Short Communication

Development of microsatellite markers and genetic diversity analysis for *Pelodiscus sinensis*

T. Li[1,2], J. Zhao[1], W. Li[1], Y. Shi[1], X.Y. Hong[1,2] and X.P. Zhu[1,2]

[1]Key Laboratory of Tropical and Subtropical Fishery Resource Application and Cultivation of Ministry of Agriculture,
Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, China
[2]College of Life Science and Technology, Shanghai Ocean University, Shanghai, China

Corresponding author: X.P. Zhu
E-mail: zhuxinping_1964@163.com

Genet. Mol. Res. 15 (3): gmr.15038346
Received December 28, 2015
Accepted February 26, 2016
Published July 29, 2016
DOI http://dx.doi.org/10.4238/gmr.15038346

ABSTRACT. *Pelodiscus sinensis* is a common freshwater soft-shell turtle found in China, and is an important aquaculture species. In this study, 20 polymorphic microsatellite primers were developed from the transcriptome. The genetic diversity of three populations of *P. sinensis* was evaluated, using 72 individuals. The number of alleles per locus ranged from 3 to 26. The observed and expected heterozygosities varied from 0.208 to 0.958, and from 0.302 to 0.963, respectively. The polymorphic information content varied from 0.283 to 0.953. No significant linkage

disequilibrium was detected. These markers will be useful for future population genetic studies and molecular breeding of *P. sinensis*.

Key words: *Pelodiscus sinensis*; Chinese soft-shell turtle; Microsatellites; Genetic diversity

INTRODUCTION

The Chinese soft-shell turtle, *Pelodiscus sinensis*, is a common and widely distributed freshwater turtle in China. It is an important aquaculture species, and the annual output reached 340,000 tons in 2014 (Bureau of Fisheries, Ministry of Agriculture, China, 2015). It is also distributed in Japan, Korea, Vietnam, and in other Southeast Asian countries (Fritz et al., 2010). Because of its wide distribution, there are many different geographical populations of *P. sinensis*, and each population has distinctive genetic characteristics. Microsatellite markers are valuable tools for use in genetic studies and for genetic resources conservation and management (Bai et al., 2011; Lin et al., 2012). In this study, 20 novel microsatellite markers were developed and used to analyze the population genetics of three geographical populations of Chinese soft-shell turtle. These markers will be useful for population genetic studies and molecular breeding of *P. sinensis*.

MATERIAL AND METHODS

Three geographical populations of Chinese soft-shell turtle, including 72 individuals, were sampled. Twenty-four individuals were collected from the Yangtze River system (YR), 24 from the Pearl River system (PR), and 24 from Japan (JAP). Genomic DNA was extracted from the nails using Omega Micro Elute Tissue DNA Kit according to manufacturer protocol.

A total of 100 pairs of candidate microsatellite primers were designed and developed from the transcriptome of the Chinese soft-shell turtle. A M13 tail was added to the 5' end of each forward primer (5'-CACGACGTTGTAAAACGAC-3') (Table 1).

Polymerase chain reaction (PCR) was performed in a 10-μL volume containing 5 μL AB Multiplex PCR Master Mix, 2 μL primer mixture with the appropriate primer ratio (forward primer: reverse primer in 1:40 ratio), 0.2 μL fluorescence labeled M13-forward primer, 1 μL 20 ng/μL DNA, and 1.8 μL deionized water. Amplifications were carried out in an Eppendorf master cycler using the following procedure: initial denaturation at 94°C for 5 min, followed by 22 cycles of 30 s at 94°C, 30 s at the annealing temperature of 55°-63°C (Table 1), and 40 s at 72°C, then eight cycles of 30 s at 94°C, 30 s at the annealing temperature of 53°C, and 50 s at 72°C. A final elongation of 10 min at 72°C was added, holding at 4°C. A sample of 2 μL of the PCR products was mixed with 9 μL Hi-Di containing Genescan 500Liz (Applied Biosystems Grange, Woolston, Warrington, UK), denatured for 5 min at 94°C, and chilled immediately on an ice plate. The mixture was separated using an ABI genetic analyzer 3130.

The alleles were detected by the Peak scanner software V1.0 (downloaded from http://www. Lifetechnologies.com). Pop gene 32 (Yeh et al., 2000) and Cervus 3.0 (Kalinowski et al., 2007) softwares were used to assess the genetic parameters of the 20 markers, including the number of alleles, observed (H_O) and expected heterozygosities (H_E), polymorphism information content, and the departure from the Hardy-Weinberg equilibrium. MEGA5.0 was used to establish the relationships between the three Chinese soft-shelled turtle populations.

