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Genetic Polymorphism of Microsatellite DNA in Two Populations of Northern Sheatfish (*Silurus soldatovi*)

QUAN Ying-Chun[1,2], SUN Xiao-Wen[1,①], LIANG Li-Qun[1]

1. *Heilongjiang Fisheries Research Institute, Chinese Academy of Fishery Sciences, Harbin* 150070, *China*;

2. *College of Aqua-life Science and Technology, Shanghai Fisheries University, Shanghai* 200090, *China*

Abstract: In this article, population variations and genetic structures of two populations of northern sheatfish (*Silurus soldatovi*) were analyzed using 24 microsatellite loci enriched from southern catfish (*S. meriaionalis* Chen) by magnetic beads. Gene frequency (*P*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), polymorphism information contents (*PIC*), and number of effective alleles (*Ne*) were determined. One population was wild, ripe individuals collected from Heilongjiang River (*HNS*); the other was cultured fry collected from Songhuajiang River (*SNS*). The Hardy-Weinberg equilibrium (HWE) was tested by the genetic departure index (*d*). The coefficient of gene differentiation G_{ST} and Φ_{ST} by AMOVA (Analysis of Molecular Variety) was imputed using Arlequin software in this study. In addition, a phylogenetic tree was constructed by UPGMA method based on the pairwise Nei's standard distances using PHYLIP. A total of 1 357 fragments with sizes ranging between 102 bp and 385 bp were acquired by PCR amplifications. The average number of alleles of the two populations was 8.875. Results indicated that these microsatellite loci were highly polymorphic and could be used as genetic markers. The mean values of the parameters *P, Ho, He, PIC,* and *Ne* were 0.165, 0.435, 0.758, 0.742, and 5.019 for *HNS* and 0.147, 0.299, 0.847, 0.764, and 5.944 for *SNS*, respectively. Although there were differences, there were no significant differentiations except for the locus *HLJcf37*. These populations to a certain extent deviated from HWE, such as excessive and deficient heterozygote numbers. The value of G_{ST} was 0.078 and above 98% of the variation were differences among individuals within the population, so the variation between populations was insignificant. Cluster analysis also showed that the relationships among individuals were very close. In conclusion, the microsatellite markers that were developed through this study are useful for genetic analysis and the genetic culture that was proposed in this study has no significant impact on *S. soldatovi*.

Key words: *Silurus soldatovi*; microsatellite DNA; genetic polymorphism

Microsatellites, also known as simple sequence repeats (SSRs), simple sequence length polymophisms (SSLPs), or short tandem repeats (STR), are regions of DNA that exhibit short repetitive sequence motifs. These motifs are often composed of 1–6 bp repeat sequences, such as CA, AGA, ATA, and the like. Their repeat numbers are variable, which makes microsatellites polymorphic. Characterized as codominant, highly polymorphic and amenable to genotyping by polymerase chain reaction (PCR), microsatellites have become instrumental as genetic markers in areas such as population genetics, evolutionary biology, QTL, conservation biology, and genetic mapping [1-3].

Northern sheatfish (*Silurus soldatovi* Nikolsky et Soin), also called Suo's/Su's sheatfish or Huai Tou sheatfish, belongs to the *Vertebrata*, *Osteichthyes*, *Siluriformes*, *Siluridae*, and *Silurus*. Few years ago, it was distributed widely in areas along the middle and lower reaches of the Heilongjiang River and the Liao River. It is well received with suitable size of the adult individual, since it is delicious, nutritious, disease-resistant, and easy to capture. It also grows quickly and can survive over wide temperature ranges.

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① Corresponding author. E-mail: sunxw2002@163.com

The features mentioned above have made northern sheatfish an excellent freshwater fish for the culturists. Since most of the captured species are wild, over-fishing and habitat degradation threaten its living condition. Consequently, in recent times it has become one of the endangered freshwater fishes in China[4]. To protect and develop the breed resource, studies on its biology and genetics are necessary. Although there are many reports of channel catfish (*Ictalurus punctatus*) [2,5,6], European catfish (*S. glanis*) [7, 8], Asian catfish (*Pangasius krempfi*, *P. bocourti*, *P. conchophilus*, *P. pleurotaenis*, and *Helicophagus waandersii* [9]. *S. asotus* Linnaeus and *S. meriaionalis* Chen [10-12]), and so on, reports about the biology [13, 14] and cytology [15, 16] of northern sheatfish are few. Application of microsatellite variations in this species has been introduced for the first time in this study.

