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TECHNICAL NOTE

Isolation and characterization of twenty-six microsatellite loci for the tetraploid fish Dabry's sturgeon (*Acipenser dabryanus*)

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Abstract To evaluate the population genetic diversity as a means of devising conservation strategies, we constructed (CA)n and (CT)n enriched genomic libraries for *Acipenser dabryanus*, a narrowly endemic and endangered species in China. Twenty-six polymorphic microsatellite markers were developed using the fast isolation by AFLP of sequences containing repeats (FIASCO) protocol. Between 3 and 13 alleles per locus were observed. Mean expected heterozygosities (H_E) and Shannon-Wiener Diversity Indices (H') per locus ranged from 0.303 to 0.806, and from 0.480 to 1.893, respectively. The microsatellite markers described here are valuable tools for the population genetics research of *A. dabryanus*.

Keywords Genetic diversity · Microsatellites · *Acipenser dabryanus* · China

The tetraploid fish Dabry's sturgeon (*Acipenser dabryanus* Dumeril, 1868) in the genus *Acipenser* had been an important commercially fish, which is mainly distributed in the mainstem of the upper Yangtze River and its tributaries (Zen 1990; Zhuang et al. 1997). The natural population has been declined drastically in the last two decades due to overfishing, damming, pollution and habitat alteration and destruction (Zhuang et al. 1997). Since 1982, only tens of specimens have been captured incidentally in the upper reaches of Yangtze River, and now the species rarely occurs in reaches below the Gezhouba Dam, which was built in 1981 across the Yangtze River at Yichang, Hubei Province (Zhuang et al. 1997; Chen 2007). Consequently, the species was listed as a First Class Protected Animal by the China Government in 1989 (Wei et al. 1997). It was also characterized as a Critical Endangered species in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List (IUCN 2010), and protected animal in the Appendix II of CITES. The need for rehabilitation of Dabry's sturgeon has been recognized, several efforts have been undertaken to support the recovery of this endangered species, including establishing a national nature reserve, controlled reproduction and re-stocking of cultured juveniles (Zhang et al. 2011). To better address the conservation status of endangered species in the Yangtze River, a management programme based on genetics is being devised to define the efficiency of these conservation measures (Zhu et al. 2005). Despite the increasing demand for preservation and management plans, no suitable high-resolution genetic data have been available for this tetraploid species.

Microsatellites or simple sequence repeats have been used widely and efficiently as molecular markers in studying population genetics, parentage and kinship analyses because of their high level of polymorphism, co-dominant Mendelian inheritance and rapid detection protocols (O'Connell and Wright 1997; Chistiakov et al. 2006). In this study, we report a set of polymorphic microsatellite loci for *A. dabryanus* that can serve as effective genetic markers for conducting further assessments of quantifying genetic diversity within and among this endangered species.

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Table 1 Primer sequences and characteristics of 26 microsatellite loci in *Acipenser dabryanus*

