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PRIMER NOTE

Identification of microsatellite loci in lake sturgeon, *Acipenser fulvescens*, and their variability in green sturgeon, *A. medirostris*

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Abstract

From $(CATC)_n$, $(GATA)_n$, $(AAAC)_n$, and $(CA)_n$ –enriched libraries for the lake sturgeon *Acipenser fulvescens*, 254 primer pairs were developed. These primer pairs resulted in the identification of 128 microsatellite loci in either *A. fulvescens* or *A. medirostris*. Polymorphic loci were identified in both sturgeon species for 48 of the primer pairs and 14 of the primer pairs amplified polymorphic loci only in *A. medirostris*. Most of the identified loci appear to be tetrasomic (79.1% in *A. fulvescens* and 64.5% in *A. medirostris*). These results offer estimates of the degree of diploidization in each of these species.

Keywords: *Acipenser*, *Acipenser fulvescens*, microsatellites, primers, sturgeon, tetrasomic

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Lake sturgeon (*Acipenser fulvescens*), along with many other sturgeon species, is faced with the challenges resulting from overfishing, pollution, and dams. Life history traits, such as late sexual maturity, make it difficult for sturgeon populations to overcome these obstacles. Within the Great Lakes Basin, a management plan based on genetics is being devised to define appropriate stocking procedures for the recovery of lake sturgeon. The project is comprised of several different phases, which include the development of polymorphic microsatellite markers that target disomic loci and the standardization of these genetic markers, along with previously developed disomic markers (May *et al*. 1997; Pyatskowit *et al*. 2001; McQuown *et al*. 2002), among laboratories working on lake sturgeon genetics. Much of the lake sturgeon genome appears to be duplicated (Ludwig *et al*. 2001), complicating interpretation of gene dosage. Therefore, efforts focus on developing disomic microsatellite markers. The selected disomic microsatellite markers will be used to gather data on the population structure of lake sturgeon throughout the Great Lakes Basin. Data will then be compiled, culminating in a basin-wide management plan accompanied with sub-basin specific plans. The development of polymorphic microsatellite markers resulted in the design of 254 primer pairs, which were subsequently screened using DNA from both *A. fulvescens* and *A. medirostris* to assess primer utility in different species.

Genomic DNA was extracted from *A. fulvescens* and libraries enriched for the repeat motifs $(CATC)_n$, $(GATA)_n$, $(AAAC)_n$, and $(CA)_n$ were designed and screened according to the protocol described in Meredith & May (2002). Purified plasmids (500 ng/μL) were cycle sequenced in a 10-μL reaction using 20 μM of pUC19 forward or reverse sequencing primer and $^1/_4$ X ABI Big Dye™ terminator reaction with 5X dilution buffer (400 mM Tris-HCl and 10 mM $MgCl_2$) under the following thermocycler conditions: 96 °C for 30 s; 30 cycles of 96 °C for 10 seconds, 55 °C for 15 s, and 60 °C for four minutes, ending with a 4 °C soak. Sequencing product was purified using magnetic bead separation (RapXtract™ by Prolinx), suspended 2:1 in a formamide/loading dye mix, and denatured at 65 °C for three minutes. Sequencing results were visualized on the M.J. Research BaseStation.

Sequences obtained were analysed for the repeat region using the SEQMAN software (LASERGENE 5.1, DNASTAR Inc.), which also compared sequences to determine the existence of duplicates. The software PRIMERSELECT (LASERGENE 5.1, DNASTAR Inc.) was then used to create 254 primer pairs flanking the repeat regions of interest. Primers were named AfuG ## (Afu representing *A. fulvescens* and G representing Genomic Variation Laboratory) and were numbered sequentially.

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Extracted DNA (using the TNES-Urea method; White & Densmore 1992) from the fin tissues of four wild lake sturgeon and four wild green sturgeon was amplified using the newly created primers. Five ng/μL of the extracted DNA were amplified in 10 μL reactions with 20 mM Tris-HCl, pH 8.4; 50 mM KCl; 1.5 mM $MgCl_2$; 0.8 mM dNTPs; 1 μM unlabelled forward and reverse primers; and 0.4 units *Taq* DNA polymerase (Gibco or Promega). Amplification parameters for AfuGs 1–147 (excluding AfuG 56) were: 95 °C for 1 minute 30 s; 30 cycles of 95 °C for 1 minute, 52 °C for 45 s, 72 °C for 2 min; ending with a 4 °C soak. Due to the amplification of a secondary locus at AfuG 56, further optimization was required, resulting in the following amplification parameters: 94 °C for one minute; 20 cycles of 92 °C for 30 s and 70 °C for 40 s with a 0.5 °C decrease in the second step each cycle; 20 cycles of 92 °C for 30 s and 60 °C for 40 s with a one second increase in the second step each cycle; ending with a 4 °C soak. Additional optimization measures are noted within Table 1. In an effort to reduce the number of initial amplification failures, different thermocycler parameters were used for AfuGs 148–254: 95 °C for 1 minute 30 s; 30 cycles of 95 °C for 1 minute, 67 °C for 45 s with a 0.5 °C decrease each cycle, 72 °C for 2 min; ending with a 4 °C soak. Amplified fragments were suspended 1:1 in 98% formamide/loading dye, denatured at 95 °C for 3 min, and separated on a 5% acrylamide gel running at 35 W for 70 min (AfuGs 1–147) or 50 W for 70 min for increased resolution (AfuGs 148–254). Products were visualized using an agarose-™ SybrGreen overlay procedure as described by Rodzen *et al*. (1998) and then scanned with a Molecular Dynamics 595 fluorimager. Those primer pairs that appeared to amplify polymorphic disomic loci in *A. fulvescens* were further screened on eight additional individuals to confirm status.