In the earlier research by the authors of this study, microsatellite DNAs were isolated from a CA/GT enriched library of southern catfish (*S. meriaionalis*) by combining biotin capture method and radioactive-labeling hybridization. Southern catfish and northern sheatfish belong to the same taxonomic species; so 40 microsatellite loci were synthesized and screened. Twenty-four microsatellite loci were polymorphic and were used to analyze the population structures and genetic diversities of northern sheatfish.

1 Materials and Methods

1. 1 Samples, primers, and reagents

All the samples of northern sheatfish were collected from Heilongjiang province on June 2005, including 22 wild individuals (9♂, 13♀) gathered from the Heilongjiang River (*HNS*) and 30 cultured fry (sex unknown) rooted in the Songhuajiang River (*SNS*).

Microsatellite primers were synthesized by Shanghai Sangon Biological Engineering & Technology and Service Co. Ltd., Shanghai, China. Enzymes and vectors were bought from Promega, USA, and other biochemical reagents were from TaKaRa, Dalian, China.

1. 2 Methods

1. 2. 1 Extraction of genomic DNA

All the fins of the samples were kept in 70% ethanol, from which genomic DNAs were extracted following the method of Geng *et al* [17].

1. 2. 2 Development and screening of microsatellite DNAs

Cloning and characterization of southern catfish microsatellite sequences were performed following the microsatellite hybrid capture technique of Carleton *et al* [18]. In brief, genomic DNA was extracted from the fin of a southern catfish (Wuhan, the middle reach of the Yangtze River) and digested with the restriction enzyme *Sau*3AⅠ. Then short (20 bp) Brown linkers [19] were attached to the DNA. DNA fragments (400−900 bp) were hybridized with biotin-labeled oligonucleotide $(CA)_{12}$ probe and enriched by the magnetic beads coated with streptavidin to capture the fragments containing microsatellites. A partial genomic library was then established in a pMD18-T vector. These clones were screened using the oligonucleotide $(CA)_{15}$ radiolabeled with [γ^{32}P]-ATP at the 5′ end. Finally, positive clones harboring strong hybridization signals would be sequenced. Primer pairs would be designed according to the flanking sequences of microsatellite loci using Primer Premier 5.0 software.

Forty pairs of microsatellite primers were synthesized and screened by PCR amplification using the mixed genomic DNAs of northern sheatfish. Twenty-four pairs successfully yielded reproducible and stable amplifications. They were registered in GenBank and were used to identify the genetic diversities of northern sheatfish.

1. 2. 3 Conditions and processes of PCR

Amplifications were performed with a reaction volume [17] of 25 μL using 50−100 ng of template DNA, 10 pmoles of each primer, 0.01 mol/L Tris-Cl (pH 8.3), 0.05 mol/L KCl, 0.0015 mol/L $MgCl_2$, 0.01% Gelatin, 0.1% Tween 20, 0.1% NP-40, 0.0002 mol/L dNTP, and 1 U *Taq* DNA polymerase. The profile of thermal cycling (Perkin-Elmer 9700, USA) was as follows: 3 min at 94℃, followed by 38 cycles of 30 s at 94℃, 30 s at the selected higher annealing temperature, 30 s at 72℃, and 5 min at 72℃.

1. 2. 4 Electrophoresis and visualization of PCR products

PCR products were separated by electrophoresis in 2% agarose gel (0.5 × TBE buffer at 5 V/cm) for 2 h with 0.02× GoldView (SBS, Beijing China) nucleic acid stain and visualized under UV light on a GDS 8000 (UVP, USA). Microsatellite alleles were identified by their size in base pairs using Gel works software package (Version 3.0; UVP, USA). Sizes of amplified fragments were determined by reference to a standard base-pair ladder, DL2000 (TaKaRa, Dalian, China).

1. 2. 5 Statistical analysis

The observed number of alleles (*A*), effective number of alleles (*Ne*), allele frequency (*P*), observed heterozygosity (*Ho*), and expected heterozygosity (*He*) were computed by the software of POPGENE (Version 3.2). Polymorphism information content (*PIC*) was computed according to the following formula[20].

$$PIC = 1 - (\sum_{i=1}^{n} P_i^2) - (\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2)$$

where n was the number of alleles at one locus; P_i and P_j were the frequencies of the ith and jth alleles at one locus, $j=i+1$.

