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Development of 30 SNP markers for the *Daphnia magna* based on restriction site-associated DNA sequencing (RAD-seq)

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ABSTRACT:

Daphnia magna belongs to the Cladocera and plays an important role in the water ecosystem. With the intensification of water pollution, the wild population of *D. magna* has declined rapidly in recent years, and insufficient molecular markers have limited effective research and conservation of this species. In this research, 30 novel single nucleotide polymorphism (SNP) markers were developed in a cultivar of *Daphnia magna* and 12 wild *Daphnia magna* using restriction site-associated DNA sequencing (RAD-seq). The minor allele frequency, observed heterozygosity, and expected heterozygosity ranged from 0.115 to 0.721, 0.073 to 0.800, and 0.077 to 0.520, respectively. The PIC ranged from 0.071 to 0.403. Six loci showed significant deviations from the Hardy-Weinberg equilibrium after Bonferroni correction ($p < 0.05$). These newly developed polymorphic SNP markers for *D. magna* are of great significance in terms of the genetic breeding of *D. magna*, identification of wild and artificially domesticated species and conservation genetics research.

KEYWORDS:

Daphnia magna; SNP markers; Genetic diversity analysis; RAD-seq

Daphnia magna belongs to the Cladocera and is an important species of zooplankton (Jeong and Simpson 2019). It mainly lives in freshwater. *Daphnia magna* helps to increase the diversity of phytoplankton in the water body and build a stable phytoplankton community structure, as well preventing the occurrence of cyanobacteria blooms (Sarnelle 2007). It is the key to balance in the water ecosystem. In China, the artificially domesticated *Daphnia magna* has been used to inhibit *Cyanobacteria* blooms and restore submerged vegetation, which has been used in more than 400 ecological restoration projects (Huo et al. 2010; Peng et al. 2011). In addition, it is widely used in water pollution monitoring and aquatic organism toxicological research (Dietrich et al. 2010; Fan et al. 2011). However, in recent years, with the rapid expansion of the population and increases in the discharge of domestic sewage, the aquatic ecosystem has been severely damaged; relevant research shows that 54% of Asian, 53% of European, 48% of North American, 41% of South American, and 28% of African rivers are eutrophic water bodies, and eutrophication of water bodies is currently the most serious problem facing rivers and lakes (Bagousse et al. 2012), as a result, the habitat of *D. magna* has been severely damaged, and its genetic diversity is also declining sharply. To date, a comprehensive study of *D. magna* population genetic variations is still lacking, and very little information is available on the genome and effective molecular markers of *D. magna*. This hinders correlational studies on this species. Thus, it is very important to investigate the genetic diversity of this species for scientific management and sustainable utilization in the future.

Genetic methods based on DNA markers are extremely efficient at revealing population distributions and the laws of evolution (Hao et al. 2017; Chen et al. 2018; Zhao et al. 2019; Liu et al. 2020a; Liu et al. 2020b). Using genetic marker

technologies, the differences and dynamics of populations can be understood. The disclosure of genetic diversity and genetic structure is also the basis of species genetic management (Manel et al. 2003; Schwartz et al. 2007). Because single nucleotide polymorphism (SNPs) are codominant, dimorphic, allelic, and widely distributed (Vignal et al. 2002; Céline et al. 2018; Honorio et al. 2019), they have become among the most advanced DNA markers. Nowadays, SNP have been widely used in genetic structure analysis, cultivar identification, genetic diversity evaluation, construction of high-density genetic linkage maps, and molecular-assisted breeding (Gupta et al. 2008; Pujolar et al. 2013; Vasemagi and Primmer 2005).

Restriction site-associated DNA sequencing (RAD-seq) is an important sequencing technology for simplifying the genome. It has a high number of markers and high density, and can be applied in many fields. For research subjects without a reference genome, RAD-seq technology is the best choice for simplifying genome establishment (Miller et al. 2007). This technology has been widely used in research in the fields of population genetics, genetic map construction, and systematic evolution (Li et al. 2018). Here, we adopted restriction site-associated DNA (RAD-seq) technology (Miller et al. 2007) and reported on the development and verification of SNP markers in *D. magna*.

The experimental materials used in this research were taken from the breeding base of Shanghai Taihe Water Environment Technology Development Co., Ltd. (DZ), Yantai (YT), Jining (JN), Yunnan (YN), Hainan (HNa), Jiangsu (JS), Beijing (BJ), Hunan (HNb), Shanxi (SX), Liaoning (LN), Guangdong (GD), Anhui (AH), Sichuan (SC) (Table 1 and Figure 1). Hundreds of *D. magna* adult individuals were randomly collected from 12 wild population sampling sites and brought back to the laboratory for cultivation. The TIANGEN DP304 kit (TIANGEN BIOTECH Co., Ltd, China) was used to extract 50 asexual reproduction cultured adult *D. magna* genomic DNA samples, and the integrity was checked using 1.5% agarose gel electrophoresis. The purity and concentration of the DNA were detected using a UV spectrophotometer to ensure the genomic DNA obtained met the quality conditions for building a database.