Of the 254 primer pairs developed, 105 (41.3%) resulted in poor or no amplification in *A. fulvescens*. Thirty-four (22.8%) of the loci that successfully amplified were monomorphic. Of the remaining 115 primer pairs, 91 (79.1%) appeared to amplify tetrasomic loci, producing banding patterns consistent with four gene doses, while 24 (20.9%) appeared to amplify disomic loci, producing symmetrical two-banded genotypes (Table 1). In *A. medirostris*, 142 (55.9%) primer pairs resulted in poor or no amplification. However, conditions were not optimized for *A. medirostris* to improve amplification success, which may account for its lower success rate when compared to *A. fulvescens*. A total of 50 [19.8% (out of the 254 initially screened)] loci were monomorphic in *A. medirostris*. Of the 62 primer pairs that successfully amplified the DNA of *A. medirostris*, 40 (64.5%) loci appeared tetrasomic and 22 (35.5%) appeared disomic. While some of the putative tetrasomic loci may actually be two disomic loci, these results give an estimate of the level of diploidization that has occurred in each of these species.

Table 1 Characterization of 128 microsatellite loci in lake sturgeon (*Acipenser fulvescens*) and green sturgeon (*A. medirostris*). GenBank accession numbers, primer sequences, number of individuals genotyped (*n*), repeat motif, number of alleles, allele size range, clone size, and observed and expected heterozygosities for *A. fulvescens* are presented. Amplification results for *A. medirostris* are given, including number of alleles and allele size range in parentheses

	GenBank accession no.	Primer Sequence (5′–3′)	*n*	Repeat motif	No. of alleles	Gel estimated allele size range (bp)	Sequenced clone size (bp)	H_O	H_E	*Acipenser medirostris* ($n = 4$)
AfuG 1	AF529444	F: TCACAGGTCGGGAGGGAGATTTAC R: CCGTGTTCGCGTGGGTTTTC	4	$(CATC)_6$	mono	232	232	—	—	5 (210–250)
AfuG 7	AF529445	F: TGCACCACTAGACAGAAAGAGTAG R: GCAGCTTCAGAATCTAAAAAGAAT	3	$(GATA)_{12}GACAGG(TA)_3(GATA)_{11}$	6	200–240	217	1.0	0.99	—
AfuG 8	AF529446	F: TGTTTATGGATACTTGGTGTCTTA R: ATTGTAGCAGGATATGGGTTCTT	4	$(GATA)_{11}GA(GATA)_{19}$	8	280–370	386	1.0	0.99	0
AfuG 9	AF529447	F: CATAATGTAAAGCAAAAGT R: ACCTGAAATGTATGTTATG	9	$(GATA)_{14}(GA)_2GATA(GA)_2(GATA)_6$	6*	130–160	152	0.67	0.81	3 (120–130)
AfuG 12	AF529448	F: TTCTGATTAAGCACTCC R: ACCTGGTTAATCATACTG	3	$(GATA)_{15}$	6	190–250	214	1.0	0.99	3 (190–210)
AfuG 15	AF529449	F: CACACCTGTATGGCTCAACT R: GAACCCCAATACATAACAATACAG	3	$(GATA)_{14}$	5	215–260	225	1.0	0.99	mono
AfuG 16	AF529450	F: CTTAGCAGACGCCCTTAT R: ATCGCAATCTGTATGTTTTT	4	$(GATA)_{14}$	4	170–190	185	0.5	0.93	—