Deviation from Hardy–Weinberg equilibrium (HWE) between pairwise loci was tested by the genetic departure index (d): $d = (H_O - H_e)/H_e$.

Coefficient of gene differentiation (G_{ST}) was computed and variation ratios of each part Φ-statistics (Φ_{ST}) was analyzed by Analysis of Molecular Variety (AMOVA) using the software of Arlequin Version 2.000 to assess the significance of population differentiation. In addition, a phylogenetic tree was constructed by UPGMA method based on the pairwise Nei's standard distances using the software package of PHYLIP 3.63.

2 Results

2. 1 Microsatellite isolation

A total of 593 positive clones were obtained through biotin capture method and radioactive-labeling hybridization. Among them, 178 were sequenced, out of which 173 (97.19%) were confirmed to contain microsatellite sequences. Of all the microsatellites sequenced, 90.60% repeated more than 10 times and 75.98% had perfect repeat motifs. There were also CT, GA, and ATG motifs besides the CA/GT motif. According to the flanking regions of these microsatellite loci, 120 pairs of microsatellite primers were designed. Forty pairs were synthesized and examined in the two populations of northern sheatfish, and 24 were polymorphic in at least one population using the 0.95-allele frequency criterion. The primer information and GenBank accession numbers are shown in Table 1.

2. 2 PCR amplification

Out of the 24 polymorphic microsatellite loci, only eight amplified in one of the two populations. The locus *HLJcf19* amplified no fragment in *HNS,* neither did loci *HLJcf9*, *HLJcf12*, *HLJcf15*, *HLJcf17*, *HLJcf25*, *HLJcf31*, and *HLJcf40* in *SNS*. Amplifications of locus *HLJcf20* were shown as representative examples in Figs. 1 and 2. For each template, 1−2 fragments were acquired. The sizes of fragments were 265 bp, 265/295 bp, 273 bp, and so on in Fig. 1, and the sizes were 242/265 bp, 242/265 bp, 234/250 bp, and so on in Fig. 2, as determined by Gel works software package. There were 6 alleles, 35 fragments in Fig. 1 and 5 alleles, 42 fragments in Fig. 2. In total, 1 357 fragments were amplified and the allele number ranged from 3−23, and the sizes ranged from 102−385 bp. Overall, 213 alleles (mean value, 8.875) were obtained.

2. 3 Genetic variations of microsatellite loci

A, *Ne*, *P*, *Ho*, *He*, *PIC* per locus and population in the polymorphic loci are summarized in Tables 2 and 3. These parameters were in the range of 3−18, 1.770−13.520, 0.056−0.250, 0.000−0.950, 0.435−0.926, and 0.435−0.926, respectively. Locus *HLJcf36* was the most polymorphic and loci *HLJcf40* and *HLJcf29* were the least polymorphic. Except for *P*, the mean values of the other five parameters of *SNS* were higher than those of *HNS*. Similar cases had been observed more for the male individuals than the female individuals in *HNS* (data not listed). There was no significant differentiation ($P > 0.05$) between *HNS* and *SNS* at all the loci except for *HLJcf*37 according to one-factor analysis of variance.

