Development And Identification of 131 SNP Markers In *Sthenoteuthis Pteropus* (Steenstrup)

Yan Zhao

Shanghai Ocean University

Congcong Wang (✉ ccwang@shou.edu.cn)

Shanghai Ocean University <https://orcid.org/0000-0001-8420-2002>

Bilin Liu

Shanghai Ocean University

Gang Li

Shanghai Ocean University

Hao Xu

Shanghai Ocean University

Research Article

Keywords: *Sthenoteuthis pteropus*, SNP markers, Genetic diversity, Double digest restriction-site associated DNA sequencing (dd-RAD)

Posted Date: December 28th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1147398/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

The orangeback flying squid, *Sthenoteuthis pteropus*, is a species of significant potential value that is widely distributed in the tropical and temperate waters of the Atlantic Ocean. There have been no reports of the population genetics and effective molecular markers for this species due to a lack of reliable information regarding its genetic structure and its many individual differences, as well as its complex and changeable life history. Therefore, the development of auxiliary molecular markers would contribute to the development, sustainable utilization, and protection of the species. In this study, 131 novel single nucleotide polymorphism (SNP) markers were developed by double digest restriction-site associated DNA sequencing (dd-RAD). The observed heterozygosity (H_o) and expected heterozygosity (H_e) ranged from 0.00 to 0.80 and 0.18 to 0.50, respectively. The polymorphism information content (PIC) value ranged from 0.18 to 0.50. None of the marker locations significantly deviated from the Hardy-Weinberg equilibrium ($p > 0.05$) after a Bonferroni correction. These polymorphic SNPs will be important in the further analysis of the population heredity of *S. pteropus* and its scientific management.

Full Text

Sthenoteuthis pteropus, is an economically important species that is widely distributed in the western Atlantic Ocean (from the Madeira Islands to the western Gulf of Guinea) and the eastern Atlantic Ocean (from the Nova Scotia Peninsula to the Gulf of Mexico and the Caribbean) (Merten et al. 2017; Chen et al. 2009). The instantaneous biomass of the species is in the range of 4.2-6.5 million tons, and the annual total biological yield is estimated to be 34-52 million tons, but a dedicated fishery had not yet been established (Merten 2016). Existing studies have mainly focused on the microelement content, age, and growth of *S. pteropus* (Lischka et al. 2018; Laptikhovsky et al. 1993; Arkhipkin and Mikheev. 1992). There have been few studies of the population genetics of the species, and corresponding molecular markers have not been developed. It is therefore necessary to study the Atlantic population of *S. pteropus*, especially with regard to its population genetics and the corresponding molecular markers.

In recent years, with the development of double digest restriction-site associated DNA sequencing (dd-RAD), single nucleotide polymorphism (SNP) markers have been widely used in population genetics due to their wide genome coverage, representativeness, and ease of automatic analysis (Xu et al. 2020; Li et al. 2021). There have been few population genetics studies of *S. pteropus*. Therefore, it is necessary to develop and screen a set of easily available SNP loci, which will contribute to the sustainable development and utilization of the species and the conservation of population diversity. In this study, a number of SNP markers of *S. pteropus* were developed and effectively identified by dd-RAD.

Muscle samples of 55 *S. pteropus* individuals were collected in the Middle East Atlantic Ocean ($n=55$, $1^{\circ}12' \text{ W}$ - $8^{\circ}00' \text{ W}$, $1^{\circ}16' \text{ S}$ - $5^{\circ}01' \text{ S}$). Genomic DNA was extracted by the phenol-chloroform method (Russell and Sambrook. 2001). The quality of DNA was determined by 0.8% agarose gel electrophoresis, and DNA was quantified using a UV spectrophotometer. Five *S. pteropus* individuals were randomly selected for database construction following dd-RAD, and Illumina NovaSeqTM and PE150 was used for sequencing. A total of 16.92 G raw data were obtained by Illumina double-terminal sequencing, with an average of 3.38 G per sample. The base quality, Q30, reached 91.37% of reads. After resetting the screening parameters, the minor allele frequencies (MAF) were 0.05-0.10, the deletion rate was ≤ 0.05 , and the minimum sequencing depth was 5, with a total of 1896 SNPs obtained. To improve the reliability, we screened SNP loci from 70 to 90 bp and retained 131 SNP markers. The 131 SNP markers were detected by a multiplex polymerase chain reaction (PCR) using 50 *S. pteropus* individuals. The PCR amplification primers and single base extension primers were designed using the Massarray Assay Design software (Sequenom, San Diego, CA, USA). The PCR reaction system included 1 μL DNA (10-30 ng/ μL), 0.25 μL of each primer, and 1.1 μL PCR mix, with ddH₂O added

to 5 μ L of the final mixture. The PCR reaction conditions were as follows: 94°C for 3 min, then 40 cycles of 94°C for 30 s, an annealing temperature of 56°C for 25 s, an extension at 72°C for 30 s, and a final extension at 72°C for 3 min, with the reaction saved at 4°C. The amplified products were detected by a Massarray Analyzer Compac Mass spectrometer (Sequenom), the results were analyzed using TYPER software, and SNP markers were detected by the SNP typing results. For the validated sequence of SNPs, observed heterozygosity (H_o) and expected heterozygosity (H_e) were calculated using the Stacks Pipeline analysis tool and the population computing program (Catchen et al. 2013). The polymorphism information content (PIC) value and Hardy-Weinberg equilibrium were estimated and tested using PowerMarker V3.25 software (Liu and Muse. 2005). The linkage disequilibrium (LD) was calculated using PLINK 2.0 Alpha software (Chang et al. 2015).

