Development of Daphnia magna SSR markers and genetic diversity analysis based on RAD-seq technology

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March 30, 2022

Abstract

Daphnia magna belongs to the Cladocera order 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 our research, 26 novel microsatellite (SSR) markers were developed in an artificially domesticated of D. magna and 12 wild population of D. magna using restriction site-associated DNA sequencing (RAD-seq). The results showed that the observed heterozygosity (Ho) and expected heterozygosity (He) ranged from 0.083 to 0.999 and 0.085 to 0.862, respectively. The PIC ranged from 0.368 to 0.805. These results indicate that the developed SSR marker is highly polymorphic. Nei's genetic identity (H) ranged from 0.0926 to 0.3462, with a mean of 0.2233. Shannon's Information index (I) ranged from 0.1333 to 0.4799, with an average of 0.3073; Shanxi province had the highest value and Hunan province had the lowest. Genetic distance and Nei's genetic identity analysis, NJ tree diagram analysis, and PCoA analysis were conducted on populations of Daphnia magna from different regions. An analysis of population genetic structure divided the test D. magna samples into two major groups. There was no absolute correlation between the gene distribution of D. magna and its geographic origin. These results indicate that the genetic diversity of D. magna is rich, and the genetic structure of D. magna differs considerably in different regions. These research results and the newly developed polymorphic SSR 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.

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Funding informatio

This study was supported by National Water Pollution Control and Treatment Science and Technology Major Project of China (2017ZX07205).

Abstract

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Keywords

Daphnia magna , SSR polymorphism, RAD-seq, Genetic structure, Genetic diversity

1 INTRODUCTION

Daphnia magna belongs to the *Daphniida* e family.*Daphnia* are an important component of the water ecosystem(Forró et al., 2008), mainly living in freshwater lakes, rivers, ponds, and reservoirs. *Daphnia magna* feeds directly on phytoplankton (Nandini and Rao, 1997), and is often used to control the population and community structure of phytoplankton in water bodies (Sarnelle, 2007). 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. *Daphnia magna* has the characteristics of wide distribution, strong adaptability, short reproduction period, allowing large-scale cultivation in the laboratory, and is highly sensitive to a variety of toxic substances in the water environment (Seda and Petrusek, 2011), so it is widely used in water pollution monitoring and aquatic organism toxicological research (Dietrich et al., 2010; Fan et al., 2011). It is also an ideal model organism in the field of life science research (Miner et al., 2013).

It is of great significance to study the genetic diversity of a population. The higher the genetic diversity of a species, the more extensive the source of variation, and the higher the probability that a good germplasm can be screened from germplasm resources. At the same time, studying the genetic diversity of species can be used as a reference for dividing heterosis groups. Heterosis groups are divided according to the distance between samples. The farther the genetic relationship, the more obvious the heterosis. An assessment of population structure can also conduct allelic mining of germplasm resources, study the genetic structure differences at the DNA level of germplasms obtained from different geographical sources, different breeding periods, different subspecies, and different breeding sources. (Chen et al., 2010). *D. magna* is an extremely important zooplankton in lakes, rivers, and reservoirs. It grows fast, reproduces well, and has a strong feeding

power on algae, effectively controlling the accumulation of primary products in water bodies (Grintzalis et al., 2017). *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. It is the key to balance in the water ecosystem. 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. In this study, we use the microsatellite marker method to understand the genetic variation, genetic structure, and gene distribution of *D. magna* , in order to better protect its genetic diversity. In addition, Shanghai Taihe Water Environment Technology Development Co., Ltd. invented a technology for ecological restoration of *D. magna*guided submerged plants, the artificially domesticated *D. magna*that can quickly filter the high biomass of phytoplankton and other particulate organic matter, increasing the transparency of the water body by 1.0-1.5 m in a short time, so that the planting of submerged plants can quickly form vegetation. *D. magna* was first introduced to water ecological restoration in the world and has achieved good ecological restoration effects in many rivers and water bodies (Huo et al., 2010; Peng et al., 2011). Therefore, it is very important to use the microsatellite marker method to study identification of wild and artificially domesticated species,too.

SSR refers to simple sequence repeat (Ding et al., 2017), also called microsatellite DNA, which is a DNA molecular marker technology based on PCR with specific primers. SSRs are short tandem repeat sequences composed of one to six nucleotides as repeat units in the genome. They are widely distributed in various regions of the genome, with the length generally between 100 and 200bp. SSRs are currently one of the most commonly used microsatellite markers. Because of their large number, codominance, easy operation, stable results, high polymorphism, and strong reproducibility, they have been widely used in research on gene mapping, genetic map construction, fingerprint analysis, genetic relationship identification, and biodiversity assessment (Panesar et al., 2015; Kim and Sappington, 2013; Chambers et al., 2014).

At present, research on *Daphnia magna* mainly focuses on reproduction ecology, toxic and toxicological effects (Rosenfeldt et al., 2014), and water pollution purification (Berger et al., 2007; Straile, 2000). Some scholars have used molecular biology methods to study the genetic diversity of some species of zooplankton. For example, Kotov and Elías-Gutiérrez (2009) conducted a phylogenetic analysis of*Ilyocryptus* . Petrusek et al. (2010), Colbourne et al. (2006), Belyaeva and Taylor (2009) used different gene marker methods to analyze 12SrDNA, COI, and ITS-2 in *Daphnia longispina* , *Chydorus sphaericus* , *Daphnia* and *Daphniapsis* . Young et al. (2012) conducted studies on *Simocephalus vetulus* , *Simocephalus vetuloides* , and *Simocephalus mixtus* based on the mitochondrial COI gene, and concluded that their genetic distance was 0.00671-0.00785. To date, there have been few studies on molecular markers of*Daphnia magna* , and analysis and development of *D. magna*SSR markers is still lacking.