Table 1. Characteristics of the 20 novel microsatellite loci identified in this study.

Locus	Primer sequence	Repeat motif	Size range (bp)	Ta (°C)
ZYF1	F-GTGGGTGTTTGGTCAAGGAT	(TG)6	86-125	63
	R-CTTCCACACACACAACCCTG			
ZYH18	F-CAGACCCAACAACCCAATCT	(ATAG)5	253-293	63
	R-TGAAAGCACACCACCCAGTA			
ZYH30	F-AGAAGAGAGGGGGTGAGAGC	(GACA)4	273-302	63
	R-GCGTGTGACTTCCTCTGTCA			
ZYF42	F-TGCTCGCATTGTCTTCAGTC	(TA)10	246-279	63
	R-AACCCCAAACACATCCTTGA			
ZYF56	F-TGCTGTGCTGTATCCCAGAG	(TC)8	276-286	63
	R-GGATTACCAGGGTTAGGGCT			
ZYH61	F-ACCCCTCACAGCATTGTTTC	(TA)8	208-243	63
	R-GGTTGCAAGGAGTCCCACTA			
ZYF68	F-GCAACACGCCACATTTACTG	(CA)6	249-266	63
	R-CGATGAGAGCATCCTGAACA			
ZYH73	F-TGTTCCCAACCAGTCATTCA	(CA)8	290-394	63
	R-GGGGAAGAACTTCCTGTTCC			
ZYR87	F-AAGCTCCAGGAACTGTGCAT	(AT)9	288-295	63
	R-TCCTTCAGCCACATTCAGTG			
ZYH101	F-CGCTCTGCTTTTGTTTTTCC	(AG)8	173-200	63
	R-CGGTGTGTGCAAAGACTAGG			
ZY52FR	F-GCCCCAGGAAACCTAAAGTAA	(GAAA)28	225-362	57.8
	R-CCACAGTCAGCAGCAAGATAA			
ZY56FR	F-CAGCAAAGGATACCATCACCA	(CA)12	112-161	54.9
	R-TTACGAGATAAGCCCTGC			
ZY60FR	F-CCCCAAGAAGGAAGACTATT	(GATA)24	265-385	57.5
	R-ATTTGAGAGGAAGCACAG			
ZY61FR	F-TGCTGTCCCCCTCTTTTGATT	(GATA)28	176-278	60.7
	R-AGCAGACAGAAGTATCCCCAA			
ZY64FR	F-ATTCCTCTTGAACGCACTCT	(AGAT)13	192-267	55.5
	R-GGGAACGCATAATGGTAAT			
ZY67FR	F-CAACAGATTGGAAAACACC	(AG)11	193-318	58.5
	R-AATCCACTGTGTCCATTGTTC			
ZY70FR	F-CTTGAAAAAACGGACTTAC	(ATAG)15	150-234	56.6
	R-AATGTCCACGGAGTGTCTAT			
ZY71FR	F-CGTATCTCGTTTTGGCTTT	(TG)10	100-156	57.5
	R-TGACTGGAATCTGTGGTGT			
ZY79FR	F-TAACAAGCAGGACCAAGAG	(CA)10	147-178	58.2
	R-TGTGCCATTCCCCGTATT			
ZY87FR	F-GTGAATGGTTTAGGAGTC	(TG)18	219-250	55

RESULTS AND DISCUSSION

Based on the polymorphic information, 20 primer sets were selected from 100 candidates. Clear and reproducible peaks were successfully produced from all 72 samples at the 20 loci. Genetic parameters of the three geographical populations of *P. sinensis* are listed in Table 2. The number of alleles per locus ranged from 3 to 22, 3 to 26, and 3 to 20, respectively, for the PR, YR, and JAP populations. The estimated heterozygosity of the microsatellite loci was high, ranging from 0.208 to 0.952 (H_O) and 0.302 to 0.957 (H_E) in the PR population, 0.208 to 0.958 (H_O) and 0.395 to 0.975 (H_E) in the YR population, and 0.208 to 0.952 (H_O) and 0.355 to 0.973 in the JAP population. The polymorphic information content for the three populations varied from 0.283 to 0.953, 0.283 to 0.934, and 0.363 to 0.950, respectively, all of which were highly polymorphic. Deviations from Hardy-Weinberg equilibrium are indicated in Table 2 by an asterisk.

Table 2. Genetic parameters of the 20 loci in 72 individuals tested.