Table 1 Microsatellite markers of *Silurus meriaionalis*

Locus	GenBank accession number	Primer sequence (5′→3′)		Core sequence	Annealing temperature (℃)	Product size
HLJcf4	DQ223146	F:caacacctgctccactca	R:tccttgcctccttcctac	$(CA)_{18}$	55	186−237
HLJcf7	DQ223150	F:cgatgtcgctttccttac	R:gtccaccacagagctttact	$(CA)_{14}$	55	218−273
HLJcf8	DQ223151	F:cccttgtctcccatctca	R:tttaggacacctggcact	$(CA)_{25}$	55	241−269
HLJcf9	DQ223152	F:ttgtggaatctgccctct	R:ggatgcctgtgctgttaa	$(GT)_{33}$	48	260−273
HLJcf10	DQ223153	F:gggtgacagactgaggag	R:acctggcactgcaagata	$(CA)_{25}$	48	191−230
HLJcf11	DQ223154	F:gtgcatcagtgagacgac	R:aggggacatttcaggtaa	$(GT)_{20}$	55	124−234
HLJcf12	DQ223155	F:tgtcagggctccagaacg	R:ttgaagcggcccatttac	$(GT)_{28}$, $(GT)_{13}$	53	258−285
HLJcf15	DQ223160	F:tcgggtgtccacatacttt	R:atggtcgtgattgattgc	$(AC)_{51}$	53	288−385
HLJcf16	DQ223169	F:ctgctaatcacagccaca	R:cccgcttgttttatcact	$(GT)_{23}$	53	157−200
HLJcf17	DQ223170	F:cgatgaggcagtgatgga	R:gcccgtatgtcagcaggt	$(GT)_{20}$	53	170−196
HLJcf18	DQ223171	F:agcttttcccgtctttgt	R:gcatactgtccacctcct	$(AC)_{20}$	57	275−361
HLJcf19	DQ223172	F:tggatgatgagcgtatgg	R:ttacaccctcaagccaca	$(CA)_{14}$	60	141−159
HLJcf20	DQ223173	F:tccaaccaccacctgata	R:gcagcccaaggtaatgta	$(AC)_{28}$	60	230−303
HLJcf25	DQ223178	F:gagggtgaatgccatagt	R:cacgggtttgacagttagt	$(GT)_{44}$	53	182−201
HLJcf26	DQ223179	F:caccgtaccctatcaccc	R:tcggctttatcctccact	$(AC)_{26}$	55	102−232
HLJcf27	DQ223180	F:ctttcatttctgcccact	R:cgagatacaggcttccag	$(CA)_{9}$	60	177−305
HLJcf29	DQ223182	F:aagtgtagctcagaagcgaaag	R:cccgcaaactggataaagac	$(CA)_{9}$	53	104−187
HLJcf31	DQ223158	F:tttgcctcttcttcaccc	R:atccgttcgtttctgtcttt	$(CA)_{16}$	53	149−212
HLJcf32	DQ223159	F:tggcacatacgacaatcc	R:gaaacatgaaggtgaggc	$(CA)_{24}$	50	368−373
HLJcf35	DQ223163	F:gacaggccatctaaggag	R:agcaggaactggaggtat	$(GT)_{27}$	60	227−286
HLJcf36	DQ223164	F:cacaatgccctgctcaca	R:ctgcctttctaaagctctatca	$(CA)_{16}$	60	149−239
HLJcf37	DQ223165	F:atggcgatgtgctgtaat	R:tccaggctctaaatgtgc	$(GA)_{24}$	57	174−239
HLJcf38	DQ223166	F:gcaccctaagccaagcct	R:gatccgacggcaacagtg	$(CA)_{33}$	57	122−178
HLJcf40	DQ223168	F:gagactggctcaggaaag	R:ctaggttgggacaagcac	$(GT)_{20}$	57	149−215

F: forward primer; R: reverse primer.

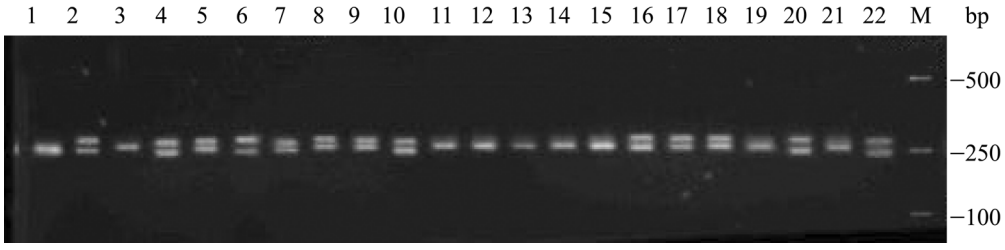

Fig. 1 Electrophoresis patterns of *HNS* in the locus *HLJcf20*

Note: 1−9 are male individuals and 10−20 are female individuals.