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). Because there is no reference genome sequence for*D. magna* , our study used RAD-seq technology to develop and screen polymorphic SSR loci of 12 wild population of *D. magna* and one species of artificially bred *D. magna* , and analyze the genetic diversity and genetic structure of *Daphnia magna* . The 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 *Daphnia magna* .

2 MATERIALS AND METHODS

2.1 Sample collection and genomic DNA extraction

The experimental samples used in this research are one artificially bred population and 12 wild populations (Table 1); the artificially bred population was taken from the breeding base of Shanghai Taihe Water Environment Technology Development Co., Ltd. (Shanghai Fengjing). 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 single-clone 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) until further use.

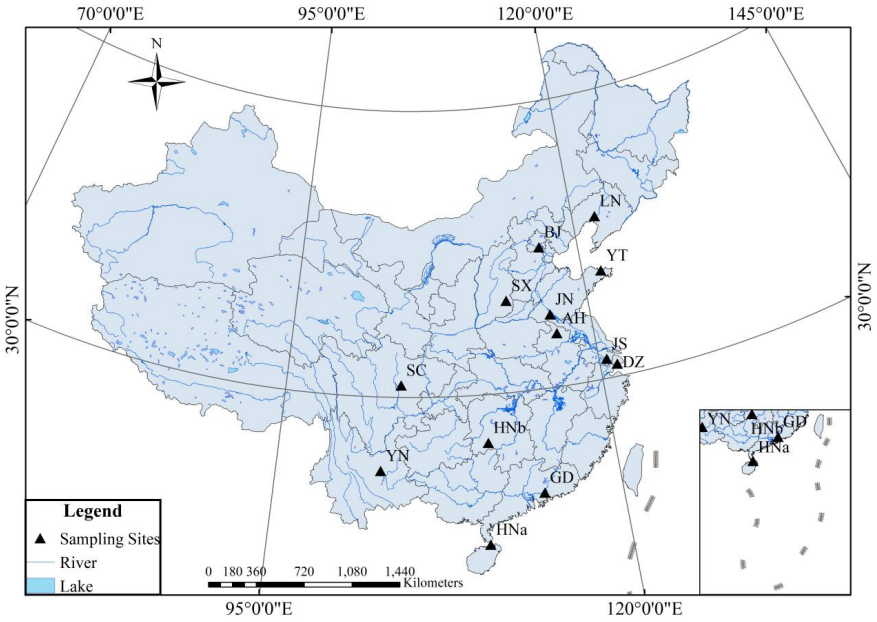

FIGURE 1 Map of the sampling locations for the 13 populations of *Daphnia magna* in the present study

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

2.2 Restriction site-associated DNA sequencing

The genomic DNA of the four populations (DZ, YN, JN, and YT) was mixed in equal amounts, and the database was constructed and sequenced by Shanghai Map Biotech Co., Ltd. (Shanghai, China). Since *D. magna*had no reference genome, it was necessary to use multiple restriction endonucleases to digest the DNA of *D. magna* separately. After digesting the genomic DNA with appropriate restriction endonuclease, P1 adapters were added. A P1 adapter contains the primer sequence required for PCR amplification, the sequence of the Illumina sequencing primer binding site, and the short tag sequence to distinguish different samples. Samples with different P1 adapters were mixed, physically breaking them into sequences of 300˜500bp, and then P2 adapters were added. Next, we performed PCR amplification and enrichment of RAD-tags, construction of a paired-end library, and sequencing using the Illumina HiSeq PE150 (Illumina, USA) system. Raw data were filtered using trimmomatic software (http://www.usadellab.org/cms/uploads/supplementary/Trimmomatic) to obtain high-quality clean data and ensure smooth subsequent analysis. We removed the linker sequence in the reads and reads without inserts because of factors such as linker interconnection. Bases of lower quality (quality value less than 20) were trimmed at the 3' end of the sequence and entire sequences containing base quality values less than 10 were removed, eliminating reads with a N ratio higher than 10%, and removing adapter and quality modification sequences of less than 75bp in length.

2.3 SSR loci analysis and primer design

MISA (http://pgrc.ikpgatersleben.de/misa) software was used to estimate the qualified SSR, and Primer 3 (Li et al., 2010)software was used to design SSR primers for better SSR loci.

