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ARTICLE *in* CONSERVATION GENETICS RESOURCES · JUNE 2012

Impact Factor: 1.17 · DOI: 10.1007/s12686-012-9834-z

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Conservation Genet Resour (2013) 5:487–490
DOI 10.1007/s12686-012-9834-z

TECHNICAL NOTE

High-throughput microsatellite marker development in Amur catfish (*Silurus asotus*) using next-generation sequencing

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Received: 12 November 2012 / Accepted: 10 December 2012 / Published online: 19 December 2012

Abstract The Amur catfish, *Silurus asotus*, is an economically important fish species in East Asia, but few genetic studies have been conducted on this species, especially those based on nuclear markers. Here, we isolated and characterized 47 novel polymorphic microsatellite loci in the genome of *S. asotus* using 454 sequencing. We screened 70 primers and 48 of them generated amplification products. Forty-seven of the amplification products were polymorphic in a population of 40 collected from the upper Yangtze River. The number of alleles varied from 3 to 15, and the observed and expected heterozygosities varied from 0.300 to 0.800 and 0.305 to 0.866, respectively. The average polymorphic information content (PIC) of all loci was 0.682, indicating high levels of polymorphism. In addition, cross-species amplification in a congener species, *Silurus meridionalis* showed a high level of transferability (79.2 %), which confirmed that the microsatellite markers developed here could be used effectively for other related catfish species.

Keywords Amur catfish · *Silurus asotus* · Microsatellite · 454 sequencing

The Amur catfish, *Silurus asotus*, is an economically important catfish species in East Asia (Froese and Pauly 2012). The annual catches of the species have continued to drop in recent years, primarily because of overfishing and habitat alterations (e.g. Ru et al. 2011; Zhang et al. 2011). To save this valuable resource, large numbers of molecular markers, such as microsatellites, are required for use in molecular marker assisted breeding in this species. However, very few such data are available for this species to date (Wu et al. 2011). In addition, traditional methods for microsatellite development are time-consuming and labor-intensive (Abdelkrim et al. 2009; Allentoft et al. 2009), which prohibits high-throughput microsatellite development on a genome-wide scale. Recent advances in sequencing technologies have dramatically increased the efficiency of microsatellite development, especially the Roche 454 Genome Sequencer FLX System. In the present study, 47 novel polymorphic microsatellite loci were isolated and characterized in *S. asotus*, using 454 sequencing. These markers will be useful for marker assisted breeding of *S. asotus* and related catfish species.

Samples from *S. asotus* were collected from Yibin ($n = 40$) in the upper Yangtze River and stored in 95 % ethanol. The genomic DNA was extracted from fins using the DNeasy Blood and Tissue kit (Qiagen). Approximately 10 μg genomic DNA from a single *S. asotus* individual was subjected to high-throughput DNA sequencing on 1/4-plate using the 454 Life Sciences Genome Sequencer FLX Titanium instrument (Roche). Newbler 2.3 software was used to assemble the raw sequence reads. All unique sequences longer than 200 bp were used to screen for microsatellites using MSATCOMMANDER version 0.8.2 (Faircloth 2008) with default parameters. Only sequences containing tri- and tetra-nucleotide repeat motifs (at least 10 repeats and eight repeats for each type, respectively) were used for subsequent primer design.

All the selected primers found to generate polymorphic PCR products in eight randomly selected individuals from the Yibin population were used for subsequent genetic

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Table 1 Characteristics of the 47 polymorphic microsatellite loci in *S. asotus* and cross-amplification in another congener species (including one monomorphic locus shown on the last row in the table)