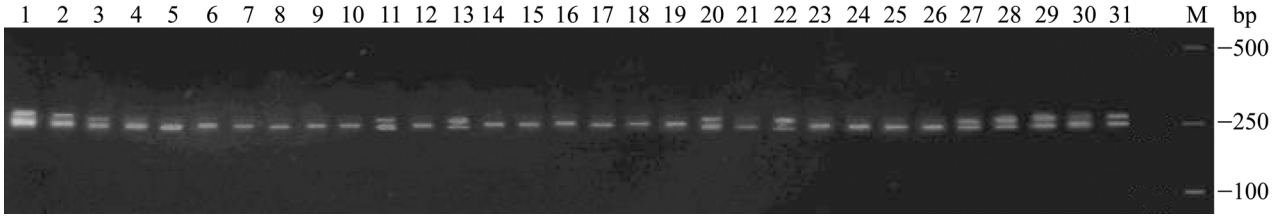

Fig. 2 Electrophoresis patterns of *SNS* in the locus *HLJcf20*

Table 2 The genetic polymorphism of *HNS*

Locus	*A*	*Ae*	*P*	*Ho*	*He*	*PIC*
HLJcf4	8	6.469	0.125	0.619	0.845	0.840
HLJcf7	8	5.742	0.125	0.650	0.826	0.825
HLJcf8	4	3.195	0.250	0.000	0.687	0.676
HLJcf9	4	2.073	0.250	0.063	0.518	0.474
HLJcf10	5	4.429	0.200	0.368	0.774	0.753
HLJcf11	6	4.831	0.167	0.476	0.793	0.778
HLJcf12	5	3.227	0.200	0.545	0.690	0.658
HLJcf15	5	4.299	0.200	0.667	0.767	0.745
HLJcf16	5	3.903	0.200	0.091	0.744	0.732
HLJcf17	5	4.566	0.200	0.000	0.781	0.766
HLJcf18	8	4.964	0.125	0.455	0.799	0.785
HLJcf20	6	3.919	0.125	0.500	0.745	0.728
HLJcf25	6	3.903	0.167	0.000	0.744	0.729
HLJcf26	15	10.640	0.067	0.455	0.906	0.905
HLJcf27	14	6.817	0.071	0.682	0.853	0.848
HLJcf29	6	4.119	0.167	0.136	0.757	0.733
HLJcf31	3	2.899	0.333	0.950	0.655	0.600
HLJcf32	4	3.374	0.250	0.000	0.704	0.682
HLJcf35	11	8.491	0.091	0.818	0.882	0.879
HLJcf36	15	11.090	0.067	0.864	0.910	0.909
HLJcf37	9	6.281	0.111	0.727	0.841	0.836
HLJcf38	7	4.433	0.143	0.450	0.774	0.759
HLJcf40	6	1.770	0.167	0.500	0.435	0.435
Mean	7.174	5.019	0.165	0.435	0.758	0.742

Table 3 The genetic polymorphism of *SNS*

Locus	*A*	*Ae*	*P*	*Ho*	*He*	*PIC*
HLJcf4	8	6.364	0.125	0.484	0.843	0.835
HLJcf7	12	8.232	0.083	0.567	0.879	0.874
HLJcf8	6	5.248	0.167	0.367	0.809	0.802
HLJcf10	4	2.886	0.250	0.000	0.653	0.596
HLJcf11	5	2.856	0.200	0.065	0.650	0.612
HLJcf16	6	3.829	0.167	0.000	0.739	0.717
HLJcf18	8	5.357	0.125	0.333	0.813	0.804
HLJcf19	5	3.672	0.200	0.000	0.728	0.707
HLJcf20	5	6.582	0.091	0.452	0.848	0.841
HLJcf26	17	12.480	0.059	0.462	0.920	0.919
HLJcf27	16	9.360	0.062	0.516	0.893	0.890
HLJcf29	4	2.525	0.250	0.033	0.604	0.535
HLJcf32	7	3.298	0.143	0.000	0.697	0.654
HLJcf35	10	6.894	0.100	0.667	0.855	0.852
HLJcf36	18	13.520	0.056	0.778	0.926	0.926
HLJcf37	4	3.302	0.250	0.000	0.697	0.655
HLJcf38	6	4.643	0.167	0.355	0.785	0.766
Mean	8.294	5.944	0.147	0.299	0.785	0.764

2. 4 Hardy-Weinberg equilibrium test

Significant deviations from Hardy-Weinberg equilibrium were tested using the parameter *d*. Heterozygote excess was found at loci *HLJcf8*, *HLJcf17*, *HLJcf25*, and *HLJcf32* in *HNS* and loci *HLJcf10*, *HLJcf16*, *HLJcf19*, *HLJcf32*, and *HLJcf37* in *SNS* (*d*=-1). Those were also found at loci *HLJcf9* and *HLJcf16* for the male individuals in *HNS*. But male heterozygotes were missing at locus *HLJcf12* in *HNS* (*d*=1) (Table 4).