2.4 SSR loci screening

SSR loci screening mainly includes unitary and polymorphism screening. Unitary screening uses PCR and agarose gel electrophoresis (1.5% agarose gel, 200V, 15 min) to determine whether there is a product and whether the product is single-stranded. Polymorphism screening uses fluorescent primer PCR and capillary electrophoresis to determine whether the peak shape is qualified and whether it is polymorphic. Six samples (DZ, YN, HNa, GD, JN, and SZ) were used for polymorphism screening, with PCR amplification using tailed primers, and the universal typing primer Common-famF (AGTCACGACGTTGTAAAACGAC). Polymerase chain reaction (PCR) amplification was performed in 20μL volumes containing 10μL Taq Master Mix (Vazyme Biotech Co., Ltd, China), 30ng/μL DNA template 1μL, typing primer 1μL, forward primer 0.2μL, and reverse primer 1μL, supplemented with ddH_2O 7μL. The PCR amplification reaction was carried out on a Mastercycler pro PCR machine (FR-180, China). The PCR thermal cycle consisted of initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30s, locus-specific annealing temperature for 30s, (Table 2 shows the annealing temperature for each primer reaction), 72°C for 30s, final extension at 72°C for 10 min, and then the temperature was maintained at 4°C. PCR products were electrophoresed with 1% agarose and stained with ethidium bromide (EB), observed, and photographed using the gel imaging system.

2.5 Data analysis

Genemapper 4.0 software was used to read the molecular weight data of the SSR amplified products, and then DataFormater software was used to determine the genotype of individual points based on the molecular weight data. The total number and number of effective alleles (Na and Ne, respectively), and observed and expected heterozygosity (Ho and He, respectively) were computed using GenAlEx 6.5 (Peakall and Smouse, 2012). Polymorphic information content was calculated using Powermaker 3.25. POPGENE software (Mir et al., 2012) was used to calculate the number of polymorphic loci (A), the percentage of polymorphic loci (P), Nei's genetic identity (H), Shannon's information index (I), and Nei's unbiased measures of genetic identity and genetic distance for each population (Nei, 1978). A neighbor-joining (NJ) dendrogram was constructed from the calculated genetic distance between populations using POPTREE software (Takezaki et al., 2010). During bootstrapping, 1000 permutations were performed to evaluate the robustness of the clusters. Principal Coordinates Analysis (PCoA) was implemented in GenAlEx 6.5 in order to analyze the genetic relationships across populations. Bayesian model-based clustering analysis was performed using STRUCTURE

2.3.4 software to estimate the most likely number of genetic clusters (Pritchard et al., 2000). This clustering method was used to identify genetically distinct subpopulations based on allele frequencies. The admixture model was applied, and the number of clusters (K value) was set from 2 to 10 with three independent runs for each fixed K number. Each run included a burn-in length of 10,000 followed by 10,000 MCMC (Monte Carlo Markov Chain) repetitions. The most likely K value was determined based on the method described by Evanno et al. (2005) by submitting all result files for K = 2 to 10 to STRUCTURE HARVESTER (Earl and vonHoldt, 2011). The run with the highest log probability value among the three independent ones was chosen and represented as bar plots (Sitther et al., 2014) using DISTRUCT 1.1 (Rosenberg, 2004).

3 RESULTS

3.1 Polymorphism of newly developed microsatellite markers

A total of 26 polymorphic SSR loci were developed in 13 populations using RAD-seq technology. The primer sequences (5'-3'), repeat motifs, size ranges, and annealing temperatures are shown in Table 2. The value of Na and Ne across all loci was highest in ZSC_320 and lowest in ZSC_7904, with an average number of 4.654 alleles per locus, and mean effective number of alleles of 3.000. The observed heterozygosity (*Ho*) ranged from 0.083 to 0.999, with a mean of 0.486. The expected heterozygosity (*He*) was 0.085-0.862, with a mean of 0.645. The Polymorphic information content (*PIC*) range was 0.368-0.805, with an average of 0.576. The Shannon's information index (*I*) ranged from 0.173 to 1.936, with an average of 1.187. The highest values for *He* , *PIC* , and *I* were found in ZSC_320, while the highest values for *Ho* were found in ZSC_3833. The lowest values for *Ho* , *He* , *PIC* , and*I* were all found in ZSC_7904 (Table 3). The *PIC* value is an important index to evaluate the degree of variation of a gene locus (on the molecular level, a sequence with genetic defects). If the*PIC* value of a gene locus is greater than 0.50, it means it is a highly polymorphic locus; if the *PIC* value is between 0.25 and 0.50, it means it is a moderately polymorphic locus; and if the*PIC* value is less than 0.25, it means there is low polymorphism. In our research, the average *PIC* value of 26 SSR markers in 13 populations was 0.576, with 17 loci having a *PIC* value higher than 0.5. These results indicate that the developed SSR marker is highly polymorphic.

TABLE 2 Detailed information on the 26 pairs of microsatellite primers

Locus	Primer sequences (5'-3')	Repeat motif	Size range (bp)	Annealing temeperature ()
ZSC_320	F:ACAACTACACCTACCCCAGCAG R:TAGCTGTGACCTGGTTTAAATG	(CAG)6	222˜239	57.0
ZSC_448	F:TACAGCAATCACATTCCAAATG R:TTTTCAGGTCATGAATAATGGA	(TAC)6	97˜114	56.5
ZSC_546	F:ACATTTCAATTGTTAGATGGGTG R:GGATGTTGCGTTTGTCAAT	(AGA)6	174˜191	55.8
ZSC_679	F:AATTCCTCGAAATCTTGGT R:GAAAATTCAAAGTTGCCTAACC	(TTG)7	29˜49	57.0
ZSC_748	F:CCGACTTGATGGAATAACCATTAT R:CACCTGTTAATGTGAGTCTTGC	(TAA)7	76˜96	58.0
ZSC_761	F:TTATTGCTTTTTGAAACTTGGAC R:GGAAGCTTTTGTATAGGCAGTC	(AAC)5	246˜260	56.9
ZSC_1372	F:CTGACATCCATCAACTATGAAGA R:AACAAATCCCTCTGCTATTCTC	(CTA)7	198˜215	57.0
ZSC_1701	F:CTGAGCAACAGCAACAATTTG R:GATTGTAAAGCGGATAGTGTGA	(CAA)5	108˜122	58.0
ZSC_18335	F:CGCTCTCTCTTTCTCTCTTTGT R:CCGAGTATTTCTTCGATGTTTA	(TCT)7	113˜126	57.3
ZSC_875	F:AATTCGATGACGGAAACTC R:CAACAGCGTCAGTCCTACAC	(AAT)5	21˜35	55.7

