAAGTGTTTCATTGA R: AACTCATTGCCCCCTGTGAG	294	60	A/C	59	0.5000	0.5304	0.0120	NS	0.4103	0.4750
258126	F: TAAGAGGGCACGGAAGAGGA R: ACGCATCTGCTTAGACAAATGC	329	60	C/G	97	0.9500	0.5063	-0.3103	***	0.3750	0.5000
258935	F: ACACCTGTAAACACGACTTGC R: AGAAAGGCCGTATAGCTGCG	270	60	T/A	47	0.5500	0.5215	-0.0542	NS	0.4244	0.3500
268378	F: TATCCGCTACCCCGCATAGA R: TGCGAAGTCTACCAGAACCG	280	60	C/T	65	0.4000	0.5051	0.0999	NS	0.3969	0.3750
270177	F: TAACTGCATTGTGTTGGGCT R: ACTGTTGATTACACCGGGCA	304	60	C/T	106	0.2250	0.2022	-0.0540	NS	0.1797	0.1125
270725	F: CGCTTGACGTGACTTCTGGA R: GGCGTTTTGAGGCCTGTTATT	263	60	C/A	67	0.0750	0.1649	0.3572	NS	0.1553	0.0625
273467	F: CAAAATGCAGCCGTATCTGGA R: CGGTTCCAATCAGTGAAACACC	343	60	T/C	73	0.1000	0.2684	0.4471	NS	0.2469	0.0500
277151	F: CTCAGCCGTGGTGCTAAGA R: CACACAAACGGAACCAACCA	317	60	T/A	62	0.0250	0.3579	0.8633	NS	0.3205	0.0625
286890	F: TCCTAAACTACAAAATAATTCAATCCT R: ACATCCTGTCGTGCCTTGTT	344	60	A/T	50	0.4000	0.5418	0.1272	NS	0.4359	0.4000
286896	F: TTTTGAGAAACCTTTTGCGAA R: TCGGAAGGTGAGGTTAAACGT	275	59	T/C	92	0.1000	0.2291	0.3782	NS	0.2138	0.0750
286910	F: TTCCATTTGTCAAATTCATCGAT R: AACTGTTGCCCAAAATGATGG	352	60	C/T	27	0.1000	0.1873	0.2897	NS	0.1769	0.0500
286921	F: CCCAGTACCCGTGTCATCAG R: GCCATTGCACTGATGACGAC	268	60	T/C	97	0.1000	0.1873	0.2897	NS	0.1769	0.0500
286938	F: TCAGTTTGAATCACTGTGACA R: GTCAGTTTTCTGCCACAGCG	299	60	G/A	86	0.1250	0.2066	0.2332	NS	0.1910	0.0875
286939	F: ATTCCCTAAGCCCTAGTGCC R: GGATCACGATAAAACGCGGC	288	60	C/T	53	0.2250	0.5282	0.3841	NS	0.4430	0.3125
286945	F: ACCATTTTCTCCACGTCCCT R: CGTTCTGCCAGGAGGTGATT	321	60	G/T	91	0.1750	0.4839	0.4562	NS	0.4298	0.1375
286946	F: TCGGACAATAGTTAGAGCAGCT R: AAACCAGCAGGATCCATGCA	255	60	A/G	97	0.1250	0.1649	0.1240	NS	0.1553	0.0625
286949	F: GTTCGAAAACAGCAGAATCCA	256	60	T/C	95	0.1000	0.2266	0.3750	NS	0.2075	0.1000

	R: AAACCAGCAGCCGATCAGAA										
286953	F: GCAACAGGGTCTTACTCGCT R: CCGCGTTGTTTCAGTCGATC	303	60	T/G	86	0.3000	0.6127	0.3154	NS	0.5270	0.3500
286957	F: ATTCATGCCCTTTGGACCCC R: TGACGGCAGGCCAGTTTTTA	332	60	T/G	45	0.4500	0.5177	0.0323	NS	0.4537	0.2250
286963	F: CTTGCCAGCTCTACACTCC R: GAGACGTGTGGAAGATGGGG	284	60	G/A	57	0.3500	0.5468	0.1898	NS	0.4662	0.3000
286970	F: TTCAGTTGTCGGGGAAGCTCG R: AACTTTTCGTTTCTGAAGCGT	332	60	A/G	47	0.2000	0.5924	0.4777	*	0.5129	0.3000
286981	F: CGGTCAGCAATTATGGAAATGCA R: CAGACGACATGCTCTCGTGT	274	60	A/C	55	0.3250	0.4617	0.1518	NS	0.3881	0.2625
286985	F: TGGGGTTTTACGACGTACTCA R: TGCTGGATGTTGCTGTTTTCC	304	60	C/T	79	0.0750	0.3256	0.6156	NS	0.2973	0.1125