All 131 SNP loci were detected in 50 *S. pteropus* individuals, and the 131 potential SNP markers were polymorphic and biallelic (Supplementary Table 1). The statistics of genetic diversity are shown in Table 1. The ranges of H_o and H_e were 0.00 to 0.80 and 0.18 to 0.50, respectively. The PIC ranged from 0.18 to 0.50. After a Bonferroni correction, all loci were in accordance with the Hardy-Weinberg equilibrium. No significant LD was detected. To the best of our knowledge, this is the first SNP marker developed for *S. pteropus*. These new SNP markers will enable further population genetics analysis and a better understanding of this important species, which will lead to the use of *S. pteropus* resources and the protection of its population.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Funding information

This work was sponsored by the National Key R&D Program of China (2019YFD0901404), the Program for Professor of Special Appointment (Eastern Scholar) at Shanghai Institutions of Higher Learning under contract 0810000243, the National Nature Science Foundation of China (41876141) and the Shanghai Science and Technology Innovation Action Plan (19DZ1207502).

Authors' contributions

Yan Zhao carried out all the experiments; Congcong Wang and Hao Xu designed the methods and experiments, interpreted the results and finished the discussion. Bilin Liu and Gang Li were responsible for overall supervision, and participated in coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Experimental protocols involving live animals were approved by the Ethics Committee for the Use of Animal Subjects of Shanghai Ocean University.

References

1. Arkhipkin A, Mikheev A (1992) Age and growth of the squid *Sthenoteuthis pteropus* (Oegopsida: Ommastrephidae) from the Central-East Atlantic. *Journal of Experimental Marine Biology & Ecology* 163(2):261–276. [https://doi.org/10.1016/0022-0981\(92\)90054-E](https://doi.org/10.1016/0022-0981(92)90054-E)

2. Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis tool set for population genomics. *Mol Ecol* 22(11):3124–3140. <https://doi.org/10.1111/mec.12354>
3. Chang CC, Chow CC, Cam TL, Shashaank V, Purcell SM, Lee JJ (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. *Giga Science* 4(1):7. <https://doi.org/10.1186/s13742-015-0047>
4. Chen XJ, Liu BL, Wang YG (2009) *Cephalopods of the world*. China Ocean Press
5. Laptikhovsky VV, Arkhipkin AI, Golub AA (1993) Larval age, growth and mortality in the oceanic squid *Sthenoteuthis pteropus* (Cephalopoda, Ommastrephidae) from the eastern tropical Atlantic. *J Plankton Res* 15(4):375–384. <https://doi.org/10.1093/plankt/15.4.375>
6. Lischka A, Lacoue-Labarthe T, Hoving H, Javidpour J, Pannell JL, Merten V, Churlaud C, Bustamante P (2018) High cadmium and mercury concentrations in the tissues of the orange-back flying squid, *Sthenoteuthis pteropus*, from the tropical Eastern Atlantic. *Ecotoxicology & Environmental Safety* 163(NOV):323–330. <https://hal.archives-ouvertes.fr/hal-02014990>
7. Liu K, Muse SV (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21(9):2128–2129. <https://doi.org/10.1093/bioinformatics/bti282>
8. Li XL, Hu XX, Lu HJ, Liu Y, Wang CC (2021) Development and validation of 107 SNP markers in *Todarodes pacificus* (Ommastrephidae). *Conservation Genetics Resources* 13417–424. <https://doi.org/10.1007/s12686-021-01227-2>
9. Merten V, Christiansen B, Javidpour J, Piatkowski U, Puebla O, Gasca R, Hoving HT (2017) Diet and stable isotope analyses reveal the feeding ecology of the orangeback squid *sthenoteuthis pteropus* (steenstrup 1855) (mollusca, ommastrephidae) in the eastern tropical atlantic. *PLoS ONE* 12(12):e0189691. <http://dx.doi.org/10.1371/journal.pone.0189691>
10. Merten VJ (2016) The trophic ecology of the oceanic orangeback squid *Sthenoteuthis pteropus* (Steenstrup) in the eastern tropical Atlantic
11. Russell D, Sambrook J (2001) *Molecular Cloning: A Laboratory Manual*
12. Xu H, Wang CC, Liu Y, Liu BL, Li G (2021) Development and characterization of 101 SNP markers in jumbo flying squid, *Dosidicus gigas*. *Conservation Genetics Resources* 13:13–20. <https://doi.org/10.1007/s12686-020-01177-1>

Table

Table 1 Summary of the 131 SNP markers in *Sthenoteuthis pteropus*

Primer ID	Primer sequences (5-3')	SNP type	Size (bp)	SNP position	PIC	H_0	H_e	P_{HWE}
SNP 1	F: ACGTTGGATGTCTCTGTCTTTTGGTGCCTG R: ACGTTGGATGATGCTAAGGCATACCTGGTG EXT: CAGGCCCCAAAACTCT	C/T	139	89	0.32	0.40	0.32	1.000
SNP 2	F: ACGTTGGATGGTTACTGAGAGAAAGACACC R: ACGTTGGATGCACATTACCAAATGTAGACTG EXT: AGAAAGACACCTCACAC	G/A	139	83	0.18	0.20	0.18	1.000
SNP 3	F: ACGTTGGATGGGAATACACCTTATGTGGAG R: ACGTTGGATGAAAAGTCAGTAAATTTTCGC EXT: GCTGAGCTGTGGATATA	C/T	139	89	0.50	0.20	0.50	0.365
SNP 4	F: ACGTTGGATGGCACTCTCCTACATCTGTTT R: ACGTTGGATGTAACGTCGTTACCGACTGAG EXT: tGGTAGGCCAAAACTGTC	T/A	139	87	0.18	0.20	0.18	1.000
SNP 5	F: ACGTTGGATGATTGGTGGGAGTGTTACTGC R: ACGTTGGATGGGCCTTTCTGGTTTTTCCAAC EXT: TTGACCCAGATAACCCTTC	G/A	140	81	0.18	0.20	0.18	1.000
SNP 6	F: ACGTTGGATGTCTGCCCAAACAAAGGACAC R: ACGTTGGATGTAAGGTTTTCCCTGAGGTCC EXT: ACACCCACCTAGATAGATT	A/G	139	85	0.18	0.20	0.18	1.000
SNP 7	F: ACGTTGGATGCTTTTCTACTCTCCTGTCCC R: ACGTTGGATGTAGCCAGACTTCATAACCCC EXT: CTCCTGTCCCCATCCTGTCC	C/T	139	83	0.32	0.00	0.32	0.111
SNP 8	F: ACGTTGGATGAGTGATAATGATGGTGGTGG R: ACGTTGGATGTTACTGTTGGGAACAGCGAG EXT: agGATGGTGGTGGTCCTAA	T/C	139	84	0.18	0.20	0.18	1.000
SNP 9	F: ACGTTGGATGGAAAATCTAAAAAGTACCG R: ACGTTGGATGGTGAAGCACCAAAAAGTCCC EXT: taTAAAAAGTACCGAATCGC	A/G	139	81	0.18	0.20	0.18	1.000
SNP 10	F: ACGTTGGATGGATGATTGGCTACCTCCTTC R: ACGTTGGATGCCTCATCCTCACATTGACAG EXT: cccaCTTCACCCAAGGGATTT	C/G	139	81	0.18	0.20	0.18	1.000
SNP	F:	T/C	140	86	0.18	0.20	0.18	1.000