DNA samples were diluted to a concentration of 30 ng/μL and stored at -20°C (Haier BCD-576WDPU, China).

Equal amounts of genomic DNA of *D. magna* were fused and Shanghai Map Biotech Co., Ltd. (Shanghai, China) performed RAD-seq to isolate and characterize SNP markers. The constructed RAD libraries were sequenced on the Illumina HiSeq 150 platform. After quality filtering, 19,631 putative SNPs with the highest scores were generated. Primer 3.0 software was used to develop SNP primers. A total of five samples, including YN, YT, JN, DZ, and JS were selected for verification using Sanger sequencing. Sequencing primer was general-purpose Common-F (AGTCACGACGTTGTAAAACGAC). We obtained preliminary polymorphic locus using Sanger sequencing, and then we used SNaPshot for group verification. After verification of the small group, the stability of the experimental system and process was confirmed. Following this, the remaining samples were verified by the large group. Allele frequency, observed heterozygosity (H_o), and expected heterozygosity (H_E) indices were calculated using GenAlEx 6.5 (Peakall and Smouse 2012). The polymorphism information content (PIC) and Hardy–Weinberg distribution (HWE) were estimated at the population level using Arlequin v3.0 (Chaves et al. 2018; Excoffier 2005).

Figure 1 The map of the sampling locations of the 13 populations of *Daphnia magna* in the present study.

Table 1 Information on samples used for genetic marker development

Sampling sites	Codes	Longitude	Latitude	Dates
Taihe Water Co., Ltd.	DZ	121°21'34.7"E	30°50'50.9"N	2019.09
Yan tai	YT	121°37'44.4"E	37°23'33.6"N	2019.11
Ji ning	JN	116°39'36.5"E	35°0'36.5"N	2019.11
Yun nan	YN	102°39'41.7"E	25°1'20.8"N	2019.12
Hai nan	HNa	110°20'7.9"E	20°3'45.2"N	2019.12

Jiang su	JS	120°36'37.8"E	31°19'18.1"N	2020.01
Bei jing	BJ	116°32'27.2"E	39°46'7.9"N	2020.04
Hu nan	HNb	110°39'59.2"E	26°45'1.9"N	2020.05
Shan xi	SX	113°4'33.4"E	36°19'56.0"N	2020.06
Liao ning	LN	122°6'44.9"E	41°12'51.9"N	2020.06
Guang dong	GD	114°25'17.7"E	23°6'7.3"N	2020.06
An hui	AH	116°58'50.9"E	33°39'21.4"N	2020.07
Si chuan	SC	104°4'58.8"E	30°48'52.5"N	2020.07

A total of 30 SNP loci were polymorphic. The name of the SNP locus, primer sequences (5'-3'), SNP type, fragment size, Minor allele frequency (MAF), observed heterozygosities (H_O), expected heterozygosities (H_E) polymorphic information content (PIC) and probability for Hardy–Weinberg equilibrium tests (HWEP) were shown in Table 2. Minor allele frequency (MAF) varied from 0.115 to 0.721, the mean MAF was 0.311. The observed heterozygosity (H_O) ranged from 0.073-0.800, and the mean H_O was 0.262. The expected heterozygosity (H_E) was between 0.077-0.520, and the mean H_E was 0.330. The Polymorphic information content (PIC) range was 0.071-0.403, with an average of 0.270. Across all samples, 6 of 30 loci showed significant deviations from HWEP after Bonferroni correction ($p < 0.05$), but no significant linkage disequilibrium was found. The results showed that 13 *D. magna* populations had good genetic polymorphism.