Locus	Repeat motif	Primer (5′–3′)	Ta (°C)	Size (bp)	Na	H_E	H'	GenBank Accession no.
Ada1-24	(GT)23	F: CCACTACAGCTTTGAATAAAAACAG	58	188	4	0.664	1.164	JX897686
		R: AGCAGCTGTGGATTTTTCGTG				(0.012)	(0.024)	
Ada2-57	(TG)46	F: GGGAGGTGGCTGATTGCAGC	58	210	3	0.570	0.918	JX897687
		R: CATCCCTGCCAACCTTACATTC				(0.010)	(0.021)	
Ada3-76	(CA)25	F: CAGGCACACACAAACAGAAACA	56	194	7	0.759	1.579	JX897688
		R: GCACTACATCACAAGCTATACAGCA				(0.008)	(0.023)	
Ada4-123	(AC)54	F: GGCCCTCGTCCCTGATAGTATTCT	58	241	13	0.785	1.893	JX897689
		R: TACTGCGCATGGGCAATTGGAG				(0.013)	(0.042)	
Ada5-236	(GT)43	F: GGACTTCAAACAAAGACTGTACCC	58	302	5	0.722	1.363	JX897690
		R: GCCAGGGGTGGATTATAACTGTTT				(0.004)	(0.017)	
Ada6-418	(GT)17	F: GGGGCTAAAAGAACCCGAGGT	58	164	5	0.678	1.202	JX897691
		R: CCAACTGACGCAAATGATTTGTAT				(0.006)	(0.017)	
Ada7-450	(TG)19	F: GGAGAAGGCTTTCAGGGCAGGC	58	215	4	0.690	1.226	JX897692
		R: CCAACCCCCTGACAGTTTTTCCTG				(0.007)	(0.020)	
Ada8-468	(CA)21	F: TCACACACACCCTCATATTCCC	58	218	7	0.791	1.697	JX897693
		R: CGTGTGTGCAGGGTTTGCATT				(0.006)	(0.021	
Ada9-477	(TG)50	F: GCATACTGCGCATGGGCAATTGG	58	231	4	0.670	1.198	JX897694
		R: GGCCCTCGTCCCTGACAGTAAGCT				(0.014)	(0.029)	
Ada10-215	(GA)69	F: CCACTGGACCACACAGCCTCC	58	249	4	0.744	1.375	JX897695
		R: GGTAAAAAGCTGTGAAAATTAGCC				(0.004)	(0.007)	
Ada11-217	(GA)70	F: GGTGAAGAATCAAAGTAGTGCTTTG	56	247	7	0.736	1.543	JX897696
		R: TGTGGGAACTGGGTTTATTCATTAG				(0.014)	(0.028)	
Ada12-229	(CT)46	F: CGGCAGCTCTGTGATACTGAGAGTG	58	378	5	0.747	1.431	JX897697
		R: CCCCCTTAGCCCTTCATCTGTAATG				(0.008)	(0.025)	
Ada13-247	(CA)66	F: GGGAGATATACAAAATGTGTGAAAG	58	248	5	0.786	1.620	JX897698
		R: CGAGAGTACAACACCGTACACGGGT				(0.007)	(0.022)	
Ada14-265	(TG)43	F: GGAGGCGCCGGAATGTG	58	183	6	0.780	1.604	JX897699
		R: ACAGAGCAATAAAATGGGCAGATTT				(0.006)	(0.020)	
Ada15-269	(CA)29	F: AGCACGTCTACACTCCTTGGGCAAC	56	361	5	0.771	1.547	JX897700
		R: GGTTCAAAGGGAGGGAGAGAGATGG				(0.000)	(0.001)	
Ada16-273	(CA)23	F: CCCCATCCCTGCCAACCTTACATT	58	220	5	0.785	1.575	JX897701
		R: CAGACTGTGGAGCCATGGATCCC				(0.005)	(0.010)	
Ada17-295	(GT)51	F: GACGCCCTTCTAAACGCCAAC	58	244	5	0.686	1.362	JX897702
		R: GGGCAAGGTGCAGGCACAC				(0.018)	(0.033)	
Ada18-307	(GT)15	F: GGGCTGGGTTGCAGGTTTTAT	58	191	3	0.630	1.040	JX897703
		R: TGTAAATGTGCAGTTCTGAC				(0.010)	(0.016)	
Ada19-255	(CA)25	F: GTTACAGGTGTTATTCACCAGCGCC	56	232	3	0.303	0.480	JX897704
		R: ATTGAAGCGCGCAGAATAGGCTATA				(0.032)	(0.037)	
Ada20-202	(TG)18	F: TGGGTACCAGTGTTGGTCCTGTG	58	295	4	0.692	1.290	JX897705
		R: AATGAGGAGCGAAGAAAAGTGTTGT				(0.010)	(0.020)	
Ada21-396	(AC)19	F: CGCACCCAGCATCACATGAAAC	58	217	5	0.722	1.40	JX897706
		R: GGATAAGTGAGTCATAAAAACCGC				(0.007)	(0.020)	
Ada22-331	(TG)36	F: ACTTTTATAACGGGTATCCTTTTTG	58	180	6	0.648	1.266	JX897707
		R: CCCAATTACACACCCGAGTAC				(0.006)	(0.014)	
Ada23-364	(TG)36	F: TTCTGTGTATTGTGAGGGTACGGGG	58	181	3	0.510	0.752	JX897708
		R: GTTTGGGCCGGGGTTCACG				(0.007)	(0.018)	
Ada24-209	(TG)16	F: AGCCAATCACTAATTTGCCTGTTTT	58	191	3	0.500	0.731	JX897709
		R: TGTGGTCAGGGACTGGGCGG				(0.005)	(0.008)	

Table 1 continued

Locus	Repeat motif	Primer (5′–3′)	Ta (°C)	Size (bp)	Na	H_E	H'	GenBank Accession no.
Ada25-249	(AC)46	F: GGCCCTCGTCCCTGACAGTAAG	56	213	10	0.806	1.818	JX897710
		R: CATGGGCAATTGCAGACAGGTG				(0.008)	(0.031)	
Ada26-245	(TC)8(TG)35	F: TGCAGTATTAAGAAGCGCGCAGATT	56	262	4	0.410	0.651	JX897711
		R: CATGTTACAGGTGTTGTTCACCAGC				(0.004)	(0.017)	

Ta annealing temperature, *Na* observed number of alleles, H_E mean expected heterozygosity H' Shannon-Wiener Diversity Indices