11	ACGTTGGATGTGACCAAGGAGGCATTTGAG R: ACGTTGGATGCATTCCGTATGCTCTTCTCG EXT: GGTGTCCCTCAACATAAAAGC								
SNP 12	F: ACGTTGGATGTCACACCAATCGTGAACGTC R: ACGTTGGATGCTGTTCCGCTGAAATTTCCG EXT: tcCGTCAAACAGCTTAAAGAA	G/A	139	82	0.18	0.20	0.18	1.000	
SNP 13	F: ACGTTGGATGGGTCAGTAGGTGTTCTTTTC R: ACGTTGGATGCCTGTACAAGACTGAAATGG EXT: ccccCTCACACCTTTGACTCGT	T/C	139	82	0.18	0.20	0.18	1.000	
SNP 14	F: ACGTTGGATGTGCGTCGCTCTAAGTTGTTG R: ACGTTGGATGCCCTCACCTCAAGTAACGAC EXT: aatcGGATTCTAAAGTTGACGC	A/C	139	88	0.18	0.20	0.18	1.000	
SNP 15	F: ACGTTGGATGTGGTGCGGTCTGTGTGTATG R: ACGTTGGATGTATGAACACCACCCTGAACC EXT: gggTGAAGTCCCATAGTATGGT	C/T	140	84	0.42	0.20	0.42	0.333	
SNP 16	F: ACGTTGGATGGGACTTTATCCTCGCTCTTG R: ACGTTGGATGAAATGTGCAGCCAGGGAATC EXT: TCTTGCTTCCTCTAGTTTACTAA	G/A	139	91	0.32	0.40	0.32	1.000	
SNP 17	F: ACGTTGGATGAGGACACAGATGACGATAAC R: ACGTTGGATGGTTGGGTGTATACTACCCTC EXT: gCGGAGGATTTTCCTTCTGAAAT	T/C	140	91	0.18	0.20	0.18	1.000	
SNP 18	F: ACGTTGGATGAGGTGTGACGTTGAAGTGAC R: ACGTTGGATGCAAGCAGTGAGCTGAGATAG EXT: aacctTCGCTCCGAAAACCTCTCTA	G/A	140	86	0.18	0.20	0.18	1.000	
SNP 19	F: ACGTTGGATGGCTTGGCGTATTCAGTATCC R: ACGTTGGATGCTTGTGCGTAATACCACAGTC EXT: gccgCGCCTGCCTCTTTGCTGTCC	G/A	139	80	0.18	0.20	0.18	1.000	
SNP 20	F: ACGTTGGATGAATTACAGTCGCAGGGACTC R: ACGTTGGATGGGGTCTTCAACAGACCTTTC EXT: taagtGACTCAAAAACCTGAGACT	T/C	139	86	0.18	0.20	0.18	1.000	
SNP 21	F: ACGTTGGATGGGCACATCCACTCATAAAAG R: ACGTTGGATGTGCACCCAATTTTGTAGCCAC	A/G	140	87	0.18	0.20	0.18	1.000	

	EXT: agtgGCCAAAAATGGCTAAAGAAC								
SNP 22	F: ACGTTGGATGAGCCGGCTTATAGACCAATC R: ACGTTGGATGGTCACAGCTTGGAAATGACG EXT: tcttcTAGACCAATCGATGCAGCAC	C/T	140	86	0.18	0.20	0.18	1.000	
SNP 23	F: ACGTTGGATGGGGCTTAAGATGAATAAATC R: ACGTTGGATGTGTTCTAAATCCTTTCCGTC EXT: ggTGATAATGTACAATGACAAAGTA	T/G	139	86	0.18	0.20	0.18	1.000	
SNP 24	F: ACGTTGGATGACATATCTTCCGTCTTCTGC R: ACGTTGGATGCTTGAAAAAACAGGAACGC EXT: TTGAGTAGATATCAATTCTAACACTG	C/T	139	85	0.42	0.60	0.42	1.000	
SNP 25	F: ACGTTGGATGAGAAACAAATAACAACCTGC R: ACGTTGGATGCCAACACGACGTGTAACAAC EXT: caaaAAATAACAACCTGCAAAGCAAAA	A/G	140	80	0.18	0.20	0.18	1.000	
SNP 26	F: ACGTTGGATGCGCGTCATCCCTATTGATTC R: ACGTTGGATGGAGTTTGTGTGTGATGTCAG EXT: cccggCCTTTTTATATACCCCCAAAAC	T/C	140	87	0.18	0.20	0.18	1.000	
SNP 27	F: ACGTTGGATGTTCCCTCAGGTCCAGAAATAC R: ACGTTGGATGCTAGGACAACATATAATTC EXT: aaaacAAAGAAATGAAATGTTTGGAG	G/A	139	83	0.18	0.20	0.18	1.000	
SNP 28	F: ACGTTGGATGCCTAGACCTAGATTCACCTG R: ACGTTGGATGAACCTCCTAAGTACAAGCGG EXT: aactcATACTCATACCACGTAGCTATA	T/G	139	84	0.18	0.20	0.18	1.000	
SNP 29	F: ACGTTGGATGTCCACAGATTTACACTCGCC R: ACGTTGGATGCTATCAGTTATTGCTTGTCTG EXT: TTGCTGTCCGTCGTCTA	G/C	139	88	0.32	0.40	0.32	1.000	
SNP 30	F: ACGTTGGATGAAAGCTGCCGAAGTAGTTGG R: ACGTTGGATGGCCTACTATATCTGCCTTCG EXT: TTAAGACAGACGGTGGA	C/T	139	89	0.18	0.20	0.18	1.000	
SNP 31	F: ACGTTGGATGAAATCGTCCCTCGGCTAATC R: ACGTTGGATGGGTATATTGGTACAGAAAC EXT: tTTGTGGTCCCCAAATTA	C/A	140	81	0.18	0.20	0.18	1.000	
SNP 32	F: ACGTTGGATGCGAGCCTATCCTTAAACACC	T/G	139	87	0.18	0.20	0.18	1.000	