Table 2 Characterization of 30 SNPs in *Daphnia magna*

SNP Locus	Primer sequences (5'-3')	SNP type	Fragment size (bp)	MAF	H _O	H _E	PIC	HWEP
SNP-01	F:TTGCCTTCTTTGCTTCACATCC R:AGTAATCAGCTTATGAGCCTCGG	C/T	112	0.115	0.194	0.173	0.152	ns
SNP-02	F:AAATTGTTTTTGCACGGATGTCC R:TGAAAACATCTACGGCGATCTCG	A/G	161	0.171	0.117	0.294	0.239	ns
SNP-03	F:ATCTGCTACACCATGGGTTTTGG R:TAGACTCAGATTGATGGCTGACG	C/T	203	0.180	0.073	0.076	0.071	ns
SNP-04	F:AACGAAGTGATATCTCCAGTGGC R:TGTATTTGGA AAAACCATCTGACGC	A/G	187	0.121	0.167	0.159	0.141	ns
SNP-05	F:TGATTGCTTTTGTGTTGTTCGC R:TGGGAATTAATTTTCCGACTGCC	A/T	248	0.329	0.077	0.473	0.350	**
SNP-06	F:GAAGTATACGCAGGGGTCCG R:TGGA AAAAGAACAAGTATGCCG	A/G	146	0.396	0.116	0.493	0.381	*
SNP-07	F:GAGAAATAGTGTCTGATCCAGGGG R:CTTGTAGCGTCCATCCAAGTCG	C/T	253	0.425	0.155	0.148	0.132	ns
SNP-08	F:ACCAATTGTTGCATATGGGAAGG R:ATTCATTACATTCAGCAAGCCCC	G/T	194	0.102	0.431	0.150	0.140	ns
SNP-09	F:TTCTTTCCCTTAATACGACGCC R:GAAATGGATGAATTTCTGGGAGGG	G/T	112	0.248	0.182	0.312	0.253	ns
SNP-10	F:ATAGACGTCTTTAAATCGCGAGC	A/T	209	0.165	0.081	0.083	0.077	ns

	R:ACTTTTTCGACAATTCTTTTGGTAGC							
SNP-22	F:TTCCTTTTGCAACACTTCGTTTCG R:CGGAATGAAGGTCGACCAGC	C/T	145	0.254	0.231	0.323	0.262	ns
SNP-23	F:TTGGGAGTAAACCAGCAATTAGC R:TATCGGGCGATTGAAAAACTGC	G/T	181	0.683	0.800	0.505	0.385	ns
SNP-24	F:CTCTTGTCCTTATGCCTTCC R:CACCCTACAGCAAAGACTAGAGC	G/T	189	0.426	0.077	0.409	0.316	**
SNP-26	F:TAATTAGGCGCGTCATCAAACG R:GAAGGGGAGTAATTCACGCTACG	A/T	197	0.467	0.500	0.479	0.372	ns
SNP-26	F:TCGGTGAAATATCGAATGATGTGC R:ATGAAGACAAATCCAACAGTCGG	A/T	201	0.355	0.455	0.507	0.387	ns
SNP-27	F:GATGCCACATTTTCAAACCAGG R:TCCTCACTATTCGATCAGACAACG	C/T	154	0.308	0.229	0.327	0.202	ns
SNP-28	F:GCTCCTAAATTGGCTTGATGACG R:CTCTAAGAAAGGCCCTCTTCAGG	A/G	246	0.332	0.426	0.441	0.403	ns
SNP-29	F:TTCGGAACTTTTGTTCCTATCC R:CCGCTGCCAGATTAAATCTAACG	A/T	208	0.551	0.464	0.321	0.388	ns
SNP-30	F:TGGCAAATTTAACAGGTGCTACG R:TATCGGTCAATCCAGCAATCAGG	C/T	185	0.086	0.189	0.086	0.177	ns

MAF: Minor allele frequency; PIC: Polymorphism information content; H_E : Expected heterozygosity; H_O : Observed heterozygosity; HWEP: probability for Hardy–Weinberg equilibrium tests, ns non-significant, *P < 0.05, **P < 0.01, ***P < 0.001.

To our knowledge, this is the first time SNP markers were developed for *D. magna*, thus, filling the gap in the field. The application of RAD-seq technology is continuously expanding, and it has been widely used for gene mapping (Guo et al. 2015), high-precision genetic map construction (Zhang et al. 2018) and assessments of genetic diversity (Pootakham et al. 2011). Our study identified 30 polymorphic SNP markers using RAD-seq, which can efficiently and accurately identify the *D. magna* populations in different geographic regions, thereby revealing the genetic structure of *D. magna* populations and providing molecular marker reference for subsequent research on *D. magna*. Our research results are of great significance for identification of wild and artificially domesticated species, genetic breeding, evaluation of genetic diversity, genetic relationship identification, construction of a genetic map, and protection of germplasm resources of this species.

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Author contributions

PMH designed the study and wrote the final draft of the manuscript. SMZ did all the practical work and data analyses and wrote the first version of the manuscript, WHH assisted in discussions and revisions of the manuscript. All authors contributed to writing.

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Data availability

Provided as supporting information.

Compliance with ethical standards

Conflict of interest The authors declare no competing interest in this work.

Ethical approval Samples were caught from wild water bodies and provided by Shanghai Taihe Water Environment Technology Development Co.,Ltd, and no specific permissions were required. All experiments were conducted after review and approval from the local Ethical Committee.

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Figures

Figure 1

The map of the sampling locations of the 13 populations of *Daphnia magna* in the present study. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.