2. 5 Population genetic variations

G_{ST} was computed to estimate the differences between the two populations. The value was 0.078, which showed that the differentiations were very small. AMOVA was applied to analyze the distributions of variation [21,22]. Above 98.43 percent of the variations were due to differences among individuals within population and Φ_{ST} was 0.608 ($P > 0.05$), so the variations between populations were not significant. The phylogenetic tree (Fig.3) was constructed by UPGMA method using the software package of PHYLIP 3.63. The radialized appearance suggested that the differentiations among different individuals were small; otherwise they should cluster into one or a few branches.

3 Discussion

3. 1 Parametric estimation of population genetic differentiations

The 24 microsatellite loci were polymorphic in at least one population using the 0.95 allele frequency criterion. According to the microsatellite selection standard, any locus that had at least four alleles would be excellent for genetic evaluation [23]. Here, 23 loci had at least four alleles except for locus *HLJcf31*. Excluding loci *HLJcf9* and *HLJcf40*, the remaining

Table 4 The genetic departure index (*d*) of *Silurus sddatovi* populations

Locus	*HNS*			*SNS*	Locus	*HNS*			*SNS*
	Total	Male	Female			Total	Male	Female	
HLJcf4	−0.268	−0.505	0.003	−0.426	*HLJcf20*	−0.329	−0.291	−0.313	−0.467
HLJcf7	−0.213	−0.028	−0.268	−0.355	*HLJcf25*	−1.000	−1.000	−1.000	
HLJcf8	−1.000	−1.000	−1.000	−0.547	*HLJcf26*	−0.498	−0.594	-0.407	−0.498
HLJcf9	−0.879	−1.000	−0.832		*HLJcf27*	−0.201	−0.432	0.030	−0.422
HLJcf10	−0.524	−0.614	−0.405	−1.000	*HLJcf29*	−0.820	−0.556	−0.892	−0.945
HLJcf11	−0.400	−0.656	0.172	−0.901	*HLJcf31*	0.450	0.474	0.500	
HLJcf12	−0.210	1.000	−0.270		*HLJcf32*	−1.000	−1.000	−1.000	−1.000
HLJcf15	−0.131	−0.189	−0.028		*HLJcf35*	−0.073	−0.087	−0.027	−0.220
HLJcf16	−0.878	−1.000	−0.745	−1.000	*HLJcf36*	−0.051	−0.128	0.0402	−0.160
HLJcf17	−1.000	−1.000	−1.000		*HLJcf37*	−0.135	−0.182	0.004	−1.000
HLJcf18	−0.431	−0.673	−0.154	−0.590	*HLJcf38*	−0.419	−0.571	−0.190	−0.548
HLJcf19				−1.000	*HLJcf40*	−0.425	−0.438	−0.359	