	R: ACGTTGGATGCTGATTTCACTCCTGTCTATC EXT: TGGAGGAATTTAGGGGTA								
SNP 33	F: ACGTTGGATGTTTGTGAATCGTGCCGATTG R: ACGTTGGATGTTCAAGATGTACTCCTCCTT EXT: GTGCCGATTGGATTGCTAC	C/T	139	89		0.18	0.20	0.18	1.000
SNP 34	F: ACGTTGGATGGGTGTCTTCTTGGGCATTTG R: ACGTTGGATGCAGCCATATCTAGACACCAC EXT: GGAATGTTAAGTGGCTTTC	C/T	139	87		0.48	0.40	0.48	1.000
SNP 35	F: ACGTTGGATGTGGTTTAAACACCACCACCC R: ACGTTGGATGAATACCTTGGATGTCTGGGC EXT: CAACAAATCCCAAAGACAAC	A/G	140	81		0.18	0.20	0.18	1.000
SNP 36	F: ACGTTGGATGAGCCTATCCAGAAATTGCTC R: ACGTTGGATGCCTAAATAAATATCGCATC EXT: ctGTCCTTTCTTTGCAAGAT	A/G	140	86		0.18	0.20	0.18	1.000
SNP 37	F: ACGTTGGATGAGCGTGTACCCTTGTGTATG R: ACGTTGGATGACCTCTCCTTTTATGGTAGC EXT: aaaGGCGGGCAGATTGTGCG	A/G	139	91		0.18	0.20	0.18	1.000
SNP 38	F: ACGTTGGATGTTCCCATTAGATTCCCCAGG R: ACGTTGGATGCTAAAGGCCTTTACCTAAACC EXT: Cggaggggtaggacttgat	A/G	140	89		0.42	0.60	0.42	1.000
SNP 39	F: ACGTTGGATGCTTTCATGTTTCTTCTCTCG R: ACGTTGGATGCGAACAAGAGCGATACCTAC EXT: TTTCTTCTCTCGTTAATTAGTT	C/T	140	91		0.18	0.20	0.18	1.000
SNP 40	F: ACGTTGGATGGGTTCACAAGCTAAGTAGGG R: ACGTTGGATGCTGTAAGCACTTTTTTTCCG EXT: cccGGGGTACTCTCCTCAAATA	A/G	139	91		0.18	0.20	0.18	1.000
SNP 41	F: ACGTTGGATGCTACAGAGGACTACCACTTG R: ACGTTGGATGTATGGCTAACGAGTACGAGG EXT: gAACGATTCTTAGTTAAAACGC	A/G	139	82		0.18	0.20	0.18	1.000
SNP 42	F: ACGTTGGATGTATGAACACCACCCTGAACC R: ACGTTGGATGTGGTGCGGTCTGTGTGTATG EXT: gaCGGTAACGGAAACTGAAAAT	A/G	140	82		0.18	0.20	0.18	1.000

SNP 43	F: ACGTTGGATGCTACTTTCAAACATAGAAGC R: ACGTTGGATGATACACTTATATCTAAATATTA EXT: ttgCAAACATAGAAGCACTGAAT	G/A	140	81	0.32	0.40	0.32	1.000
SNP 44	F: ACGTTGGATGGGTAACATTATCCCGAACGA R: ACGTTGGATGCAACGTTTTTGTGAAGGCTG EXT: cgggggATTGTAATCCTGGACGGG	G/A	139	91	0.18	0.20	0.18	1.000
SNP 45	F: ACGTTGGATGCCTCCATGTGTTATTCTATCC R: ACGTTGGATGACATGGGTACTAGCTTAGAG EXT: caTCTAAACTTTCTACTCCGTATT	A/G	140	91	0.18	0.20	0.18	1.000
SNP 46	F: ACGTTGGATGCCGAGTATATGACCCACATC R: ACGTTGGATGGTTGCAAATCATACGACGAC EXT: cccacGACCCACATCAGTGAAAAAT	C/T	139	80	0.18	0.20	0.18	1.000
SNP 47	F: ACGTTGGATGGCCAGAAATAGTGAGATAGC R: ACGTTGGATGCTGCATCGTATTCGTTCTGG EXT: cccGTGAGATAGCTTTGGAAAATA	T/A	140	87	0.18	0.20	0.18	1.000
SNP 48	F: ACGTTGGATGTTTCATGTCCACAGCTACCC R: ACGTTGGATGGTGATTAACTTTACCTCGC EXT: ccccaCTGGAACAACAATAGTTACT	A/C	140	86	0.18	0.20	0.18	1.000
SNP 49	F: ACGTTGGATGGTTGCCACGTAAAAATAGC R: ACGTTGGATGACTAAGGTTTTTAAGTTGGC EXT: cccctTTAAATAGCTAGCTGGTTG	G/A	139	84	0.18	0.20	0.18	1.000
SNP 50	F: ACGTTGGATGACGGTCTTCTTCGGAAGG R: ACGTTGGATGCTATATGAAGTGGCTGTGTG EXT: gggacGGGTTTACATACGGAATA	C/A	140	82	0.18	0.20	0.18	1.000
SNP 51	F: ACGTTGGATGGCCTCTGTTGTGTGTTAAAG R: ACGTTGGATGAATCCGGAAGAAGTCCAAAG EXT: tcGTTAAAGTTACCGATAGTTTTTTT	A/T	140	81	0.50	0.60	0.50	1.000
SNP 52	F: ACGTTGGATGGGGCTTAAGATGAATAAATC R: ACGTTGGATGCCGTCAATTTTAATGGCTTGG EXT: aagCAATGATAATGTACAATGACAAA	C/A	139	89	0.18	0.20	0.18	1.000
SNP 53	F: ACGTTGGATGCTTATTAACCTTTCCGACAC	C/T	139	80	0.32	0.00	0.32	0.111