Table 1 *Continued*

	GenBank accession no.	Primer Sequence (5′–3′)	*n*	Repeat motif	No. of alleles	Gel estimated allele size range (bp)	Sequenced clone size (bp)	H_O	H_E	*Acipenser medirostris* (*n* = 4)
AfuG 19	AF529451	F: AAACAAGCGCCCACAAAA R: TGAGCGAAATAACACAACAAAAC	3	$(GATA)_{13}$	4	240–260	233	1.0	0.96	mono
AfuG 20	AF529452	F: TTCACAACGGGTCATTCA R: TATTTTGTACTGGGTGGTTCATC	10	$(GATA)_{12}$	4	280–310	307	0.70	0.93	mono
AfuG 21	AF529453	F: TAAATGCAATACAATCTG R: CAACAATGCCTTTTACT	12	$(GATA)_{12}$	6	200–240	206	1.0	0.99	4* (190–220)
AfuG 22	AF529454	F: ATTGGGCCGTCTGATTG R: AGCCCTCCTCGTCTCCTT	3	$(GATA)_{11}$	7	135–170	146	1.0	0.99	—
AfuG 23	AF529455	F: AAGCTCCATTTTCACAG R: TTTACATAATTACAACAGAT	4	$(GATA)_{11}$	mono	148	148	—	—	2* (150–160)
AfuG 24	AF529456	F: GCAGCAGCAAGAGGTTAT R: GTGTGGCTGGCGATTTT	4	$(GATA)_{10}$	6	340–400	369	1.0	0.99	5 (340–400)
AfuG 28	AF529457	F: GCGAGATTAACTAAAGATGAAAAA R: ATCTAGCCACGTAAAAACAAGTCT	3	$(GATA)_{10}$	4	220–245	224	1.0	0.98	2* (185–220)
AfuG 30	AF529458	F: GTAAACGCTCAAGCTATCACACC R: GACGGCGTTCTCCATTCTAA	6	$(GA)_5GTA(GATA)_{10}$	3*	150–170	171	0.83	0.54	mono
AfuG 34	AF529459	F: GAAGGTCAGGGGCTCAACACA R: ACGTATTCATCCCAGCATCCATC	8	$(GATA)_2AATA(GATA)_9$	—	—	134	—	—	5 (190–215)
AfuG 35	AF529460	F: CCATTACCATATTCAAAACCAT R: AAAAATATTCAAAACAGAGGACTT	10	$(GATA)_{10}$	4	200–220	217	0.50	0.64	—
AfuG 37	AF529461	F: CAGGGAATCATGAGCACACG R: TGGCGCAGGATTTTGACAC	3	$(GATA)_9$	5	160–190	140	1.0	0.98	7 (170–200)
AfuG 39	AF529462	F: TGTCTCATGCTTCAGCTCTTTTGT R: CTCTGCTTTATTGCTCTGCTTCC	8	$(GATA)_9$	—	—	182	—	—	2* (135–145)
AfuG 41	AF529463	F: TGACGCACAGTAGTATTATTTATG R: TGATGTTTGCTGAGGCTTTTC	3	$(GATA)_9TA(GATA)_3$	8	200–260	209	1.0	0.99	7 (170–240)
AfuG 42	AF529464	F: GGTCCTGGCATGCACTAAAAT R: AAGGGCAGCAGAGATGTCAAAG	3	$(GATA)_9$	5	230–255	242	1.0	0.98	5 (380–450)
AfuG 43	AF529465	F: TTCCATGGTATTGCTGTAAAA R: GGGGGTTGACGCCACTA	4	$(GATA)_8$	mono	195	195	—	—	5 (190–230)
AfuG 46	AF529466	F: CACTTTGTTCTGCATCTTCTC R: ACGGGTTGTCTATTTTTGTG	3	$(AAAC)_8$	7	200–230	215	1.0	0.99	6 (180–210)
AfuG 51	AF529467	F: ATAATAATGAGCGTGCTTTCTGTT R: ATTCCGCTTGCGACTTATTTA	2	$(AAAC)_6(AC)_2(AAAC)_8$	3	230–260	251	1.0	0.92	mono
AfuG 52	AF529468	F: TCTCTAAAACTTCAGGATAAAA R: TAAACCATGGCAGTAAACA	3	$(AAAC)_{11}$	5	115–135	132	0.67	0.97	4 (130–150)
AfuG 53	AF529469	F: ATATCATTTTAAAGGCAGACAGTA R: TCAATGACAGAAAGGGTAAAC	2	$(AAAC)_{11}$	4	280–300	289	1.0	0.97	6 (260–300)
AfuG 54	AF529470	F: CCGTTTTATAGTGTTGGTCA R: CTGGCAGATTTGGTTATTTA	3	$(AAAC)_{10}$	4	240–265	247	1.0	0.93	3 (240–260)