ZSC_1797	F:AAATAAAACAACC[illegible]AATGG R:CAAGACGTGTCCTGTGTCACT	**(GAA)5**	250~264	57.2
ZSC_1890	F:AGAAACGATGTCT[illegible]ACTGT R:CGGTTAATCACTGCATCACTAT	**(GAT)5**	39~53	56.3
ZSC_2050	F:AACGATTTCACTT[illegible]ATTAG R:GAGACGGACAAGAACAGTAACA	**(TGC)6**	165~128	57.0
ZSC_2586	F:ATTCACCGATCGT[illegible]AAT R:GTCACTCCCTCCTAGACAACTC	**(CAG)8**	26~49	57.0
ZSC_2799	F:AAAGAAATGCCTC[illegible]ATCAT R:ATTGTAGGTCATCGGGAGGT	**(AGG)5**	148~162	57.0
ZSC_2828	F:CACTGAACAAAGG[illegible]AAGAA R:GATGAGTGTCAATGCTGACTT	**(TGA)5**	62~76	57.0
ZSC_3113	F:ATCTTCATATCGA[illegible]GTT R:TGAATCAGTTCTCCAATAATCG	**(AAC)5**	142~156	56.9
ZSC_3761	F:TCATCATAGCGTC[illegible]CC R:GGAAGAGGCCTGAGATCC	**(CTG)6**	256~273	57.2
ZSC_3833	F:GCGCCTTTCCTAT[illegible]CTAA R:GCAAAGAAGAAGGAGAACAAGT	**(AAC)6**	67~84	57.0
ZSC_4037	F:CTACGGTCCAGGT[illegible]CTGAT R:GAGATGGTTACGACTGAATTTG	**(AGA)6**	141~158	57.4
ZSC_4319	F:GTCCAGGCTATTT[illegible]TCTAC R:AAAACTCGCTTGAAAAATTGTA	**(CTT)7**	87~107	57.0
ZSC_10218	F:CTCTTGACCAGAA[illegible]AGAC R:ATTCGACTGGAGGTATGGATT	**(GTG)6**	40~57	56.5
ZSC_6980	F:GCGTCACTATGTC[illegible]CATTA R:GGAAGTCCATACCAAGAAAAAC	**(ACC)6**	196~213	57.8
ZSC_7904	F:TCATTTTCCAAAA[illegible]AAACC R:GTATATGTCAATGTTTGAGCGG	**(AAC)6**	117~134	57.4
ZSC_8717	F:AGTTTCATCAACA[illegible]CTTC R:AGGAAGGCGTTTTTGGAC	**(GCC)6**	179~196	56.9
ZSC_9631	F:AGAAGAGGAAGAG[illegible]AGTTG R:TGTTTTTAATGAGCCGATGAT	**(AAG)6**	143~160	57.1

TABLE 3 Characterization of 26 novel microsatellite loci in 13 populations of *Daphnia magna*

Locus	Number of alleles (*Na*)	Effective number of alleles (*Ne*)	Observed heterozygosities (*Ho*)	Expected hetero
ZSC_320	9	5.760	0.417	0.862
ZSC_448	3	2.198	0.700	0.573
ZSC_546	6	3.646	0.583	0.757
ZSC_679	6	4.400	0.455	0.810
ZSC_748	6	3.507	0.727	0.749
ZSC_761	3	2.051	0.778	0.543
ZSC_1372	5	2.439	0.600	0.621
ZSC_1701	5	2.793	0.231	0.668
ZSC_18335	6	2.432	0.308	0.612
ZSC_875	6	3.485	0.615	0.742
ZSC_1797	3	2.602	0.636	0.645
ZSC_1890	6	4.500	0.750	0.812
ZSC_2050	3	1.852	0.400	0.484
ZSC_2586	4	3.270	0.636	0.727
ZSC_2799	3	2.268	0.333	0.583

ZSC_2828	3	2.283	0.364	0.589
ZSC_3113	3	1.986	0.250	0.518
ZSC_3761	6	2.522	0.231	0.628
ZSC_3833	4	2.455	0.999	0.628
ZSC_4037	3	1.811	0.250	0.467
ZSC_4319	3	2.160	0.556	0.569
ZSC_10218	5	2.704	0.539	0.655
ZSC_6980	6	4.546	0.600	0.821
ZSC_7904	2	1.087	0.083	0.085
ZSC_8717	5	4.000	0.167	0.783
ZSC_9631	7	5.236	0.417	0.844
Mean	4.654	3.000	0.486	0.645