	R: ACGTTGGATGCGTCGTCTGATTGCTCACTT EXT: cccACACTTTTAATAAACATTTCAAACA								
SNP 54	F: ACGTTGGATGCCATAACATGCAAGGAAAAC R: ACGTTGGATGCTATCCCCATTATTATTACCACC EXT: cttcAAACATAATACTGACACTACTTC	G/A	140	80		0.18	0.20	0.18	1.000
SNP 55	F: ACGTTGGATGATTTTATGGCGCCAAGCCAG R: ACGTTGGATGGAACCAACACGCATATTGATG EXT: CCAAGCCAGTAACATCA	A/T	140	83		0.32	0.00	0.32	0.111
SNP 56	F: ACGTTGGATGGTGAAGCACCAAAAAGTCCC R: ACGTTGGATGGAAAATCTAAAAAGTACCG EXT: AAAGTAGGCCCGGTA ACT	C/T	139	80		0.18	0.20	0.18	1.000
SNP 57	F: ACGTTGGATGAATACACAAGGCCATAGCCC R: ACGTTGGATGGTGTTACTACACCAAGGCAG EXT: aAAGAGAGAAAAGTCCCC	C/A	139	81		0.32	0.00	0.32	0.111
SNP 58	F: ACGTTGGATGTTTGTGAGATGGAGGGTCAG R: ACGTTGGATGCTCAAAATCAAGGAAAATTG EXT: cAGGGTCAGTTGGTTGGT	G/A	140	83		0.32	0.00	0.32	0.111
SNP 59	F: ACGTTGGATGGATTTTCGTAATTGTGGTCCC R: ACGTTGGATGCTACTGCTAACAATAACATGG EXT: ccATTGTGGTCCCCAAATC	T/A	140	80		0.18	0.20	0.18	1.000
SNP 60	F: ACGTTGGATGAAAAAAAAAATTGGGTGGC R: ACGTTGGATGGAGGTAAAATCTGGTCGAGG EXT: AAAATTGGGTGGCATGCTT	A/G	139	84		0.18	0.20	0.18	1.000
SNP 61	F: ACGTTGGATGGGAGGGCGTGTGATGAAAAG R: ACGTTGGATGTTCTTCTTCTCCATGCATTC EXT: aaGGTTGTCATTGCTGGTG	G/A	139	89		0.18	0.20	0.18	1.000
SNP 62	F: ACGTTGGATGCGATTTCATACCCA ACTGTGC R: ACGTTGGATGGAGAGACTGATATCACAAT EXT: acGTGCTAGAATTTTACGCC	C/G	140	91		0.18	0.20	0.18	1.000
SNP 63	F: ACGTTGGATGTTCTCAGGTCCAGAAATAC R: ACGTTGGATGCTAGGACAACATATAATTC	C/T	139	88		0.18	0.20	0.18	1.000

	EXT: tTGAAATGTTTGGAGGAAAG								
SNP 64	F: ACGTTGGATGGGAAACTCGATTTTTTCAGGG R: ACGTTGGATGCTTCCCGAATATAGACTTTGC EXT: acccTTCTGCTTCGCGTTTTT	T/C	140	87	0.32	0.00	0.32	0.111	
SNP 65	F: ACGTTGGATGACGTTTCGGCCGTTGTAATC R: ACGTTGGATGAAGGTTTCGGTTCGTTACTCC EXT: gggaTTGTAATCTACGGCCTT	G/A	140	83	0.18	0.20	0.18	1.000	
SNP 66	F: ACGTTGGATGGGAATAGGGTATGCATCCAA R: ACGTTGGATGAACGACAACCACAAGAGACG EXT: gtGGTATGCATCCAAATTAGT	A/G	139	82	0.18	0.20	0.18	1.000	
SNP 67	F: ACGTTGGATGGCGATTATGTTAAGCGGTTC R: ACGTTGGATGGCACTTTTTTTTACCGGGTTA EXT: gctGGATAAACCCCTCCTCAAAT	A/T	139	91	0.18	0.20	0.18	1.000	
SNP 68	F: ACGTTGGATGGAATTTTTGTGACCAAAATC R: ACGTTGGATGTATCGCTTTCTACTTGCTG EXT: atcTTTACATGGTTGGAAAGAG	G/T	139	81	0.50	0.20	0.50	0.365	
SNP 69	F: ACGTTGGATGTCCCAACCTAAAACGTTCTC R: ACGTTGGATGGTTAGCGGCAACGTCACAAC EXT: cccccTTAACGGTCGTTTTTCATC	G/A	140	81	0.18	0.20	0.18	1.000	
SNP 70	F: ACGTTGGATGCCCAAACCCCAAAGACTTC R: ACGTTGGATGATGGGACTCACTCCTTCTTG EXT: gtccaAAAGACTTCCGGGTTCCG	G/A	140	84	0.18	0.20	0.18	1.000	
SNP 71	F: ACGTTGGATGTGGTGCGGTCTGTGTGTATG R: ACGTTGGATGTATGAACACCACCCTGAACC EXT: aaggaAAGTCCCATAGTATGGTG	C/A	140	83	0.18	0.20	0.18	1.000	
SNP 72	F: ACGTTGGATGCCGGTGTCACGTTATTCAAG R: ACGTTGGATGATGAGTGGCTTCAGAAGACC EXT: aacTCAAGTTTTTTCTCTTACTCA	C/T	139	80	0.18	0.20	0.18	1.000	
SNP 73	F: ACGTTGGATGTGCACCCAATTTTATGCCAC R: ACGTTGGATGGGCACATCCACTCATAAAAG EXT: cCAATTTATTGTCTTTTTCTGGTG	A/G	140	85	0.18	0.20	0.18	1.000	
SNP 74	F: ACGTTGGATGTGACCAAGGAGGCATTTGAG	A/T	140	83	0.18	0.20	0.18	1.000	

