ONLINE RESOURCES

Identification and characterization of seventeen novel microsatellite markers for Dabry's sturgeon (*Acipenser dabryanus*)

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Introduction

Dabry's sturgeon, *Acipenser dabryanus*, is a critical endangered species in the Red List in IUCN since 1996 (IUCN 2013). In the present study, we report 17 novel microsatellites loci isolated from *A. dabryanus*. All 17 loci were amplified in a sample of 22 individuals. Sixteen loci were polymorphic and only one locus showed monomorphism. At these 16 polymorphic loci, 91 alleles were observed, with an average of 5.69 alleles per locus. The number of alleles, mean expected heterozygosity (H_E) and Shannon–Wiener diversity indices (H') varied from 2 to 11, 0.127 to 0.852 and 0.247 to 2.064, respectively. These markers will be useful for population genetics and conservation management in *A. dabryanus*.

Dabry's sturgeon is also an endemic freshwater fish and does not undertake long distance migrations except for spawning (The Yangtze Aquatic Resources Survey Group 1988). Historically, it has widely inhabited the upper and middle sections of the Yangtze River and its large tributaries (Zhuang *et al*. 1997). *A. dabryanus* was an important species in commercial fisheries of the upper reaches of the Yangtze River before the 1980s (Zhu *et al*. 2009). However, due to construction of dam, overfishing and pollution, natural populations have declined sharply in the past decades (Zhu *et al*. 2008). Natural production of *A. dabryanus* is so small and scattered that no exact account of total production has been reported since 1995 (Zhang *et al*. 2011). To save *A. dabryanus*, its commercial capture was prohibited in the early 1980s, and it was listed nationally as class I state protected animal since 1989 (Wang and Xie 2009). Moreover, the biggest national reserve, three months seasonal fishing ban and hatchery-release efforts have been initiated successively in the upper stream of the Yangtze River since 2000 (Zhang *et al*. 2011).

Genetic investigation can provide managers with critical information to address important rehabilitation issues, especially when survival of this species is entirely reliant on restocking efforts. Microsatellites are often the choice for studying population genetics and are of great utility in conservation and management of endangered fishes (O'Connell and Wright 1997). Two studies were reported (Zeng *et al*. 2013; Zhang *et al*. 2013) and in total 34 polymorphic microsatellite loci in total were developed for this species. But these loci may not be sufficient for conservation assessment in this critically endangered species. Here, we isolated 17 novel microsatellites loci from *A. dabryanus* using fast isolation by AFLP of sequences containing repeats (FIASCO) protocol (Zane *et al*. 2002). These markers will be useful for parentage analysis, evaluation of supportive breeding, and genetic management on captive population of *A. dabryanus* in the long run.

Materials and methods

Genomic DNA was extracted from the muscle of a pool of three wild individuals using a proteinase K/phenol–chloroform method (Sambrook and Russell 2001). Enriched partial microsatellite genomic libraries for repeat motifs $(GATA)_n$, $(AAG)_n$ and $(GT)_n$ were obtained, following essentially FIASCO protocol (Zane *et al*. 2002) with a few modifications developed by Zhu *et al*. (2005). Finally, 200 clones were sequenced by an ABI3730XL automated DNA sequencer (Tianyi Biotech, Beijing, China). Sequences obtained were analysed for the repeat region using software 'Tandem Repeats Finder' (Benson 1999). Fifty- seven sequences with simple sequence repeat (SSR) motifs were

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Table 1. Characteristics of 17 microsatellite loci in *A. dabryanus*.

Locus	GenBank accession number	Primer sequence (5′–3′)	Repeat motif (s)	T_a(°C)	Allele size (bp)	N	Number of alleles per locus	H_E(SD)	H' (SD)
DS01	JX311718	CCGAAGACCCTGACAAAGAT TGCGGTAGTCTGACATAGTGAA	$(TACA)_{14}$	54	112–152	22	6	0.801 (0.003)	1.677 (0.008)
DS02	JX311719	ATTGGTTGTTGAGGTGGGAT CGACCGCAAGTATTTCATT	$(AG)_4(TG)_2$	54	181–203	22	6	0.655 (0.015)	1.284 (0.042)
DS03	JX311720	ATCAATCGCTGGTAGTAATG GAGACGGGACCTTCTAATT	$(TG)_6$	54	113	22	1[a]	0.000 (0.000)	0.000 (0.000)
DS05	JX311721	CTGGCAAGGCAAGGAGA GGAGGCTACGAGCGACT	$(CCT)_7$	56	174–183	22	4	0.504 (0.033)	0.966 (0.051)
DS07	JX311722	CACCTGAGAACACGATTGA GCAGTATTGGGCACCTT	$(TG)_{13}TT\ (TG)_5(CTGC)_4$	54	207–237	22	11	0.828 (0.004)	1.983 (0.011)
DS08	JX311723	CTGGCTGGCGGATTGATT AGTAGGGCGGCACGGTTT	$(AAG)_5AG\ (AGG)_3$	52	172–236	22	4	0.686 (0.002)	1.207 (0.002)
DS09	JX311724	GTCCAGAGTTTGGGTGAA ACCCGCACTCAGTATTTG	$(TC)_{23}$	54	186–208	22	7	0.816 (0.004)	1.782 (0.013)
DS11	JX311725	AGCGGTTTACAGTTAGCAT TCTGAACGGTGATTGAGC	$(TATC)_3(TA)_3(TCTA)_4(GA)_9$	52	119–127	22	4	0.682 (0.015)	1.256 (0.030)
DS12	JX311726	TCAAGTGTTGAAATCAAGCAA TATTTATTCTGGCATCTGGT	$(TACC)_3(TATC)_3(TAATC)_2$	54	210–216	17[b]	3	0.441 (0.031)	0.749 (0.042)
DS13	JX311727	CTGCTGTGCAATGGGAGT GTTTCGGAGCTGCTGGAT	$(TCCTC)_2TCCAC\ (TAATC)_2$	58	186–222	22	7	0.763 (0.013)	1.655 (0.035)
DS14	JX311728	AGTTGGCAGGTGGAGGAC ATGGCAATAATGTTACATGAGC	$(GATA)_{14}$	54	208–256	22	11	0.852 (0.003)	2.064 (0.018)
DS15	JX311729	GCAGTGGCACATACTCACC TCAGCCGTCCCTCTAACAT	$(TCTA)_6TC(TCTA)_{15}(TATG)_2\ldots(TCTG)_3$	52	232–280	22	6	0.768 (0.003)	1.605 (0.007)
DS16	JX311730	TCCAGTCTTGTGCATAGTTT TAATGAGCAGCATAAGGTCT	$(GATA)_6$	54	154–188	22	9	0.793 (0.012)	1.808 (0.040)
DS18	JX311731	CAATGCTTTCCCCGAGTT CCTCATTCTTCCAAGGGTGTT	$(GATA)_{12}\ldots(TTGCA)_4$	58	274–282	22	2	0.373 (0.031)	0.560 (0.034)
DS22	JX311732	CATTGTAAATATGGTGGGC CGGACCTGGTTGGAGAC	$(TG)_{13}$	58	195–227	22	6	0.691 (0.028)	1.449 (0.060)
DS25	JX311733	TGAGCGTATTGGGATGGAC CAACGGACTTAGCGGACAC	$(AC)_4$	56	129–135	22	2	0.127 (0.028)	0.247 (0.042)
DS27	JX311734	TCCAGGGACAGGTTGAGAA CCACCACAAGCCCAGTTTA	$(CA)_7\ldots(AAG)_3$	56	193–216	22	3	0.571 (0.012)	0.926 (0.023)