3.2 Genetic diversity of *Daphnia magna* populations

Amplification results for the 26 SSR markers in 13 *Daphnia magna*populations showed high polymorphism. At the population level, the 26 SSR markers detected that the number of polymorphic loci ranged from 2 to 18 in the 13 populations, with an average of 9.923. The percentage of polymorphic loci ranged from 7.69% to 69.23%, with an average of 41.12%. The SX population amplification results showed the highest level of polymorphism, with a polymorphic loci percentage of 69.23%, followed by GD (65.38%), DZ (61.54%), and LN (61.54%) populations, while the JS population amplification results showed the lowest level of polymorphism, with a polymorphic loci percentage of only 7.69%. Furthermore, Nei's genetic identity (H) ranged from 0.0926 to 0.3462, with a mean of 0.2233. The value for SX was the highest and that for HNb was the lowest. In addition, the values for LN, GD, and DZ also exceeded 3.000. Shannon's Information index (I) ranged from 0.1333 to 0.4799, with an average of 0.3073. SX had the highest value, followed by GD, while HNb had the lowest value (Table 4). The above results show that among the 13 regions, genetic diversity of the*Daphnia magna* populations in Shanxi, Guangdong, Liaoning, and Taihe Water is relatively high. Genetic diversity of the *Daphnia magna* populations in Shanxi Province is the most abundant, while that in Jiangsu Province is relatively low. There are obvious genetic differences among different provinces and cities.

TABLE 4 Genetic diversity index for 13 populations of*Daphnia magna* analyzed using SSR markers

Populations	Number of ploymorphic loci (A)	The percentage of ploymorphic loci (P)	Nei's genetic identity (H)	Sha
AH	11	42.31%	0.2115	0.29
BJ	5	19.23%	0.1569	0.20
DZ	16	61.54%	0.3077	0.42
HNa	4	15.38%	0.1176	0.16
GD	17	65.38%	0.3269	0.45
HNb	5	19.23%	0.0926	0.13
JN	14	53.85%	0.2692	0.37
JS	2	7.69%	0.1111	0.15
SC	7	26.92%	0.1667	0.23
SX	18	69.23%	0.3462	0.47
YN	9	34.62%	0.1800	0.24
YT	5	57.69%	0.2885	0.39
Mean	9.923	41.12%	0.2233	0.30

3.3 Genetic structure analysis of *Daphnia magna*populations

In order to further explore the genetic structure differences of*Daphnia magna* populations in different regions,

we calculated Nei's genetic identity and genetic distance for the 13 sampling sites. Nei's genetic identity and genetic distance is an important indicator that reflects the distance between populations. When the genetic distance is 1 (the genetic identity is 0), it indicates that the*Daphnia magna* of the two populations are completely different and there is no genetic relationship; when the genetic distance is 0 (the genetic identity is 1), it indicates that the germplasm of the two populations is identical (Nei, 1972). The genetic distances for the 13 different sources of *D. magna* were 0.0034-0.9783, with an average value of 0.6349. The genetic distance between YT and JS was the largest, and the genetic distance between AH and HNb was the smallest. Nei's genetic identity for *D. magna* in the 13 different regions was 0.0034-0.9966, with an average value of 0.3969. AH and HNb had the highest Nei's genetic identity, and JS and AH had the lowest (Table 5). This shows that there are certain differences in the genetic structure of *Daphnia magna* in different regions, and the genetic diversity of *Daphnia magna* is extremely rich. Jiangsu and Anhui populations are relatively distantly related, while Anhui and Hunan populations are relatively closely related.

TABLE 5 Nei's genetic identity and genetic distance for 13 populations of *Daphnia magna*

Populations	AH	BJ	DZ	HNa	GD	HNb	JN	LN	SC	SX	JS	YN	
AH	****	0.1686	0.6650	0.1528	0.2897	0.9966	0.6378	0.8275	0.2434	0.8351	0.0220	0.9691	0
BJ	0.8316	****	0.1495	0.7346	0.3485	0.1230	0.0629	0.1122	0.4469	0.1573	0.0264	0.1343	0
DZ	0.4080	0.8508	****	0.1765	0.2334	0.6303	0.5002	0.6522	0.2904	0.6825	0.0922	0.6591	0
HNa	0.8482	0.3084	0.8324	****	0.3833	0.1387	0.0970	0.1349	0.5229	0.2018	0.0954	0.1426	0
GD	0.7585	0.6994	0.7669	0.6651	****	0.3358	0.3682	0.2118	0.3969	0.1193	0.0943	0.4318	0
HNb	0.0034	0.8771	0.4616	0.8613	0.7026	****	0.5324	0.7778	0.2639	0.7859	0.0759	0.9396	0
JN	0.4497	0.9505	0.5468	0.9035	0.6669	0.5068	****	0.7037	0.4343	0.7038	0.0221	0.6322	0
LN	0.1893	0.8888	0.4274	0.8652	0.7983	0.2513	0.3514	****	0.2976	0.9290	0.0461	0.7763	0
SC	0.7666	0.6145	0.7159	0.5361	0.6584	0.7664	0.6516	0.7158	****	0.3449	0.0223	0.2844	0
SX	0.1802	0.8432	0.3826	0.8205	0.8812	0.2409	0.3513	0.0737	0.7025	****	0.0483	0.7971	0
JS	0.9783	0.9739	0.9080	0.9048	0.9061	0.9243	0.9780	0.9542	0.9779	0.9517	****	0.0896	0
YN	0.0314	0.8657	0.4169	0.8577	0.6340	0.0623	0.4586	0.2532	0.7305	0.2268	0.9174	****	0
YT	0.3648	0.8904	0.5992	0.9089	0.8195	0.4210	0.3951	0.2664	0.7839	0.2003	0.9564	0.4381	*