	R: ACGTTGGATGCATTCCGTATGCTCTTCTCG EXT: ggggtTGGTGTCCCTCAACATAAA								
SNP 75	F: ACGTTGGATGGGGTCTTCAACAGACCTTTC R: ACGTTGGATGAATTACAGTCGCAGGGACTC EXT: caCTTTCATTAATCAGTCTGAAATT	C/T	139	80		0.18	0.20	0.18	1.000
SNP 76	F: ACGTTGGATGACTGTCCCGAACTATGTTGC R: ACGTTGGATGTCGCCAGAGTTGGCTAAAAG EXT: aactAACTATGTTGCCAAATTTTAC	C/T	140	85		0.18	0.20	0.18	1.000
SNP 77	F: ACGTTGGATGTCCCTTTACTGGATTTCTC R: ACGTTGGATGCGTGTAATCTGCAGAAAATG EXT: cGTTTGGGTTGTAATACGTATAGAA	T/G	140	87		0.18	0.20	0.18	1.000
SNP 78	F: ACGTTGGATGAGTAGATGCCACGTCTATGC R: ACGTTGGATGGAGTCTCAACAAAATTTGAT EXT: agattCCACGTCTATGCGGTGTTTTT	A/G	140	83		0.18	0.20	0.18	1.000
SNP 79	F: ACGTTGGATGTCCGAGAGGTTGAGGATAAG R: ACGTTGGATGTCAAAGAAACTCGACTCCC EXT: agaagATGTTCTTCCATTTACTGTGT	T/C	140	82		0.48	0.80	0.48	0.429
SNP 80	F: ACGTTGGATGGATTGCCATGGTTTACTCAG R: ACGTTGGATGCCTCTGTAACACCAATTGAC EXT: ctctCAATCACAAATGCTTTTATTTAT	A/G	140	83		0.32	0.40	0.32	1.000
SNP 81	F: ACGTTGGATGTATTGCTGGGTGCGTAATGG R: ACGTTGGATGATGACAGTCAGACTAAATCG EXT: cccAATGGTTTTTACTATTTCTTACAT	G/A	139	86		0.32	0.00	0.32	0.111
SNP 82	F: ACGTTGGATGGTAGACATCTCCGTTTTTCC R: ACGTTGGATGGATTTTCGACACTTTTTCCCG EXT: TCCGTTTTTCCGTCAAC	G/A	139	82		0.18	0.20	0.18	1.000
SNP 83	F: ACGTTGGATGGCTATGTTGGTATTGCTCCG R: ACGTTGGATGCGACCACGGAAAGTGTTATG EXT: TTGCTCCGTTTACAATA	T/C	139	87		0.18	0.20	0.18	1.000
SNP 84	F: ACGTTGGATGAAGCCAGAGGAAGAGGAAAG R: ACGTTGGATGTTACAAACAAGGCTGCAAGG EXT: GAACATAAGGGCAGCTA	C/T	140	87		0.18	0.20	0.18	1.000

SNP 85	F: ACGTTGGATGATTTTATGGCGCCAAGCCAG R: ACGTTGGATGGAACCAACACGCATATTGATG EXT: GCGCCAAGCCAGTAACAT	A/G	140	85	0.18	0.20	0.18	1.000
SNP 86	F: ACGTTGGATGCCTCATCCTCACATTGACAG R: ACGTTGGATGGATGATTGGCTACCTCCTTC EXT: tGGTTAGAGTTGGTGCCG	T/C	139	80	0.18	0.20	0.18	1.000
SNP 87	F: ACGTTGGATGGCCAGGCTATTCCTGCAAAT R: ACGTTGGATGAGACTTACAGACTGGTACCC EXT: CTCAATTCGTCTTTCCAT	G/T	140	84	0.48	0.00	0.48	0.051
SNP 88	F: ACGTTGGATGAATTACAGTCGCAGGGACTC R: ACGTTGGATGGGGTCTTCAACAGACCTTTC EXT: ACTCAAAAACCTGAGACTA	A/G	139	85	0.32	0.40	0.32	1.000
SNP 89	F: ACGTTGGATGACATTCTGGTCTGAGCTCTG R: ACGTTGGATGGCTACCATGTGGTTAAGAAC EXT: CAGGAAAAATTCCGCCCATC	T/C	139	87	0.18	0.20	0.18	1.000
SNP 90	F: ACGTTGGATGCTATCATCCCACAGAGCATC R: ACGTTGGATGAGAAAAAATGCCCCAAAAC EXT: TGGAAGGCAGTGACGTTCTC	T/C	140	91	0.32	0.40	0.32	1.000
SNP 91	F: ACGTTGGATGCATATAATAGCTTTGAAATC R: ACGTTGGATGTACAACAACATAGGTCTGGG EXT: cTTTACATTTTAGGCCATTCA	T/C	139	85	0.18	0.20	0.18	1.000
SNP 92	F: ACGTTGGATGAAGCTCCTCCCTTGCTTCC R: ACGTTGGATGCATCTATCGTTGGTTCCTCC EXT: aacaAGGACCCTTTTTAGGGA	A/C	140	87	0.18	0.20	0.18	1.000
SNP 93	F: ACGTTGGATGGAGCGAACAAGTCGAATCTG R: ACGTTGGATGTGTTCTTTTACCTTCGTTG EXT: gaagGAGATATCTGGTAGCCG	A/T	139	83	0.18	0.20	0.18	1.000
SNP 94	F: ACGTTGGATGCGTAAAGAGAAGTCACAGGG R: ACGTTGGATGACGTCACTTCAACGTCACAC EXT: cAAAGCTTTAAGCTTCAACAAC	C/T	139	91	0.18	0.20	0.18	1.000
SNP 95	F: ACGTTGGATGACCCTTTTCCGAAGAAGACC R: ACGTTGGATGCGGAACCTTGGAGGAAGAAT	T/C	139	83	0.32	0.00	0.32	0.111