Table 1 *Continued*

	GenBank accession no.	Primer Sequence (5′–3′)	*n*	Repeat motif	No. of alleles	Gel estimated allele size range (bp)	Sequenced clone size (bp)	H_O	H_E	*Acipenser medirostris* (*n* = 4)
AfuG 55	AF529471	F: CAATTGTTTCTCTGTGATAA R: TGACTGCAATAAAATAATGT	2	$(AAAC)_{10}$	mono	275	275	—	—	4 (250–280)
AfuG 56	AF529472	F: ACTAAACCCAGCACAGAAAATCAG R: GAAGCCCATCCCACAGGTT	10	$(AAAC)_9$	4*	260–275	266	0.50	0.61	mono
AfuG 60	AF529473	F: GAAGCGCAAATGGATGGAGA R: CCGCGTTATAATGAGGTAGCACT	7	$(AAAC)_8$	3	190–220	218	0.71	0.93	2* (170–190)
AfuG 61	AF529474	F: ATGAGCGATTTTGTTTTT R: ATTCTGTGCATTAGGTGAT	9	$(AAAC)_8$	2*	200–210	207	0.78	0.49	0
AfuG 63	AF529475	F: TCCTGGCTAGCGAACGAA R: CTTTTAAATGGGGGACAGACTAT	11	$(AAAC)_8$	6*	120–150	139	0.78	0.79	4* (130–150)
AfuG 64	AF529476	F: ACCTTAGTTCGTATGGGATGTTAT R: AAATTGCAAGCCGAAGGAA	9	$(AAAC)_7$	4	120–160	159	0.78	0.83	2* (130–145)
AfuG 65	AF529477	F: CTCTGTGTATTGCGCTATTATTCA R: GTAGGGTGCGTGTATCATTTTGTT	8	$(AAAC)_7$	6	150–170	163	1.0	0.99	6 (160–190)
AfuG 66	AF529478	F: GGCAGCAACTTTACCAA R: GAGATATCTGCGTTCGTT	3	$(AAAC)_7$	2*	300–320	306	0.33	0.28	—
AfuG 67	AF529479	F: CAAAGCTAGAACAAGTAAAGAGAA R: GGGGTGTCCTATAATAAAAGTGC	6	$(AAAC)_7$	2*	280–290	288	0.14	0.17	2* (160–170)
AfuG 68	AF529480	F: AATGGCTTATCTTTTATCTTGACT R: AGCTTTTCTGGACTGTGTATGTT	2	$(AAAC)_6$	4	170–190	210	1.0	0.80	4 (160–170)
AfuG 71	AF529481	F: CTGACGGGAGACTGATTTACAC R: ATTTTGCCTTTACGCTTTATTTAG	10	$(AAAC)_6$	2*	230–240	236	0.50	0.46	mono
AfuG 72	AF529482	F: CCGCCGCCCCTGGAAAAACTA R: CAGGAGAACGCACGGGTATCAACT	3	$(AAAC)_6$	4	280–320	289	1.0	0.96	6 (280–330)
AfuG 74	AF529483	F: CTACAAAGACGGGTTACG R: AGCGACTGTCTGGTTTTC	11	$(AAAC)_6$	4*	200–230	226	0.73	0.69	0
AfuG 75	AF529484	F: TTTCTTGATTACTATGTGCGTTAC R: GTGGGGGTTTTATGTTTAGTTTAG	2	$(AAAC)_5$	4	210–240	222	1.0	0.97	5 (210–240)
AfuG 79	AF529485	F: GACGCAATCTGTTCCTTCATA R: TGCAGCCCTAAACAATAAACAC	2	$(AAAC)_2A(AAAC)_3$	3	300–330	300	1.0	0.84	mono
AfuG 81	AF529486	F: GCGGTCTTCTAGCCAATAAAAT R: AGACACAAAGACAACTCAAATAAA	3	$(GATA)_{13}$	3	260–280	266	0.67	0.95	5 (240–280)
AfuG 83	AF529487	F: TGCTTTTCTGGGGCCACTACTCC R: GGCCCCACCTTCCGCTACAAA	3	$(CA)_{19}$	2*	200–220	219	0.67	0.44	mono
AfuG 84	AF529488	F: ACAAGTGTCTTTCATATCATTATG R: CAAGCCTTTTTAAATAACAATATC	2	$(CA)_{16}$	3	155–180	168	1.0	0.84	3 (140–170)
AfuG 88	AF529489	F: GGACCTGCCCCATCTACCTG R: TGGCGCACTCCCCTGACTT	8	$(CA)_{14}$	—	—	—	—	—	5* (165–200)
AfuG 94	AF529490	F: GCGGAAACCATATAGCAAAACT R: ATAAAAGCCATTCCCACCTGT	2	$(CA)_{11}$	mono	145	145	—	—	2* (120–140)