Locus	Repeat motif	Primer sequence (5′–3′)		Yibin population (N = 40)						Cross-amplification (N = 8)
		Forward primer	Reverse primer	N_A	H_O	H_E	PIC	P_{HW}	GenBank Accession No.	*S. meridionalis*
SA1	(GAT)10	GGTTACTCCAGAAGTGCCAAAG	AAGGAGACCGAACCTGAGC	9	0.750	0.697	0.641	0.771	JX999736	M
SA2	(AAC)11	ACATGTATTGGTCACAACGGTC	GTCACCAGCCACTGTTCTC	7	0.500	0.662	0.604	0.020	JX999737	P
SA4	(GGT)10	GCGTTTTAGCGCGTTTTGG	TGTTCGCTGTTCGATCCAC	6	0.475	0.727	0.673	0.008	JX999738	M
SA5	(AAAT)10	ACCCCGGTCTGTCCTAAAAG	AGAAGAAGGTGTGTCCAAACTG	12	0.775	0.866	0.840	0.162	JX999739	M
SA6	(ACAG)11	GAGAGAGCTACAGACAAAGAGC	GCTGTGGTTTGCTGAGTGG	6	0.650	0.703	0.651	0.759	JX999740	P
SA8	(ACTT)10	TGTCCTATAAAGCCAACGACTG	TTGCTCCTCTAATTAACCTCAAC	3	0.350	0.666	0.584	0.000*	JX999774	M
SA12	(ACC)10	CCTGCATGTCCTCCAAAACC	GTACTGCTGTCCAAGCCAC	4	0.650	0.753	0.696	0.168	JX999741	M
SA14	(CTT)10	CCTGCATTCTCGATTCCTCG	ATGGCTGTGGTGAGGGAAG	6	0.410	0.554	0.512	0.014	JX999742	M
SA15	(CTT)17	GTGTGAGCACCAAGGAACAC	TGGTGTGGGAAGGACATGC	10	0.700	0.854	0.827	0.017	JX999743	M
SA16	(GAT)12	TGGGATGAGTCTCTGGCAC	ACCAGGGCTTCACCAACTG	7	0.500	0.659	0.608	0.020	JX999744	P
SA18	(AGAT)20	GGCGATCGATGAGAGGGAG	GAGTCCTAATGGGCGGAGG	12	0.825	0.883	0.859	0.283	JX999745	P
SA19	(ATCC)10	CAGCAGACACAGTTTTCCCC	GACTGCAAGTGACCGAACAG	3	0.600	0.652	0.570	0.677	JX999746	F
SA21	(ATCT)20	CATGCTGGCACTGATATGGC	CTGTGGTTCTGCCTGTAGC	9	0.525	0.823	0.788	0.000*	JX999747	M
SA22	(ATTT)9	TATGGGTGTGGGCTGTGTG	CACTCACCTGGCATGAACG	8	0.550	0.714	0.668	0.005	JX999775	F
SA23	(CTGT)12	GTGGTGTTGTTTAGGTCAGGG	GCTAGCAGGTCACAAGAGTG	8	0.725	0.792	0.748	0.918	JX999748	F
SA24	(AAT)10	GGGCTTGAAAGGTTGAGCG	CAGGCCGGGATCTGTCTG	5	0.325	0.728	0.668	0.000*	JX999749	P
SA26	(AAAC)8	CCTCCAGAGGGCAGATAGC	GCTAGCAGGCAGCACAAAC	6	0.600	0.587	0.534	0.905	JX999750	P
SA27	(AAAT)13	CCGGAAATACTTTAGGACCAGAC	TGGGTTCTTTCTTTCTGGGAAC	9	0.650	0.808	0.769	0.116	JX999751	P
SA28	(ACAT)14	CAGATAGAAATGTCTGCATGGC	AGCTGTGGAAGGTTTACTCC	8	0.750	0.766	0.730	0.451	JX999752	F
SA29	(AGAT)15	TGATCCCAAGAAGGAGGTGTC	GAGGTGTGGTCAAAAGGGG	7	0.725	0.750	0.696	0.025	JX999776	P
SA32	(ATCT)10	AGAGCAGCACTAGGAAGCTG	GCGATTCCGATTGGCTAGAAG	8	0.775	0.813	0.777	0.017	JX999753	P
SA34	(ATTT)10	CTCACCTACTTGTGCATACGG	CTTGTCTGTGCTGTGTCCC	8	0.750	0.834	0.799	0.088	JX999754	P
SA35	(CATT)8	AGATTGTACCCCGTCAGCG	TGGACATGGACACAAGGAC	5	0.500	0.622	0.563	0.010	JX999755	M
SA36	(CTGT)8	GCAGGCTGAGAAAACCTCAC	AGCAAAACCAAGCTGCCAC	3	0.300	0.305	0.276	0.745	JX999756	F
SA37	(CTTT)16	CTGGCTCTGAGTACTGGGC	TGAGAGAGGGCGTTCCTTG	7	0.775	0.815	0.776	0.000*	JX999757	P
SA39	(GGAT)11	GGTTGATGAGGGTTGCAGTC	GGCCAACACAGACAATACCC	3	0.450	0.559	0.486	0.041	JX999758	P
SA40	(AAC)18	AGACTTTGCTGGTGTGATGC	TGGAAGGCAGGTTATGCGG	7	0.775	0.795	0.751	0.125	JX999759	P
SA42	(ATT)12	CTGCTACCTCCAGCTCCAC	GGCCTCTTCCTCCTCATCC	8	0.625	0.824	0.790	0.084	JX999760	P
SA44	(GTT)14	TGGCTGTGTTCAGGACTGG	ACAAACTGGGTGGTTGACAC	5	0.625	0.727	0.678	0.040	JX999761	P
SA46	(ATCT)16	CCCCTCAATGACCTACTGACG	GGTGTGGCTCCTACCTGATG	15	0.725	0.916	0.897	0.001	JX999762	M
SA47	(ATGT)13	TTAACCCACCCACACCCAG	GCAGCACTGGAGAGAATGC	8	0.725	0.807	0.770	0.095	JX999763	M
SA49	(CTGT)11	TGTGGCTCTGGATAAGGGC	AGACAGGCTAACAGACAGGAC	10	0.725	0.832	0.799	0.285	JX999764	F