	EXT: GACCGTAAAACGTCGAAAATAT								
SNP 96	F: ACGTTGGATGTTGGCGAACAATAGCCAACC R: ACGTTGGATGATAGCCCGTAACACTTCGTC EXT: tctccCAATAGCCAACCCAGACA	A/G	140	89	0.18	0.20	0.18	1.000	
SNP 97	F: ACGTTGGATGGGAAAGTATTCGTGGCCTTC R: ACGTTGGATGACCCTGTCCTTTTCGATTTTG EXT: GTAAATACTTTTTGCTATAAGCG	T/A	139	83	0.18	0.20	0.18	1.000	
SNP 98	F: ACGTTGGATGCCATAACATGCAAGGAAAAC R: ACGTTGGATGCTATCCCCATTATTATTACCACC EXT: ttttcACTGACACTACTTTCGAATA	C/T	140	85	0.18	0.20	0.18	1.000	
SNP 99	F: ACGTTGGATGGCCTCAAGATGAATAAATC R: ACGTTGGATGTGTTCTAAATCCTTTCCGTC EXT: TGATAATGTACAATGACAAAGTAA	G/A	139	88	0.18	0.20	0.18	1.000	
SNP 100	F: ACGTTGGATGTCTCTCGTTTTATAAGCAG R: ACGTTGGATGAACACCAAACTCCCTCCTC EXT: gaAGAATGATACTGGTTTTCAAAA	T/C	140	89	0.18	0.20	0.18	1.000	
SNP 101	F: ACGTTGGATGATTTGGCAGAGCAGTCGAAC R: ACGTTGGATGATGGTACATACCACACCACG EXT: ctcACACTACTCTAATTTTTTTCAGG	T/G	139	82	0.18	0.20	0.18	1.000	
SNP 102	F: ACGTTGGATGCAGTGAGCGATATTACTGGC R: ACGTTGGATGGACCTACTTTGACATGTGAC EXT: gATAAGGAAGTATTACGATATGTAG	G/A	140	88	0.18	0.20	0.18	1.000	
SNP 103	F: ACGTTGGATGCCGCAGTAATTTCAACCCAC R: ACGTTGGATGCCATGTTGTTTTCTTAAT EXT: ggtcaACAAAAATTATAACCACCGCC	C/A	139	80	0.18	0.20	0.18	1.000	
SNP 104	F: ACGTTGGATGAGGCTTCCAAAAAGAGAGGG R: ACGTTGGATGCAAGCGCTATTTACAGAGAG EXT: cccgaGTGACAACACTGGTTCAAATG	C/T	140	84	0.18	0.20	0.18	1.000	
SNP 105	F: ACGTTGGATGAGCAGATGTGTTGTGCAATC R: ACGTTGGATGATGGTGAAAGCCCATTATC EXT: gATTATTGAGTAACCAATAGAAATTC	T/C	139	91	0.18	0.20	0.18	1.000	
SNP 106	F: ACGTTGGATGCAAGCTCGCAAATGATGATG R: ACGTTGGATGTATTTTTGCCCGTGGCTTTG	A/T	140	86	0.18	0.20	0.18	1.000	