286986	F: TTCGCTGATCTGGGTGCTAC R: AAGAAGAAACGGCGCTGTCA	337	61	A/C	98	0.3250	0.4560	0.1387	NS	0.4068	0.1625
286993	F: GCAATTGTTGATTCCAGCGC R: TGAGAGTTTGGCGCTGGAAT	283	60	G/A	110	0.1500	0.3430	0.3798	NS	0.3114	0.0750
286997	F: TCGTTTTCCATAATACATTCATCGA R: TGATTGAGGTTTTCCGCCGT	335	60	G/A	34	0.1000	0.2266	0.3750	NS	0.2075	0.1000
287004	F: ACAATAACACGCTTTAACACACA R: CGATCGCATCGCCAACAATT	278	60	A/T	82	0.0750	0.1655	0.3590	NS	0.1569	0.0375
287008	F: CGACACTTGTTAACGTTTCGTCC R: GGTGGAGAAACCCCTGGAAG	329	60	C/T	40	0.1500	0.2684	0.2670	NS	0.2469	0.1000
287013	F: TCATTGCATTAGCCAACAGC R: AGCTTCTGATGTTGCTGTTG	296	59	T/C	55	0.1000	0.3430	0.5375	NS	0.3114	0.1250
287019	F: ACGATTAGAGTGGGACATGGA R: GCTCTGCCACGCTTGTTTAG	292	60	T/A	74	0.0750	0.2079	0.4553	NS	0.1942	0.0375
287020	F: TTAACCACCAGCTGCGACAA R: GCTTCGCTTGTCCTCCTCTT	328	60	G/A	69	0.1250	0.2491	0.3171	NS	0.2308	0.0875
287033	F: TGGTTTCTCCTTCCATAAGTGT R: AGCTCTTGAGGGATGTGC	282	60	T/C	40	0.3250	0.3668	0.0541	NS	0.2966	0.2375
287041	F: ACTTCAGCTTCACCACCCAG R: CGTGTGTGATGGACCGGTAA	298	60	A/G	90	0.1500	0.4101	0.4499	NS	0.3680	0.1500
287045	F: AGTCAAACAGCAACAACGGC R: CCGAAGCCTGTAATCCAGACA	262	60	T/C	66	0.2000	0.6367	0.5050	*	0.5501	0.4000
287057	F: ACGAGTTACTATTTTATATGTTGAACG R: ACACCACAGCGTGCAATAGA	250	60	A/G	110	0.2000	0.3759	0.2862	NS	0.3372	0.1500
287062	F: ACAGCATATCATGACAAAGGTGT R: GCGTTTGCAATTGAGTACGGT	334	59	C/G	99	0.0500	0.1873	0.5516	NS	0.1769	0.0500
287065	F: ACATTGTCATTTGCACCACA R: TCTGAAGAACAAGGGTGCCC	285	60	A/C	96	0.3500	0.4772	0.1383	NS	0.3813	0.3250
287083	F: TTCGCCGAAATACAGCAAAT R: TTCGGCTGAAACTTGACGGA	353	60	C/G	49	0.0000	0.4101	0.9966	NS	0.3680	0.1500

287083	F: TCGCCGAAATACAGCAAATT R: TCTCGATTGTGCAAGCATGG	328	59	T/C	106	0.1750	0.4782	0.4479	NS	0.4191	0.2125
287138	F: GGGGCCCCATAGCGTGAATT R: TATAAATCCGCCAGTCCGCC	307	60	T/A	79	0.3750	0.5453	0.1626	NS	0.4378	0.4125
287182	F: TGAACGCTCTACTTTATAACCTGCT R: GTTGTCCACCTAGCCACGAA	337	60	G/C	64	0.1000	0.4266	0.6118	NS	0.3651	0.2250
287192	F: TTCCACCAACTAGCGTCTG R: TGAGGAAAGTTTGGACCATTTGA	313	60	A/T	107	0.0500	0.1873	0.5516	NS	0.1769	0.0500
287224	F: TCCAGGCAATATAATAAGTGGCGA R: TATACAACAGTGCCGCGTGT	311	60	C/T	94	0.0750	0.1206	0.2179	NS	0.1155	0.0375
287240	F: TCTGTCCTCGAAGACCACCA R: TGCCATATATTCTTGCCCCGG	273	60	C/A	47	0.0500	0.0978	0.2936	NS	0.0949	0.0125
287283	F: TGCAAGGCATAACATAAAAGAAA R: TCTGGCGTTGGTTTCTGAGA	259	59	G/A	71	0.1000	0.1861	0.2875	NS	0.1736	0.0750
287292	F: AGGTCATAAATATAACGGATGACT R: ACTTCGAGTTTAGGTGCCTGA	310	59	G/A	69	0.0750	0.0731	-0.0102	NS	0.0696	0.0375
287298	F: TCCCCAATCGTCAAAATGCA R: ACATTCGGTATTCCAGAACCTGT	355	60	G/T	45	0.2500	0.4595	0.2735	NS	0.3981	0.2250