Table 1 continued

Locus	Repeat motif	Primer sequence (5′–3′)		Yibin population(N = 40)						Cross-amplification (N = 8)
		Forward primer	Reverse primer	N_A	H_O	H_E	PIC	P_{HW}	GenBank Accession No.	*S. meridionalis*
SA50	(CTTT)13	TGGTCTTGTCACCAACAGG	ATGCTGGTCTGAAAGAGGC	7	0.675	0.827	0.792	0.005	JX999777	F
SA52	(CATT)11	ACCTGAACCTCAGCCTTGG	GCTGAAGGTAGCATCATATAGAAAGG	7	0.605	0.739	0.688	0.122	JX999765	F
SA53	(CTGT)18	TGATGTCATTACACCCCATACAC	TTGTGGTCTGTCTCACTGC	6	0.625	0.748	0.698	0.041	JX999766	P
SA54	(ATGT)8	TTTTGGGGTGGCCATTCAG	GTGTCCCTACCCGTCCTAC	5	0.325	0.460	0.434	0.000*	JX999767	M
SA55	(ATTT)12	TGAGTGTCATGGCAAAGGC	CATCTGCCCACCTACAATGC	6	0.675	0.753	0.704	0.038	JX999768	M
SA56	(ATTT)15	CAAATGTGTCCACAAATGCAGG	GGTAGGATGGACTGGACAGG	5	0.625	0.728	0.670	0.047	JX999769	M
SA58	(ACTC)12	CCCTGTGAACAACAATTTTGGG	AGTGATGAGTCGGTGCGTG	11	0.800	0.821	0.790	0.538	JX999770	P
SA60	(AATG)9	TACCACCCCGTAGCTTGTC	TGCTTCTGAGCTGGGGATAC	3	0.400	0.522	0.424	0.010	JX999771	P
SA61	(AAAC)9	TACAGTGCCACAAGGTCAG	CTGCCTTGAATAAATGCCAATCC	3	0.359	0.588	0.485	0.006	JX999778	P
SA63	(AAAT)12	AGTGTCAGGAGCAGAAAATGAC	TGTTTGCACGTTTGTGGAAG	10	0.625	0.816	0.781	0.011	JX999779	F
SA65	(AATC)9	TGAATCTGCCGTGCTTTTC	CAGCATAGGCATGTGGAGG	7	0.575	0.787	0.742	0.003	JX999780	F
SA66	(AATG)8	GGTGATCAAAATGAGGAGGGG	ACTTATGTGAGTGTACCTGGTTTC	6	0.450	0.621	0.574	0.033	JX999781	P
SA69	(AGAT)13	TGAGCAAATGATCTGTATGGGTC	AGCCACTGGGTTAGTCTGG	7	0.625	0.749	0.709	0.187	JX999782	P
SA70	(AGAT)16	GCCTAGCTGGCATCCCC	TCAGTGTGGCAGCATTGTC	9	0.875	0.819	0.784	0.201	JX999772	M
SA71	(AGAT)19	TCAGACAAGGTGATTTTCGGG	GCCTCCTTGGGCATTCAAC	6	0.632	0.791	0.748	0.001	JX999773	P
SA17	(ACAG)14	TGGGAGACAGAGAAAGAGGG	TGGCTGACTACCGACTGATG	1	0.00	0.00	M			M