Table 1 *Continued*

	GenBank accession no.	Primer Sequence (5′–3′)	*n*	Repeat motif	No. of alleles	Gel estimated allele size range (bp)	Sequenced clone size (bp)	H_O	H_E	*Acipenser medirostris* (*n* = 4)
AfuG 95	AF529491	F: CAGCAGAGTCTCCAGCCTTCAC R: CACAACCGTAACCTAGCAACACTG	2	$(CATC)_6$	2*	220–230	229	0.50	0.38	3* (210–230)
AfuG 96	AF529492	F: GGGTGTAGTCCTGCCTTGTGC R: CTTCCCATTTCCGTGTAGTTCTTG	4	$(CATC)_7$	mono	218	218	—	—	2* (210–220)
AfuG 99	AF529493	F: CAAAGCCACAGACACGTAAATAAA R: GGCTCACCGCAACCCTCTA	4	$(CATC)_5$	3	160–180	176	0.75	0.84	4 (155–180)
AfuG 101	AF529494	F: ACCTAAAGTCTGCGTGCCTCTA R: GTGGACCGGTTGATTTTGTATT	11	$(CATC)_{10}$	5	180–220	211	1.0	0.97	4 (180–210)
AfuG 102	AF529495	F: TGTAATGTGTTTGTGAGATGAGAT R: AAATTGCCCCTTAGTTGC	11	$(CATC)_2AATC(CATC)_5$	3	200–225	222	1.0	0.91	mono
AfuG 104	AF529496	F: TGACTCATTTTCTGTATCTCG R: GCTTATCTGCTTTGTATTGAA	12	$(CATC)_6$	5	200–220	207	1.0	0.98	mono
AfuG 109	AF529497	F: TACAATGGGGTCCAGTGGAGA R: ATCGAAAAGGCAGATAGACCTCTC	3	$(CATC)_7CACC(CATC)_5$	2*	210–230	277	0.67	0.44	0
AfuG 110	AF529498	F: ACCCGATGCTAACTTTGTAAT R: CACTTTTTGGCTGTAGACTTTT	4	$(GATA)_{21}$	2*	280–300	319	0.25	0.22	—
AfuG 112	AF529499	F: TATTGTTCCTTTATGGTTATG R: TATTTCACTGTCTGTTGTATGTA	9	$(GATA)_{12}GACA(GATA)_6$	4*	240–260	260	0.89	0.62	0
AfuG 113	AF529500	F: CGGGTTGGTTAATGAGAGGA R: CCAATATTCGGAAGCGTGTG	4	$(GATA)_{17}$	7	300–380	345	1.0	0.98	3 (290–360)
AfuG 115	AF529501	F: TTGCAAAATTGAAACAGAAAAA R: TGATAGACGGGCAGCAGAC	4	$(GATA)_5GATG(GATA)_{13}$	8	200–250	251	1.0	1.0	—
AfuG 116	AF529502	F: TTAACTCCCAATACACATCACTTC R: AGCTTTCTACGTCTCGGTTTTA	11	$(GATA)_{14}$	4	255–270	254	0.73	0.87	mono
AfuG 122[1]	AF529503	F: AACACGACAACAAACTTATTCA R: TGTGTTTCTATGTCTGTCTGTCTA	7	$(GATA)_{13}$	4*	160–180	175	0.43	0.64	0
AfuG 123	AF529504	F: GAGCCGCTCTATACCTGGAAACAT R: ACAGACACGCTGAGAGGCACAC	4	$(GATA)_3GACA(GATA)_{12}$	8	200–240	224	0.75	0.94	—
AfuG 124	AF529505	F: AATGCCCAAAGCCACAATAGTCA R: TCTGTCTGTCTGTCTCTGCCTGTC	8	$(GATA)_{12}$	5	130–155	149	1.0	0.97	mono
AfuG 125	AF529506	F: ACCGGGCGAGTGCAGGAC R: CGTCATTTTGCATGTTCCTATACA	2	$(GATA)_{13}$	5	130–180	132	1.0	0.98	0
AfuG 126	AF529507	F: TCCAATCTGTTATCTGACCA R: AAGCTTACTCCCCAAACAA	2	$(GATA)_{12}$	4	320–400	325	1.0	0.93	0
AfuG 127	AF529508	F: TGTGTAATATGGATCAGTCTTT R: TATAGCCCCTTTTAGCAC	2	$(GATA)_{11}$	4	200–225	206	1.0	0.97	0
AfuG 128	AF529509	F: CTTATCAGCCAATCAGGA R: CAGTATCAGTCTAATGTTCTAATC	2	$(GATA)_{11}$	4	245–300	258	1.0	0.85	5 (230–280)
AfuG 130	AF529510	F: CACACCTCCACTTTCAA R: AGCTTACTTTCCACAATA	2	$(GATA)_{11}$	4	145–160	145	1.0	0.85	0