	EXT: agattATGATTTCAGAGTGGAAAGAAA								
SNP 107	F: ACGTTGGATGAATACACAAGGCCATAGCCC R: ACGTTGGATGGTGTACTACACCAAGGCAG EXT: AGAGAGAAAAGTCCCCC	C/A	139	82	0.18	0.20	0.18	1.000	
SNP 108	F: ACGTTGGATGATTCTTGCCGCGAGAAAGTG R: ACGTTGGATGTTTCCACTTTTAGGCGCGTC EXT: GTGAAGACCTCGCTGAG	T/A	140	80	0.18	0.20	0.18	1.000	
SNP 109	F: ACGTTGGATGCAAGCCAGGACCCTTTTGTAG R: ACGTTGGATGCACCTCGCCATCTATCGTTG EXT: cCCCTTTTTAGGGAACCC	T/G	140	91	0.32	0.00	0.32	0.111	
SNP 110	F: ACGTTGGATGCAACGTTTTTGTGAAGGCTG R: ACGTTGGATGGGTAACATTATCCCGAACGA EXT: ccCTATCAGGTCCCCGTCC	T/A	139	83	0.42	0.06	0.42	1.000	
SNP 111	F: ACGTTGGATGGAGCAATTAATAAGGCGAACC R: ACGTTGGATGGCCAAAATGCGTATTCAGAG EXT: GCGAACCTAATCTCTAAGA	T/C	139	83	0.32	0.00	0.32	0.111	
SNP 112	F: ACGTTGGATGCAGACGATTAAGTCCGAAG R: ACGTTGGATGCCATTGTCTGCCTTAATGGG EXT: GAAGTAGTTGGGATTTACC	A/G	140	88	0.18	0.20	0.18	1.000	
SNP 113	F: ACGTTGGATGTACGTAAACGGTGTGGCTC R: ACGTTGGATGAGATGATAGACTGTCCAAAG EXT: cgTCACTACTCTGCTGTATC	T/A	139	88	0.18	0.20	0.18	1.000	
SNP 114	F: ACGTTGGATGAAATGTGCAGCCAGGGAATC R: ACGTTGGATGGGACTTTATCCTCGCTCTTG EXT: tGGGAATCGAACTTTTTGAA	A/G	139	82	0.32	0.40	0.32	1.000	
SNP 115	F: ACGTTGGATGGCAAAGCTGTAGCCGACAAA R: ACGTTGGATGTTGTCCTGTGTACTGTGGTC EXT: GGCTACACATCATTATTTGGC	G/A	140	91	0.18	0.20	0.18	1.000	
SNP 116	F: ACGTTGGATGAGACAACCGTACTTCAGAGC R: ACGTTGGATGCACCCACGGGGATACATTAA EXT: gAGAGCATAATGAATGAACAG	A/G	140	87	0.50	0.60	0.50	1.000	
SNP 117	F: ACGTTGGATGTTAGCCAATCCGAGTTGACC	A/G	139	80	0.18	0.20	0.18	1.000	

	R: ACGTTGGATGCCCCACTCCCCGATTTTAC EXT: tccttGGAAACCCCCACCTAAC								
SNP 118	F: ACGTTGGATGGTGGCCAGTTTCCAGAGAAT R: ACGTTGGATGTCGTGACCACACACAGTAAC EXT: ctcacCTGGAAGGCACTTTTGC	C/T	139	87	0.32	0.40	0.32	1.000	
SNP 119	F: ACGTTGGATGGAGAAGTGTGCACGTAGAAG R: ACGTTGGATGCCTATTCTCTCTAACTTTTCG EXT: AGTAGTATCGTAGCTAAACTTG	A/G	140	84	0.18	0.20	0.18	1.000	
SNP 120	F: ACGTTGGATGGATTAAC TTCCGAATACTC R: ACGTTGGATGCACACCATATTTGGAGATAC EXT: CTTCCGAATACTCATATATCCAC	A/C	139	86	0.18	0.20	0.18	1.000	
SNP 121	F: ACGTTGGATGCTGTTTCGGTAAGTCTATTC R: ACGTTGGATGTCCAGGACATAGTTAGTAGC EXT: ATTCTTATTCTTCTTATGTGACA	G/T	139	85	0.32	0.00	0.32	0.111	
SNP 122	F: ACGTTGGATGGGGCTTAAGATGAATAAATC R: ACGTTGGATGCCGTCAATTTTAATGGCTTG EXT: AATGATAATGTACAATGACAAAG	A/T	139	88	0.18	0.20	0.18	1.000	
SNP 123	F: ACGTTGGATGTCTGTAATCCGGCAGCAAAG R: ACGTTGGATGAGAAGAAAGACGAGGGTAAC EXT: tccGCAAAGTTAATAAATTGCTCC	C/A	140	82	0.18	0.20	0.18	1.000	
SNP 124	F: ACGTTGGATGATCAATGTAATAAAAGTGTG R: ACGTTGGATGCTGATGAATGTATTCTTTTT EXT: cAATAAAAAAAAAACACAACAATGT	G/T	140	85	0.18	0.20	0.18	1.000	
SNP 125	F: ACGTTGGATGCCAGTTTTTCACTGTCCATC R: ACGTTGGATGCTGCTCCAATTATCAATCGAC EXT: ggaggTGATTCAGGCGGAATAACT	G/T	139	88	0.18	0.20	0.18	1.000	
SNP 126	F: ACGTTGGATGCACAAGTACAAAACACAACC R: ACGTTGGATGATTCTATTAGGCACTAAAC EXT: acATGACAATGTTTATAAATTCTCC	C/T	140	86	0.32	0.00	0.32	0.111	
SNP 127	F: ACGTTGGATGGTGGGTCATCAGAGCTCTAA R: ACGTTGGATGCCATAAGACCTCCTTGGAAC EXT: GGGATTGTGAATTATTTATGTATCT	C/A	139	88	0.18	0.20	0.18	1.000	

SNP 128	F: ACGTTGGATGGACTGGTATCATACTTCTCTC R: ACGTTGGATGCCTTGTCCGTCCTAGTTATT EXT: cgACTTCTCTCTAAAGACAAATTTTA	C/A	140	83	0.18	0.20	0.18	1.000
SNP 129	F: ACGTTGGATGTAGCAAGCAGATGTGTTGTG R: ACGTTGGATGTGCTGAAAGACCATTTATC EXT: acTTATTGAGTAACCAATAGAAATTC	T/G	139	9	0.18	0.20	0.18	1.000
SNP 130	F: ACGTTGGATGGGGTTTGAATGAAAGCTGAC R: ACGTTGGATGTTTCCCGTTGTCAGTTAGGC EXT: gggTGAAAGCTGACGAAATATATAAT	A/G	139	82	0.18	0.20	0.18	1.000
SNP 131	F: ACGTTGGATGCAAGTTTTGGACACTTGTACG R: ACGTTGGATGACCAAACCGTGATTGGTCG EXT: cccccACACTTGTACGAAAAAACATCT	T/A	139	91	0.32	0.00	0.32	0.111

Primers for each locus including a forward primer (F), a reverse Primer (R) and an extension primer (EXT), H_o observed heterozygosity, H_e expected heterozygosity, P_{HWE} the P value of the departures from Hardy-Weinberg equilibrium.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable1.docx](#)