Locus	Chinese soft-shelled turtle (PR N = 24)			PIC	Chinese soft-shelled turtle (YR N = 24)			PIC	Chinese soft-shelled turtle (JAP N = 24)			PIC
	*N*a	*H*O	*H*E		*N*a	*H*O	*H*E		*N*a	*H*O	*H*E	
ZYF1	3	0.958	0.749	0.690**	3	0.833	0.693	0.617**	3	0.708	0.627	0.548
ZYH18	8	0.625	0.664	0.628	4	0.542	0.588	0.515	9	0.750	0.784	0.744
ZYH30	7	0.208	0.824	0.783**	6	0.250	0.777	0.729**	8	0.292	0.706	0.669**
ZYF42	9	0.750	0.902	0.871	9	0.417	0.823	0.788*	10	0.708	0.765	0.731**
ZYF56	3	0.333	0.324	0.286	3	0.208	0.395	0.363**	3	0.417	0.531	0.488
ZYH61	6	0.583	0.766	0.711	8	0.667	0.790	0.742	5	0.583	0.658	0.579
ZYF68	7	0.375	0.730	0.673	6	0.250	0.522	0.496**	5	0.208	0.492	0.438
ZYH73	13	0.875	0.906	0.932	14	0.792	0.963	0.940**	8	0.833	0.854	0.867**
ZYR87	2	0.208	0.302	0.283**	3	0.458	0.426	0.393	2	0.208	0.355	0.316**
ZYH101	6	0.625	0.614	0.577	9	0.667	0.904	0.875	6	0.708	0.689	0.643
ZY52FR	22	0.958	0.956	0.934	26	0.958	0.975	0.953*	20	0.917	0.953	0.929
ZY56FR	10	0.667	0.727	0.673	13	0.708	0.904	0.875	8	0.417	0.713	0.670**
ZY60FR	19	0.958	0.957	0.934	17	0.875	0.958	0.935	19	0.952	0.957	0.934
ZY61FR	12	0.417	0.905	0.880**	17	0.667	0.943	0.919**	13	0.583	0.855	0.822**
ZY64FR	13	0.958	0.895	0.866	16	0.917	0.941	0.916	12	0.750	0.846	0.810**
ZY67FR	18	0.917	0.947	0.923	19	0.958	0.958	0.935	20	0.917	0.973	0.950
ZY70FR	16	0.833	0.919	0.893	14	0.958	0.911	0.885	13	0.917	0.925	0.899
ZY71FR	3	0.208	0.397	0.369*	3	0.583	0.732	0.677	3	0.333	0.457	0.424**
ZY79FR	6	0.625	0.620	0.586	7	0.375	0.791	0.754**	5	0.542	0.744	0.682**
ZY87FR	7	0.917	0.835	0.794	8	0.750	0.927	0.901*	7	0.542	0.552	0.528

*N*a Number of alleles; H_O Observed heterozygosity; *Deviation from Hardy-Weinberg equilibrium, $P \leq 0.05$; **Deviation from Hardy-Weinberg equilibrium, $P \leq 0.01$.

Based on the genetic distances calculated from the microsatellite loci, a dendrogram of the three populations was established using the MEGA5.0 software (Figure 1). The PR and YR populations were genetically close and distant from the JAP population. The results are consistent with the geographical distribution of *P. sinensis*. (Liu et al., 2012).

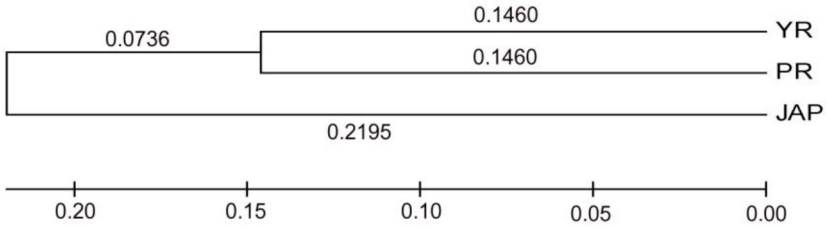

Figure 1. Dendrogram of three populations of *Pelodiscus sinensis.*

Recently, microsatellites were developed and characterized in this species (Que et al., 2007; Bu et al., 2011; Ma et al., 2014). The markers reported in the present study are novel and present a higher degree of polymorphism than those described previously. In addition, these new markers can be used to calculate the genetic distance. Furthermore, we have expanded the database to provide more selection for the study of population genetics and conservation efforts in Chinese soft-shelled turtles. Moreover, we have used different geographical populations of *P. sinensis*. These new markers should be used for population genetic studies, and for the conservation and management of *P. sinensis* and other related species.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the Marine Fishery and Industrial Development Special Scientific & Technological Research and Development Projects of Guangdong Province (#A201501A01), "The genetic improvement and cultivation of new varieties of the Chinese softshell turtle".

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