We collected the fins of 24 individuals from the Institute of Rare Hydrobiology in Yibin City, Sichuan province, China. The total genomic DNA was extracted by a universal and rapid salt-extraction method (Salah and Iciar 1997). Microsatellite-enriched genomic library for the repeat motif (CA)n and (CT)n was constructed essentially following the fast isolation by AFLP of sequences containing repeats (FIASCO) protocol (Zane et al. 2002) with the following modifications: (1) 400 ng DNA from pre-amplification step was mixed with 1 μM of a 5-biotinylated oligonucleotide probe in a total volume of 100 μL (6×SSC, 0.1 % SDS) at 55 °C for 30 min. (2) DNA is separated from the beads-probe complex by three times washes with 50 μL of TE buffer (Tris–HCl 10 mM, EDTA 1 mM, pH 8.0). Enriched fragments were ligated into pMD18-T vector (TaKaRa) and propagated in the DH5α strain of *Escherichia coli* (TaKaRa). After polymerase chain reaction (PCR) confirmation, 400 positive clones with insertion of 200–600 bp were sequenced with ABI PRISM 3,700. The repeat regions were analysed using software Tandem Repeats Finder (Benson 1999), and 67 primer sets were designed based on flanks of putative microsatellite sequences. The polymorphism of these microsatellite loci was examined with 24 individuals collected. The PCR amplification was carried out in a mixture of 25 μL volume on a PTC-100 thermocycler (Biorad, USA), containing 1×PCR buffer (TaKaRa), 50–100 ng genomic DNA, 0.25 μM for each primer, 150 μM dNTPs, 1.5 mM MgCl2 and 0.25 U Taq DNA polymerase (TaKaRa). Thermal cycle was conducted with an initial denaturation at 94 °C for 5 min, followed 35 cycles including a denaturation at 94 °C for 40 s, an annealing at the proper temperature (Table 1) for 40 s and an extension at 72 °C for 40 s, and a final extension at 72 °C for 10 min. PCR products were visualized on 8 % polyacrylamide gel stained with silver staining. A 50 bp DNA ladder molecular marker (TaKaRa) was used as standard to identify size of alleles. The analyses of polymorphism, including number of alleles (Na), expected heterozygosity (H_E), and Shannon–Wiener Diversity Indices (H') were performed using ATetra 1.2 (Van Puyvelde et al. 2010), which was developed for analyzing tetraploid microsatellite data. In this study, we constructed (CA)n and (CT)n enriched genomic libraries for *A. dabryanus*. 42 pairs of primer were proved to have specific amplification products, but only 26 primer sets were polymorphic in the population of 24 Dabry's sturgeon individuals (Table 1). The number of alleles per locus ranged from 3 to 13 with an average of 5.2, and mean expected heterozygosities (H_E) and shannon-Wiener Diversity Indices (H') per locus ranged from 0.303 to 0.806, and from 0.480 to 1.893, respectively (Table 1). These identified microsatellite markers can be employed effectively to examine fine-scale population structure and genetic diversity to allow parentage analysis for Re-stocking management of *A. dabryanus.*

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References

Benson G (1999) Tandem Repeats Finder: a program to analyze DNA sequences. Nucleic Acids Res 27:573–580

Chen XH (2007) Biology and resources of *acipenseriform* fishes. China Ocean Press, Beijing, China

Chistiakov DA, Hellemans B, Volckaert FAM (2006) Microsatellites and their genomic distribution, evolution, function and applications: a review with special reference to fish genetics. Aquaculture 255(1–4):1–29

IUCN (2010) IUCN red list of threatened species. Version 2010.3. Available online: http://www.iucnredlist.org/apps/redlist/details/231/. Accessed on 24 Oct 2010

O'Connell M, Wright M (1997) Review: microsatellite DNA in fishes. Fish Biol Fish 7:331–363

Salah MA, Iciar M (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res 25(22):4692–4693

Van Puyvelde K, Van Geert A, Triest L (2010) ATETRA, a new software program to analyse tetraploid microsatellite data: comparison with TETRA and TETRASAT. Mol Ecol Resour 10:331–334

Wei QW, Ke FE, Zhang JM, Zhuang P, Luo JD, Zhou RQ, Yang WH (1997) Biology, fisheries, and conservation of sturgeons and paddlefish in China. Environ Biol Fish 48:241–255

Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. Mol Ecol 11:1–16

Zen QZ (1990) Fisheries resources in Yangtze valley. China Ocean Press, Beijing, China

Zhang H, Wei QW, Du H, Li LX (2011) Present status and risk for extinction of the Dabry's sturgeon (*Acipenser dabryanus*) in the Yangtze River watershed: a concern for intensified rehabilitation needs. J Appl Ichthyol 27:181–185

Zhu B, Liao X, Shao Z, Rosenthal H, Chang J (2005) Isolation and characterization of microsatellites in Chinese sturgeon, *Acipenser sinensis*. Mol Ecol Notes 5:888–892

Zhuang P, Ke FE, Wei QW, He XF, Cen YJ (1997) Biology and life history of Dabry's sturgeon, *Acipenser dabryanus*, in the Yangtze River. Environ Biol Fish 48:257–264