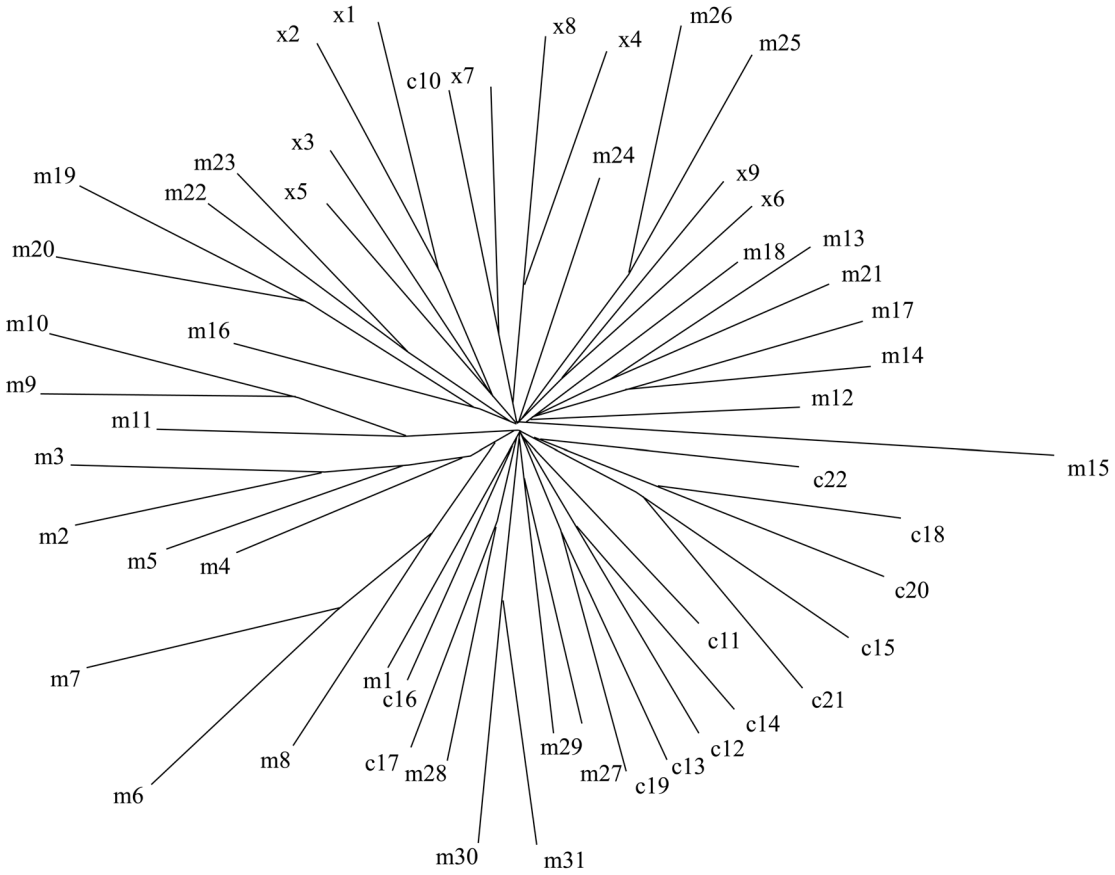

Fig. 3 Dendrogram of *Silurus soldatovi* constructed using UPGMA method based on matrices of pairwise Nei's genetic distances

'c' stands for the female samples of *HNS*, 'm' stands for the samples of *SNS*, 'x' stands for the male samples of *HNS*, and numbers that follow are serial numbers of the samples.

21 loci were highly polymorphic (*PIC*>0.5) [20,24]; the loci that were used here were very efficient in measuring the genetic variations.

P, *Ho*, *He*, *PIC,* and *Ne* are parameters of genetic

variations. The results obtained in this study showed that the levels of genetic diversity were relatively high in these populations, compared to previously published surveys of population variability. The statistical data of approximately 40 000 individuals and 524 microsatellite loci of 78 species reported by DeWoody *et al*.[25] revealed that *He* and *A* of freshwater fishes were 0.46 and 7.5, respectively, whereas the mean values of northern sheatfish were 0.77 and 7.65, respectively. The results obtained in this study were similar to the report of Triantafyllidis *et al.*[1], which analyzed the genetic population structure of native and translocated Aristotle's catfish (*S. aristotelis*) using the microsatellites of European catfish and found that *He* and *Ne* were 0.562−0.734 and 7.5−13.6, respectively. Meanwhile, it found in this study that no significant differentiation occurred between the two populations, which suggested that their relationships were very close. As a matter of fact, they were the same species and their habitats Heilongjiang River and Songhuajiang River were geographically related.

PCR amplifications at loci *HLJcf19*, *HLJcf9*, *HLJcf12*, *HLJcf15*, *HLJcf17*, *HLJcf25*, *HLJcf31*, and *HLJcf40* were population-specific as described in 2.2. So these loci can be used to distinguish the two populations, although they have the same appearance. But it was not known whether this was the result of the loss of these loci or sequence divergence at the primer binding sites.

3. 2 Analysis of HWE and genetic structure

It is often suggested that the population is close to HWE when the value of *d* is closer to zero. If the value of *d* is zero, the population is at HWE. If the value of *d* is above zero, the population lacks heterozygotes. If the value of *d* is below zero, the population is increasingly of heterozygous. The value ranges between −1 and 1; However, there is no recognized standard. As shown in table 4, heterozygotes were either in excess or were deficient at several loci, as described in 2.4.