Note: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

In order to more intuitively show the genetic distance differences of different populations of *D. magna* , POPTREE software was used to construct a neighbor connection (NJ) tree diagram. The results showed that AH and HNb clustered first, and then clustered with YN. LN and SX are clustered, and then clustered with YT, JN, and DZ, respectively. BJ clustered with HNa, and then clustered with SC. Among these 13 sampling sites, the genetic structures of GD and JS are quite different from the genetic structure of the other regions, and the genetic relationship between AH and HNb, and between BJ and HNa, is closer. The clustering results are consistent with Table 5.

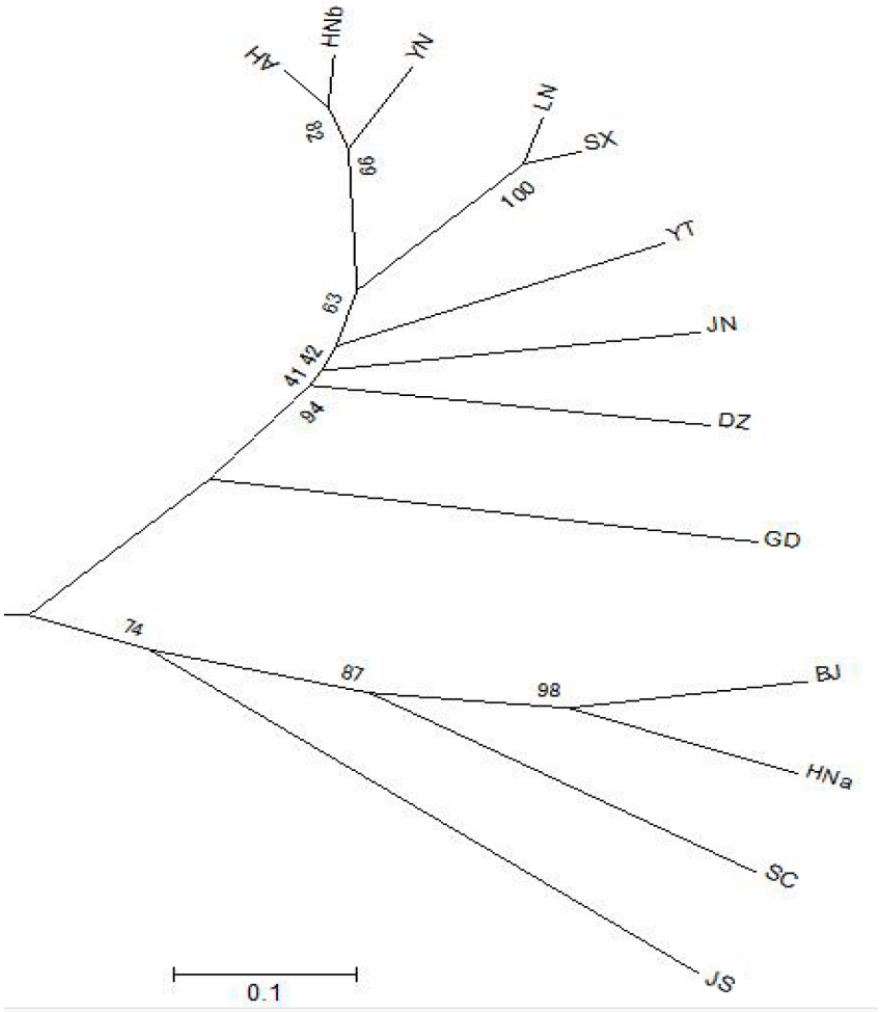

FIGURE 2 Neighbor-joining dendrogram based on genetic distances among different populations of *Daphnia magna* .

Principal coordinates analysis (PCoA) is a powerful tool for evaluating the genetic structure of a population. Based on the screened SSR loci, GenAlEx 6.5 software was used to perform principal coordinates analysis on *Daphnia magna* in different regions, and PCoA cluster maps of experimental samples in 13 regions were obtained (Fig. 3). It can be seen from the PCoA cluster maps that AH, HNb, YN, LN, JN, SX, YT, and DZ are clustered together. BJ and HNa are clustered together but they are obviously scattered from the other populations, while the *D. magna* populations for GD, JS, and SC are distributed separately and relatively scattered. These results are basically consistent with the results from population genetic distance analysis and clustering results, indicating the consistency of the clustering results.