Table 1 *Continued*

	GenBank accession no.	Primer Sequence (5′–3′)	*n*	Repeat motif	No. of alleles	Gel estimated allele size range (bp)	Sequenced clone size (bp)	H_O	H_E	*Acipenser medirostris* (*n* = 4)
AfuG 131	AF529511	F: ATATGTATAACATCAGTCTTACTA R: ATCTGTCTATCTATCTATCTATAT	8	$(GATA)_{16}$	5	250–280	257	1.0	0.99	0
AfuG 132	AF529512	F: TAGACCCAGGGAAGACGAGTG R: CTGAGGGCAAGGAAAACCAT	4	$(GATA)_9$	7	260–320	279	0.75	0.99	mono
AfuG 133	AF529513	F: CTGTATATCCAAAAGAAAAC R: GTGTGAGTGGATGGAATG	7	$(GATA)_8$	5	175–200	186	0.71	0.96	0
AfuG 135	AF529514	F: GCCAATTCCTGAAATATACCAG R: CGAAACCGCTTCAGACCTT	12	$(GATA)_7$	4	180–200	183	0.67	0.72	2* (180–190)
AfuG 137	AF529515	F: AAACGTGGCTGGGCAAAAGTG R: GCGCCCTCCCAAAAACATACC	4	$(GATA)_6$	3	215–240	236	1.0	0.93	mono
AfuG 138	AF529516	F: AGAACAGGGGGAACACAAATGAA R: AAAGAGAAGCTGCGGGAGTTACA	4	$(AAAC)_{12}$	3	180–200	170	1.0	0.93	3 (145–160)
AfuG 140	AF529517	F: CTCCCCATCCTGCTTCTCTG R: TGTTGCTCGATGCCACTTG	3	$(CA)_{15}$	mono	371	371	—	—	3* (360–380)
AfuG 141	AF529518	F: CGTTCTCGGCTTTCTTAT R: AGCCTGAGCCTTGTGATG	4	$(CATC)_6$	3	180–200	225	1.0	0.93	3 (155–165)
AfuG 142	AF529519	F: TCCCATCAATATTAAGGTCTACTA R: CTCTGGGTTTAAAATGCTGTCA	10	$(GATA)_7$	5	240–320	236	0.70	0.82	0
AfuG 147	AF529520	F: GCCACAACAGCAGAAAACAC R: AAGCTTGCAGGAGATACACAGT	3	$(GATA)_6$	5	195–240	195	1.0	0.98	0
AfuG 151	AF529521	F: CTATCGGCAGTGTCTTGTA R: ATCGCGCCTTTCATAGT	4	$(AAAC)_8$	5	180–215	188	1.0	0.98	5 (210–225)
AfuG 153	AF529522	F: AAAACGCAAATGCAATCACAA R: ACCTCCTTTCAAACCTACAACTCC	4	$(AAAC)_7$	4	160–180	159	0.75	0.90	3 (160–180)
AfuG 154	AF529523	F: ATGCCATGAACCAATACACTTTT R: ATTACAGATGCCGGCTTAGAGGT	4	$(AAAC)_{10}$	5	170–190	182	1.0	0.98	mono
AfuG 155	AF529524	F: ATCCAATCCGGGTTTTAC R: ACACAGTCGCTTTATTTTCTC	10	$(AAAC)_6$	2	340–350	345	0.30	0.26	0
AfuG 159	AF529525	F: TTTTTACTGGAAGCAAGAACAA R: GTCAAAACAAAAATGCCTACAAG	11	$(AAAC)_7$	2	300–320	316	0.45	0.82	mono
AfuG 160	AF529526	F: CCGCAGCATTAGGTCAAA R: CCCCAGTGGAAATAATAATGTA	7	$(AAAC)_8$	3*	130–150	135	0.71	0.50	0
AfuG 161	AF529527	F: CCTCCCCCGGCTTGAACTT R: ACAGCCGATCGTGGTGACAGGT	12	$(AAAC)_6$	2	180–190	186	0.25	0.38	0
AfuG 162	AF529528	F: CAGCCCAAGCAAAACACATACAAA R: CCCGCCACTCGCAGGAA	4	$(AAAC)_7$	3	260–280	274	0.75	0.92	0
AfuG 163	AF529529	F: AGCTGCTTGGGTCTCTT R: AGGCGTACTGAAAATAACAA	4	$(AAAC)_7$	3	165–180	167	0.75	0.92	mono
AfuG 164	AF529530	F: GTTGGGGGTGGGTTGTTC R: AAGATGGCTGAGTGGGTGAC	4	$(AAAC)_7$	3	260–290	295	0.75	0.94	—
AfuG 165	AF529531	F: GGACCTGCCTCACCAAAAC R: TTCCCTCCCTGTAGCAAAAAC	12	$(AAAC)_6$	2	190–210	209	0.83	0.61	mono