Assuming that several possible causes of deviations from HWE such as selective mating, population substructuring [26], shortage of samples, low levels of polymorphism, and linkage with sex chromosome [27] were not a major concern in the populations that were analyzed in this study, the presence of inbreeding caused by over-fishing and environmental degradation could provide a reasonable explanation for the lack of agreement with HWE observed in this study. According to the historical records of fish capture, northern sheatfish were captured anywhere along the Heilongjiang River and Songhuajiang River 40 years ago and divided into two populations only recently because of over-fishing and habitat degradation. Their genetic differences may be attributed to their diminishing population size. This means that overfishing and habitat deterioration diminish the population size and lead to reproductive isolation of unattached groups, which led to decreasing gene flow among the populations.

The value of G_{ST}, a relative measurement of genetic differentiation among subpopulations, changes from 0 to 1. The higher the value, the larger the discrepancy and vice versa. This value of northern sheatfish was 0.078. The value of *Φst*, another measurement of genetic discrepancy, was 0.608. Both indicated that the genetic diversity was small. The UPGMA tree shown in Fig. 3 had no obvious clustering, which also indicated that there was no significant differentiation between the wild and cultured populations of northern sheatfish. This meant that culture did not result in remarkable changes to northern sheatfish.

Northern sheatfish is an important aquaculture species not only in northern China but also in southern China, for example in Sichuan, Hunan, and Hubei provinces. Nowadays, more research has been carried out on the biology and cytology of northern sheatfish, whereas very few reports are available on the genetics at the DNA level. So, there are no previous research results to confirm the results obtained in this study. The microsatellite loci used here will become instrumental as useful genetic markers in areas such as population diversities, genetic structures, confirmation of species classification, and marker-assisted

selection (MAS).

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东北大口鲇 2 个群体的微卫星 DNA 多态分析

全迎春[1,2]，孙效文[1]，梁利群[1]

1. 黑龙江水产研究所 北方鱼类生物工程育种重点开放实验室，哈尔滨 150070；

2. 上海水产大学生命科学与技术学院，上海 200090

摘 要：利用磁珠富集法克隆制备的 24 个大口鲇（*Silurus meriaionalis* Chen）微卫星标记，对黑龙江野生群体与松花江养殖群体 2 个东北大口鲇（*S. soldatovi*）的地理种群的等位基因频率（*P*）、观测杂合度（*Ho*）、期望杂合度（*He*）、多态信息含量（*PIC*）和有效等位基因数（*Ne*）等进行了遗传检测，以遗传偏离指数（*d*）检验 Hardy-Weinberg 平衡，并以 Nei 氏遗传分化系数（G_{ST}）和 AMOVA 分析（Φ_{ST}）群体遗传变异的来源。同时，使用 PHYLIP3.63 软件绘制基于 Nei 氏遗传距离的个体间 UPGMA 系统树。结果表明：24 个微卫星标记在东北大口鲇的 2 个群体中共扩增出 1357 条多态性片段，片段长度为 102～385 bp，总体平均等位基因 8.875 个，可以用于东北大口鲇遗传多样性的评估。并发现 8 个可区分这 2 个种群的遗传标记；黑龙江群体的 *P*、*Ho*、*He*、*PIC* 和 *Ne* 依次为 0.165、0.435、0.758、0.742 和 5.019，松花江群体为 0.147、0.299、0.847、0.764 和 5.944，在这些多样性参数上，方差分析也显示 2 地理种群差异不显著，在大多数位点并无显著差异，仅 *HLJcf*37 位点具有显著差异；在多个位点偏离 Hardy-Weinberg 平衡，2 群体呈现不同程度的杂合体过度，纯合体完全缺失现象，其原因有待证实；群体遗传变异分析证实 2 群体间遗传分化较弱，其 98%以上的变异是由群体内个体间的遗传变异引起的，群体间的变异对总变异影响不显著。UPGMA 系统树也显示出个体间遗传距离小，亲缘关系很近。结果表明，人工繁殖没有对东北大口鲇的遗传多样性产生影响，该种群遗传分化小，种质资源状况良好。

关键词：东北大口鲇；微卫星 DNA；遗传多样性

作者简介：全迎春（1981−），女，湖北人，硕士，研究方向：水产动物遗传与育种。E-mail: quan_928@163.com