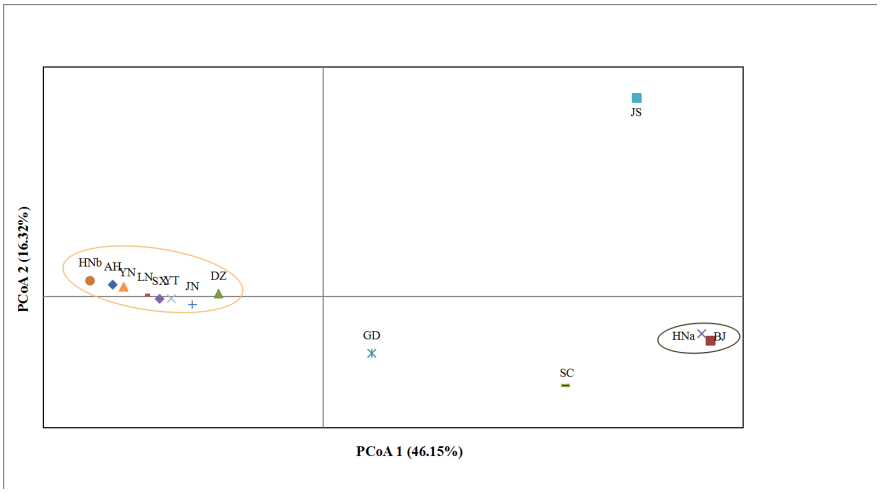

FIGURE 3 Scatter plots of principal coordinates analysis (PCoA) revealing the genetic relationships between *D. magna* in 13 regions

From the tree diagram and PCoA scatter diagram, we can intuitively understand the classification relationship between populations, but in order to determine how many subgroups a certain group has, whether there is gene exchange between groups, and the degree of hybridization of each individual, we need a structure diagram to analyze the structure of the populations. The preset number of population subgroups is equal to 1-10, that is, K=1-10, and the grouping situation and ancestry composition of the population are calculated based on the Bayes algorithm. Simulation results for each K value will produce the maximum likelihood. The larger the value of ln likelihood, the closer the K value is to the real situation, which means we need to determine the simulation result with the largest likelihood and smallest K value (Evanno et al., 2005). It can be seen from Fig. 4 that as the value of K increases, the value of Ln(K) also gradually increases, but when K=2, the value of ln likelihood enters a plateau, and the value of ΔK appears at its maximum with an obvious peak, indicating that *D. magna* in the 13 regions can be divided into two groups. Each individual is represented by a vertical colored bar, and the proportion of the color in each bar represents the probability of membership in the relevant cluster. It can be seen from Fig. 5 that *D. magna* at the eight sampling sites (AH, DZ, HNb, JN, LN, SX, YN, and YT) can be put into one group, and they may have a common ancestor, while BJ, HNa, and JS are divided into another group, their genetic structure is relatively simple. The colored bars for GD and SC are composed of two colors, indicating that the genetic background of the populations for GD and SC is relatively complicated, and it is likely to be derived from a cross of two ancestral subgroups, with the ancestral subgroup of GD accounting for between 40% and 60% of the pedigrees, and SC mostly belonging to the red group.

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FIGURE 4 Distribution of K values with L(K), L'(K), |L"(K)|, and ΔK

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FIGURE 5 Estimated genetic structure resulting from Bayesian model-based analysis using STRUCTURE 2.3.4 for populations of*Daphnia magna* . (Each number in the figure represents a population. 1: AH, 2: BJ, 3: DZ, 4: HNa, 5: GD, 6: HNb, 7: JN, 8: LN, 9: SC, 10: SX, 11: JS, 12: YN, 13:YT)

4 DISCUSSION

In this study, 26 polymorphic SSR loci were developed using RAD-seq technology, and genetic diversity analysis was performed on 12 wild*D. magna* populations and one artificially selected *D. magna* population in China. The average number of alleles for the 26 pairs of SSR primers is 4.654. With an average of more than four alleles, one can better assess the genetic diversity of a population (Wajid et al., 2014), so the developed microsatellite markers show good polymorphism and can provide help in further molecular research on*D. magna* . Diversity analysis of *D. magna* populations in the 13 regions shows that the mean values of Nei's genetic identity and Shannon's Information index are 0.2233 and 0.3073, respectively, indicating that the genetic marker diversity of *D. magna* is relatively high. The genetic diversity of *D. magna* is relatively high in Shanxi and Guangdong and is the lowest in Jiangsu. In addition, it also showed good genetic diversity in artificially domesticated*D. magna* (DZ). Genetic distance and Nei's genetic identity analysis, NJ cluster analysis, PCoA analysis, and STRUCTURE genetic structure analysis were conducted on germplasm samples from*Daphnia magna* in different regions; the results were basically consistent and complementary. The research results showed that the genetic diversity of *Daphnia magna* from different sources was quite different. The genetic relationship between Anhui and Hunan's*D. magna* germplasm is the closest, and the genetic relationship between Anhui and Jiangsu is the furthest. NJ cluster analysis and genetic distance analysis gave the same results. PCoA analysis results showed that *D. magna* in Anhui, Hunan, Jining, Liaoning, Taiheshui, Yantai, and Yunnan can be clustered into one group. The differences in *D. magna* in Beijing and Hainan are also small, while *D. magna* in Guangdong, Sichuan, and Jiangsu are quite different from other regions, and the clustering results have no correlation with geographic origin. The STRUCTURE genetic structure plot divides *D. magna* from the 13 regions into two groups. The genetic backgrounds of *D. magna* in Anhui, Hunan, Jining, Liaoning, Shanxi, Yunnan, Yantai, and Taihe Water are relatively simple, and the genetic structure of *D. magna* in Beijing, Hainan, and Jiangsu is also very simple but significantly different from the previous group, while the genes of *D. magna* in Guangdong and Sichuan are shared with the two groups.