Table 1 *Continued*

	GenBank accession no.	Primer Sequence (5′–3′)	*n*	Repeat motif	No. of alleles	Gel estimated allele size range (bp)	Sequenced clone size (bp)	H_O	H_E	*Acipenser medirostris* (*n* = 4)
AfuG 166[2]	AF529532	F: AGTTTGGGGGTTCAGATTACAG R: CTCATTGGCTCAGACACTTG	9	$(AAAC)_6$	4*	135–160	151	0.67	0.66	3 (150–170)
AfuG 169	AF529533	F: GAAACTGTATGCTGCCACTGT R: TACCCCACCAAGATAAGATGC	12	$(AAAC)_6$	3	250–265	259	0.75	0.93	mono
AfuG 172	AF529534	F: CAGCTCCGGATAGTGTTAGTAGTC R: AGGCGTCTCGGCTGAAA	2	$(AAAC)_6$	4	180–230	201	1.0	0.85	3 (195–220)
AfuG 174	AF529535	F: CAATGGGGTGGGCAAAAA R: ATTAGGAGTATGGCAGTGTAGAAC	6	$(AAAC)_6$	3	150–160	154	0.67	0.68	2 (140–150)
AfuG 175	AF529536	F: CCATAATTCAAAAGAACAGATTAA R: TGGGCCAATACTTTTGTCA	3	$(AAAC)_5$	2*	195–215	199	0.33	0.50	0
AfuG 177	AF529537	F: AAGGGTGCACGTTTGATAATGTAA R: ACTGGCCCCTAGCTTGGTGAG	3	$(AAAAC)_3(AAAC)_4$	3	230–240	240	1.0	0.68	mono
AfuG 178	AF529538	F: TAATTAGAATATGATAGAGTTGTC R: CAGGCTTGCAGATTGGT	3	$(AAAC)_5$	4	380–450	383	1.0	0.67	mono
AfuG 180	AF529539	F: TAAAAACCAGCACGATACTTGTCA R: ACGCACTTTTCTTTCCTGGTTC	3	$(AAAC)_3$	4	240–260	245	1.0	0.96	0
AfuG 182	AF529540	F: AGCCGCAAAAATGACTTAAAC R: CTGATGGAAAGTGCACATTAACT	3	$(AAAC)_7$	2	260–280	272	0.67	0.68	2* (260–280)
AfuG 184	AF529541	F: TGATACGCTGGCTGTGTCCTTA R: CTGTTGGGTCTCCGTTGGTG	4	$(AAAC)_{12}$	9	180–220	201	1.0	0.99	mono
AfuG 185	AF529542	F: CAGTCCTGTGCCTTGTGTTC R: CATTGCTTCCTGGTCTGTGA	9	$(AAAC)_{10}$	3*	360–380	370	0.55	0.66	0
AfuG 188	AF529544	F: CGCGCACGCCCCCTCTG R: TGAAAGGGTGCACACTTTTGTTGG	3	$(AAAC)_{10}$	5	150–170	166	0.67	0.80	mono
AfuG 189	AF529545	F: GCTGTTGTGTTGATGATGATGATA R: CAGGGAACAGAGCAACCAGGAC	2	$(AAAC)_6$	3	260–280	274	0.50	0.68	4 (280–300)
AfuG 190	AF529546	F: GAATCCCAGCACGTGAATGAAT R: CTGCGTAAGGGTGTCTGCTAATAA	8	$(AAAC)_7$	3	310–335	316	0.50	0.56	0
AfuG 193	AF529547	F: TGCTGAGCCCAAGCAATTC R: AAAAGAATCCAGTGGGTTTTT	16	$(AAAC)_7$	5	240–265	258	0.88	0.94	0
AfuG 195[3]	AF529548	F: ATTCCTCCAGCCGTATTATTA R: AAGCAGTTAGTTTATGTGGTTGTG	10	$(AAAC)_7$	3*	160–175	165	0.40	0.52	0
AfuG 197	AF529549	F: AAAGAGGACAGTTACAGCGAAGAA R: CAGAAGGCAGAGGGCGATGATA	4	$(AAAC)_7$	4	240–270	272	1.0	0.96	—
AfuG 198	AF529550	F: GTCTCCTCCCTCATGTTTTATTTG R: GCGTACTGAGACTTGGCTTTGA	4	$(AAAC)_7$	5	200–230	224	1.0	0.96	4 (190–220)
AfuG 204[4]	AF529551	F: TGACCAGGCACCGTAACTTTG R: TAATGTCGCCGGCTCTGGTCTA	12	$(AAAC)_5$	4*	130–150	141	0.83	0.62	0
AfuG 211[5]	AY135384	F: ACGCCCGTTCCAGTCTCTGAT R: ACGTTCTCGTGCTTCATGTTGAG	13	$(CA)_{11}$	3*	210–225	211	0.54	0.42	mono
AfuG 212	AF529552	F: GAGAGCTGCGGCTCCTC R: TGTGGGTCTGTATTTGTATCTGAC	3	$(CA)_{14}$	4	250–265	262	1.0	0.96	0