287301	F: ACACCTCCATTTTACAACTGTCA R: GCGGCGTTAAACCCCAATC	348	60	A/T	106	0.0750	0.2079	0.4553	NS	0.1942	0.0375
287340	F: TCCAACGTGTACAGATATACATCAACT R: AGAACCCACTGAAATTTGTTGCT	282	59	C/A	50	0.2250	0.4136	0.2775	NS	0.3564	0.2125
287355	F: AGCATTTGCAAAGTGGAGTGC R: GCTGGCAGATCTAGCTGTCC	330	60	C/G	86	0.1500	0.4899	0.5176	NS	0.4275	0.2250
287387	F: TTGTGCGATTTTTAGAAAATGGA R: ACACATTTCTTAACGTTGGGT	314	56	T/C	90	0.1500	0.4342	0.4726	NS	0.3803	0.2000
287412	F: TCCAAAGACAGCCTTAAGTGACT R: ACAACTGAACCTGCCCGTAC	278	60	G/A	81	0.1000	0.1430	0.1640	NS	0.1359	0.0500
287413	F: ATCCACCGTTTCGAAAGCAGT R: TAATTCAATCCGCCTGGGCT	310	60	A/C	108	0.2000	0.3392	0.2403	NS	0.3029	0.1500
287424	F: TCGAGCAAAAATTAATTGAAAGCT R: GTGTTAAGGCTGGGGAGGAC	283	60	A/G	109	0.2250	0.4250	0.2863	NS	0.3796	0.1625
287438	F: TCCTTGAAAGTTTCACGGGA R: GGCACGAGATGTCCAAGGAA	321	60	C/A	91	0.0500	0.1434	0.4509	NS	0.1367	0.0125
287440	F: TTCTTTCTGTAAACATATGCCCA R: ACCATAACAAAAACAGAAGCCA	314	57	T/C	71	0.1750	0.3554	0.3243	NS	0.3150	0.1625
287443	F: ACTGATCAGCGTAACTACAGGT R: AGCTTGCCAGAAACATCCA	353	60	A/G	97	0.2000	0.5025	0.4137	NS	0.4267	0.2750
287446	F: ATTCACTGTTTACTGTTTTGTGTTT R: GATTGTGTCATCACGCGGTC	284	60	G/T	84	0.1000	0.1873	0.2897	NS	0.1769	0.0500
287478	F: GGTGTTGGTTTCATGCGACG R: ACGTCACACAAAGGGGCATA	329	60	A/G	104	0.1250	0.5953	0.6454	***	0.5226	0.2125
287512	F: TTCCTGACCCTGCCAACATC	345	60	G/T	27	0.0250	0.2079	0.7469	NS	0.1942	0.0375

	R: ACACCAGTCAAAAGATCTTCTGA										
287513	F: ACAGTAAGATTAAGTTTACCTGAGCT R: GGCCCATAAATGGAGCTCGAA	336	60	G/A	48	0.0750	0.1206	0.2197	NS	0.1155	0.0375
287514	F: TTGCTGGTCATCTGAGGTGT R: ACAAGTGACAGTGACCATCA	341	59	C/T	51	0.1000	0.1405	0.1617	NS	0.1291	0.0750
287523	F: GGTTTAAACCCATGCGCGTT R: GCTGACGGTGTTGTTGTTGG	281	60	T/C	53	0.3500	0.5051	0.1662	NS	0.3969	0.3750
287534	F: AGAGTGAAGGGACTCAACCA R: TCGCATCCATGGCTTATCT	304	60	T/G	110	0.0750	0.2497	0.5247	NS	0.2323	0.0625
287536	F: ACCTTATCTTTTAAATGCACCTGT R: TCGTTCATGCAGACAAGAGATGA	259	60	G/A	100	0.2000	0.4658	0.3805	NS	0.4120	0.2000
287578	F: TGTGCGCCCTGAATTACAGT R: CGTGGTGCACAGTCAATTCC	331	60	T/G	54	0.1000	0.0962	-0.0164	NS	0.0905	0.0500
287581	F: TTCCAGTGGTGAATCCCGAA R: ATAGCACTTTCGCCTGGAGC	353	60	A/G	55	0.1250	0.4206	0.5300	NS	0.3706	0.1875

T_m annealing temperature, H_o observed heterozygosity, H_e expected heterozygosity, F_{IS} inbreeding coefficient, HWE results for Hardy–Weinberg Equilibrium test, PIC polymorphism information content, MAF minor allele frequency, NS non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$