* Significance of deviation from Hardy-Weinberg equilibrium at significance at $P < 0.001$ for loci in HWE after Bonferroni correction

N_A Number of alleles, H_O observed heterozygosity, H_E expected heterozygosity, P_{HW} probability of Hardy-Weinberg equilibrium

PIC, Polymorphic information content, *P* polymorphic, *M* monomorphic, *F* failed to amplify or multiple non-specific amplification

analysis in the 40 individuals. The PCRs were performed in 12.5 μl of a reaction mixture consisting of approximately 20 ng of genomic DNA, 3 pmol of forward and reverse primers, 1.25 μl of 10 × buffer, 25 mM $MgCl_2$, 2.5 mM of dNTP, and 0.5U of rTaq polymerase (TaKaRa). The basic thermocycling program was one cycle at 94 °C for 5 min, 36 cycles at 94 °C for 30 s, 52–56 °C for 30 s, and 72 °C for 40 s; followed by one cycle at 72 °C for 10 min. The molecular size of each PCR product was determined in comparison with the pBR322 DNA/MspI molecular weight marker (TIANGEN) on 6 % denaturing polyacrylamide gel. Gel images were obtained and saved with Gel Doc XR System (BIO-RAD). In addition, cross-species amplification was tested for all 48 successfully amplified microsatellite markers in another congener species, *S. meridionalis*, using eight individuals.

Allelic variation at the microsatellite loci in the 40 individuals from the Yibin population were determined using POPGENE version 1.3.1 (Yeh and Boyle 1997) for the number of alleles (N_A), expected and observed heterozygosity (H_E and H_O, respectively), and the linkage disequilibrium (LD) between loci. The program Arlequin 3.5 (Excoffier et al. 2005) was used to infer the most probable cause of the Hardy-Weinberg equilibrium (HWE) departures. The PIC was calculated according to CERVUS version 3.0.3 (Kalinowski et al. 2007). Various genotyping, typographic errors, and null alleles were tested using MICRO-CHECKER v2.2.3 (Van Oosterhout et al. 2004). The significance level of HWE departures and LD were corrected using sequential Bonferroni correction (Rice 1989).

Of the 70 primers screened, 48 successfully amplified a PCR product and 47 were polymorphic. The number of alleles varied from 3 to 15 (mean = 7.02), and the observed and expected heterozygosities varied from 0.300 to 0.800 (mean = 0.610) and 0.305 to 0.866 (mean = 0.729), respectively. Of the 47 polymorphic loci, five showed significant departure from the HWE after applying Bonferroni correction ($P < 0.001$) (Table 1). Null alleles were observed at four of the five loci, with the exception of SA37, suggesting the main reason for the observed deviation from the HWE may be the presence of null alleles. No linkage disequilibrium was detected between the loci after sequential Bonferroni correction. The PIC ranged from 0.276 to 0.897 (mean 0.682), suggesting that *S. asotus* Yibin population has a naturally high genetic diversity. A cross-species amplification test showed that 38 (including 22 poly- and 16 monomorphic loci) of these 48 loci could be successfully cross-amplified from a congener species, *S. meridionalis* (Table 1). The high success rates (79.2 %) of cross-species amplification confirmed that the microsatellite markers developed in *S. asotus* could be used effectively for other related catfish species. The polymorphic microsatellite markers described here will provide a valuable resource for future work on genetic diversity, population structure, and marker assisted breeding of this species.

Acknowledgments We thank Bin Li and Qing Zeng for the sample collections. This work was supported by the grants from the National Natural Science Foundation of China (31071903, 31272283) and the Chongqing Natural Science Foundation (CSTC, 2010BB1015).

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