Table 1 *Continued*

	GenBank accession no.	Primer Sequence (5′–3′)	*n*	Repeat motif	No. of alleles	Gel estimated allele size range (bp)	Sequenced clone size (bp)	H_O	H_E	*Acipenser medirostris* (*n* = 4)
AfuG 213	AF529553	F: TCAGTCCCGAAATAATAATAACA R: AGCATCAAACCCGAACTG	2	$(CA)_{12}CG(CA)_{12}$	4	165–180	178	1.0	0.97	mono
AfuG 215	AF529554	F: TGCTGAAAGCCTTAAGGAGAGTG R: ATTCGGGTGGCGTTCTGTT	4	$(CA)_{18}$	5	270–310	293	1.0	0.98	5 (230–310)
AfuG 216	AF529555	F: CATGTGCTGCAGCTAGGATTGTG R: GAGGGTAGACTGCAGTTTGATTTG	8	$(CA)_{17}$	7	200–230	221	1.0	0.99	0
AfuG 217	AF529556	F: TAAAGCGTGGTGTCGAATTA R: CTGCATCCAACATTTGTATGA	7	$(CA)_{16}$	4	185–210	195	1.0	0.89	4 (195–260)
AfuG 218	AF529557	F: AAGCTGATGTGGTTTGGTCTGTA R: AGTAATTGAGCTGCCCCTGTTTCT	11	$(CA)_{16}$	6	310–390	366	1.0	0.97	3 (600–650)
AfuG 226	AF529558	F: CACAAATACATGCCCCAAAATAA R: CTAGCGATGCCTGACAAAACA	10	$(CA)_{13}$	3	280–305	293	0.60	0.88	mono
AfuG 229	AF529559	F: AGAGAATGCGGAGAATGAGGAC R: GCACAGATACACGCAGACAAACA	10	$(CA)_{14}$	4*	300–330	320	0.70	0.67	3* (310–430)
AfuG 230	AF529560	F: GCACGCACATTCATTTAGTTCTT R: GATGGTTTTCAGTTGGGATAGG	6	$(CA)_{13}$	3	260–275	265	0.83	0.92	mono
AfuG 232	AF529561	F: CCCGTGGACAGACAGACCTC R: AATGCTTGCCTATGCTTTATCA	3	$(CA)_{12}$	3	300–340	336	1.0	0.96	5 (330–370)
AfuG 234	AF529562	F: GGAGCGGCACAAAAGCACT R: TGAAAACCAGGGACATACAGC	4	$(CA)_{11}$	mono	303	303	—	—	4 (280–300)
AfuG 237	AF529563	F: GCCTGCTTCTGCTGATGG R: CCCCTGCTGGTGAGACG	3	$(CA)_9$	4	230–260	214	1.0	0.97	3* (190–210)
AfuG 238	AF529564	F: GCGGTCCATGCCAGTAT R: CTTGATCAGCCCCCAGTG	5	$(GA)_{33}$	3	230–290	261	1.0	0.95	3 (260–290)
AfuG 240	AF529565	F: ACTGGGGGCTGATCAAGCTG R: CAGTGTCTCACAATATGGGAACAT	4	$(CA)_8$	mono	123	123	—	—	2* (125–140)
AfuG 241	AF529566	F: CAGAACATGCCGGGTGAGTA R: ATCCAGGGCTTGTCTTGTATTTTA	9	$(CA)_{13}$	3*	230–260	243	0.89	0.57	2* (255–290)
AfuG 242	AF529567	F: TATGACTTACCCTGCTCTTGTGTT R: ACCCTGAATTGTCCTCCTCCTT	4	$(CA)_7GA(CA)_3GA(CA)_6$	mono	275	275	—	—	2* (260–270)
AfuG 243	AF529568	F: AATAGGACCTCGTTTGTTTG R: CTCAGGAGATGCAGTGTGGT	3	$(CA)_{23}$	4	180–200	200	0.67	0.68	0
AfuG 245	AF529569	F: AACTGTCACATATCCACCCTAACC R: AATAAACGAACTCCGGCTACTCTC	3	$(CA)_{18}$	3	145–160	153	1.0	0.94	2 (120–140)
AfuG 247	AF529570	F: CAGGGTGCAAACTTGCTGTGT R: AGGGACGGCATCTGCTGTATT	8	$(CA)_{44}$	4	195–210	262	0.87	0.96	2* (170–190)
AfuG 248	AF529571	F: ACCCTGTGTTGAGTTTTGTCTG R: TTTATTGGCTATTCCTCTCACTGT	4	$(CA)_{16}$	—	—	277	—	—	2* (260–290)

*Potential disomic locus; '—' Poor amplification; '0' No amplification. [1]AfuG 122: 1.0 mM $MgCl_2$; [2]AfuG 166: 58° annealing temperature and 1.75 mM $MgCl_2$; [3]AfuG 195: 55° annealing temperature; [4]AfuG 204: 55° annealing temperature and 1.25 mM $MgCl_2$; [5]AfuG 211: 58° annealing temperature and 1.0 mM $MgCl_2$.

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