From the sampling site distribution and clustering results, there is no absolute correlation between the gene distribution of *D. magna*and its geographic origin, but on the whole, there are still large differences in the genetic structure of *D. magna* in the north and south of China, which may be a result of differences in temperature, climate, precipitation, and water environment. However, there are exceptions; the genetic distance between *D. magna* in Beijing and Hainan is relatively small, which may be due to similarities in the water environment causing *D. magna* in the two places to evolve in a similar direction. Human interference is an extremely significant activity affecting gene exchange in species, with the high similarity of*D. magna* genes in different regions likely to be the result of artificial introduction. Meanwhile, zooplankton have weak swimming ability and are highly susceptible to factors such as water level fluctuations, tidal currents, and water erosion (Robinson et al., 2013). Ingestion by large aquatic animals such as fish, shrimps, and crabs will also accelerate gene exchange between *D. magna* from different regions. However, based on our research, the genetic structure of*D. magna* in different regions is quite different. Human construction activities destroy the habitats of *D. magna* , which fragment the habitat and spread populations, resulting in low genetic similarity between *D. magna* in different regions. In addition to its weak swimming ability, *D. magna* mainly reproduces asexually, which inevitably affects gene exchange between populations due to long-term geographic isolation, which may lead to obvious genetic differentiation between populations, increase in genetic distance, and decrease in population size, leading to population selfing and genetic drift, which ultimately leads to a decrease in genetic diversity. Therefore, we should raise awareness of environmental protection, protect the safety and integrity of the water environment, and also cultivate and introduce *D. magna* into different regions and rivers in order to increase gene flow between populations, prevent the aggravation of genetic differentiation, and increase genetic diversity. A high level of genetic diversity can provide a source of genes to optimize the germplasm resources of *D. magna* . As a natural open bait for high-level aquatic animals such as fish and shrimps, high-quality *D. magna* germplasm resources can improve the quality of aquatic products, shorten the breeding cycle of aquatic products, and improve the economic benefits of aquaculture (Feng et al., 2018). In addition, artificial domestication of *D. magna* (Taihe Water sampling site) can quickly

remove algae and other organic particles, increasing the transparency of the water body by 1.0-1.5 m in a short time, so that submerged plants can be transplanted and survive. Submerged plants quickly reduce the content of nutrients such as nitrogen and phosphorus in the water body through nutrient competition, and inhibit the growth and reproduction of blooms and algae (Huo et al., 2010), playing a major role in the restoration of water ecosystems. Therefore, studying the genetic structure of *D. magna* in different regions can not only protect its genetic diversity at a molecular level, but it can also enrich the *D. magna* gene bank and increase our understanding of its evolutionary potential.

The life history of *D. magna* is unique; it consists of parthenogenetic generation and bisexual reproduction, and these two reproductive methods can alternate with changes in the external environment (Buchberger et al, 2020). When the external environment is suitable, such as suitable temperature, sufficient food, and sufficient biological space, *D. magna* reproduces mainly by parthenogenesis, and the egg cells produced by females can directly develop into female offspring without fertilization. This is the most important way for it to reproduce. Once external conditions deteriorate, such as lack of food, high or low pH, the amount of dissolved oxygen in the water drops sharply, metabolic wastes accumulate, population density increases, or the light cycle shortens, the reproductive mode of *D. magna* will change from asexual to bisexual. When environmental conditions improve again, the reproductive mode of *D. magna* will switch from asexual reproduction to bisexual reproduction (Hebert, 2010). All *D. magna* samples used in this experiment must be the offspring of asexual reproduction, so it is necessary to ensure a suitable culture environment. In wild waters environment, *D. magna* reproduces asexually under most conditions, but in some harsh environments, some individuals will reproduce with both sexes and produce fertilized eggs covered by a saddle to resist the poor external environment. Therefore, there will still be differences in the genetic structure of *D. magna* in the same water area, even if the difference is very small. In addition, the sampling sites in this study are evenly distributed, but only a few sampling sites were chosen, China has a vast territory and a large north-south span. The sampling sites of a certain river cannot fully reflect the genetic diversity of all *D. magna* in a province or city. Although the polymorphic SSR markers developed in this experiment are abundant in quantity, provided reliable results, and co-dominant markers, the SSR markers still have co-migration problems. Today, the rapid development of new molecular markers such as EST, SNP, and gene chips have brought genetic diversity research more efficient and rich results and will be the focus of our next research.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

Shengman Zhang: Formal analysis (lead); Investigation (lead); Methodology (lead). Writing-original draft (lead); Writing-review & editing (lead); **Haisu Zheng:** Investigation (lead); Writing-review & editing (lead); Methodology (lead). **Meiqin Wu:** Methodology (supporting). **Meng Tan:** Methodology (supporting). **Siwei Chen:** Resources (supporting). **Zheng Han:** Resources (supporting). **Jinlin Liu:** Methodology (supporting). **Peimin He:** Conceptualization (lead); Funding acquisition (lead); Project administration (lead); Writing-original draft (equal); Writing-review & editing (equal); Supervision (equal).**Wenhui He:** Supervision (equal); Conceptualization (lead); Funding acquisition (lead); Project administration (equal); Resources (equal).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The *Daphnia magna* used in this study were caught from wild water bodies and provided by Shanghai Taihe Water Environment Technology Development Co.Ltd and no specific permissions were required.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article and its supplementary information files. The raw reads produced in this study were deposited in the DRYAD database.

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