T_a, annealing temperature; N, number of individuals successfully genotyped; H_E, mean expected heterozygosity; H', Shannon–Wiener diversity indices, SD, standard deviation.
[a]Monomorphic locus in *A. dabryanus.* [b]Five individuals failed PCR amplification in this locus.

identified and 28 primer pairs were created by software Primer Premier 5.0 (http://www.premierbiosoft.com).

Genomic DNA for a test panel of 22 *A.dabryanus* individuals from Institute of Rare Hydrobiology (Yibin, China) was amplified with these newly created primers. The 5′-end of the forward primer of each pair for microsatellite PCR amplification was labelled with a fluorescent dye (6-FAM). Amplification conditions were optimized for each primer set, based on detection and intensity of PCR products. About 30–50 ng genomic DNA were amplified in 10 μL reactions with 1 μL 10 × PCR Buffer (Takara, Dalian, China), 0.5 unit *Taq* DNA polymerase (Takara, Dalian, China), 0.5 μM of forward and reverse primers, 1.5 mM $MgCl_2$ and 2 mM dNTPs (Takara, Dalian, China). The reaction mixture was subjected to amplification using a T-Gradient AmpCycler (Takara, Dalian, China) with the following conditions: an initial denaturing step of 5 min at 94°C and 35 cycles of 94°C for 40s, 52–58°C annealing temperature for 30 s (table 1), 72°C for 40 s and a final extension at 72°C for 10 min. Amplification products were loaded on a 96-capillary ABI3730XL DNA Analyzer with a ROX-500 internal size standard and allele sizes were scored using ABI Genemapper ver. 4.0 (New York, USA). The statistics of polymorphism including expected heterozygosity (H_E) and Shannon–Wiener diversity indices were performed using software ATetra 1.2 (Van *et al*. 2010).

Results and discussion

A total of 57 sequences containing microsatellite repeats were finally found, and 28 of them were selected for designing primer pairs. Seventeen primer pairs out of 28 yielded amplified unambiguous PCR products, whereas the remaining 11 primers resulted in poor or no amplification. All 17 loci amplified products in each individual except that one locus (DS12) has failed amplification in five individuals (table 1). All loci were totally different form those reported previously by BLAST in the GenBank. Sixteen loci showed polymorphic with 2 to 11 alleles per locus, while only one locus (DS03) showed monomorphism with one allele. Characteristics of 17 microsatellite loci of *A. dabryanus* are given in table 1. Ninety-one alleles yielded in all 16 polymorphic loci with an average of 5.69 alleles per locus and mean expected heterozygosities and Shannon–Wiener diversity indices per locus ranged from 0.127 to 0.852 and 0.247 to 2.064, respectively (table 1). Due to the polysomic nature of these loci, Hardy–Weinberg equilibrium was unable to test (Börk *et al*. 2008).

On ploidy level in *A. dabryanus*, only one document inferred that *A. dabryanus* was octoploid by comparing DNA contents with American paddlefish (*Polyodon spathula*) (Zhang *et al*. 1999). However, DNA content of American paddlefish (*P. spathula*) varied from 3.17 to 4.89 picograms per nucleus (Birstein *et al*. 1997). Moreover, there is no literature about chromosome numbers and karyotype for *A. dabryanus*. In present study, no more than four alleles in each amplified locus were found among all 22 individuals. The result is also consistent with Zeng *et al*. (2013) and most polymorphic loci (75%) exhibited tetrasomic banding patterns, which probably suggested *A. dabryanus* was a tetraploid species. Microsatellites loci may provide a way to further study ploidy level of *A. dabryanus* which was successfully applied on white sturgeon (*Acipenser transmontanus*) by Schreier *et al*. (2011) owing to its neutral characterization which can be used directly to provide evidence of duplicate genes (Mable *et al*. 2011).

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