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PRIMER NOTE

New microsatellite loci for suckers (Catostomidae): primer homology in *Catostomus*, *Chasmistes*, and *Deltistes*

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Abstract

Herein we describe the development of microsatellite markers for five Catostomid species: *Catostomus snyderi*, *Catostomus rimiculus*, *Chasmistes brevirostris*, *Deltistes luxatus*, and *Catostomus occidentalis*. A total of 89 primer pairs were developed from enriched $(CA)_n$ and $(GATA)_n$ libraries created from the *D. luxatus* genome. The successful amplification of 82 polymorphic loci across three Catostomid genera indicates that the microsatellite markers described in this study will be broadly applicable.

Keywords: Catostomidae, *Catostomus*, *Chasmistes*, *Deltistes*, microsatellites, suckers

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The Catostomid family of freshwater fishes comprises over 60 species that are widely distributed throughout North America (Miller 1959). Catostomids, or suckers, are believed to have an allotetraploid genetic heritage and retain the expression of some duplicate gene systems (Uyeno & Smith 1972; Ferris & Whitt 1980). Several Catostomid species are currently listed as threatened or endangered under the U.S. Endangered Species Act and have become the focus of recovery efforts in many western states. Notably, four sucker species are native to the Klamath River Basin, Oregon: the Klamath largescale (*Catostomus snyderi*) and smallscale suckers (*Catostomus rimiculus*) and federally endangered shortnose (*Chasmistes brevirostris*) and Lost River suckers (*Deltistes luxatus*). Currently, no information exists on the population structure or levels of gene flow occurring among populations in the Klamath Basin. In this paper we describe the development of microsatellite markers for Klamath Basin suckers and demonstrate their suitability for use in the Sacramento sucker (*Catostomus occidentalis*).

Genomic DNA was extracted from dried fin samples of *D. luxatus* using the TNES-urea procedure (Belfiore & May 2000). Two subgenomic libraries were constructed by Genetic Identification Services (Chatsworth, CA) by partially digesting genomic DNA with *Bsr*BR 1, *Eco*R V, *Hae*III, *Pvu*II, *Sca*I, and *Stu*I. An oligonucleotide linker containing a *Hin*DIII site was ligated to fragments in the range of 300–700 bp. These fragments were enriched by magnetic bead capture (Jones *et al*. 2000) to create two separate libraries for the repeat motifs $(CA)_n$ and $(GATA)_n$. The captured fragments were ligated into the *Hin*DIII site of pUC19 and the products electroporated into *Escherichia coli* DH5α. Transformed DH5′ cells were plated on LB ampicillin plates. Approximately 500 recombinant clones (sampled with a toothpick) were amplified directly in 15 μL reactions containing: (20 mM Tris-HCl, pH 8.4, 50 mM KCl), 5 mM $MgCl_2$, 1.5 mM dNTPs, 0.5 μM pUC19 forward and reverse sequencing primers, and 0.5 units *Taq* DNA polymerase (GIBCO). Reaction mixtures were amplified in an M.J. Research PTC-100 96 V thermocycler using the following procedure: 94 °C for 3 min, 25 cycles of (94 °C for 30 s, 57 °C for 30 s, 72 °C for 30 s), and 72 °C for 5 min. Approximately 1 μL of polymerase chain reaction(PCR) product was run on a 3% TAE-agarose gel stained with 0.01× ™ Vistra Green nucleic acid stain to identify inserts of 300–800 bp. Colonies containing the desired inserts were grown overnight in LB and plasmids were purified using the QIAprep Spin Miniprep Kit (Qiagen). A total of 220 clones from both libraries were sequenced using the ABI Big Dye™ Terminator cycle sequencing protocol and visualized on an ABI 377 DNA sequencer (Applied Biosystems).

PCR primers were developed for 36 dinucleotide and 76 tetranucleotide loci using *PrimerSelect* software (Lasergene 5.1, DNASTAR Inc.). For all samples screened, a total of 5 ng genomic DNA was amplified in 15 μL reactions containing: (20 mM Tris-HCl, pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, 0.5 mM dNTPs, 0.5 μM primers, and 0.5 units *Taq*

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Table 1 Characterization of 82 microsatellite loci in the Lost River sucker (*Deltistes luxatus*) based on three individuals from the Klamath River Basin: GenBank accession nos, repeat motif, observed and expected heterozygosity (H_O and H_E from Genes in Populations Version 2.2, May *et al*. 1995), number of alleles, allele size range, clone size, and primer sequences.

Locus names	GenBank accession no.	Repeat motif	H_O	H_E	No. of alleles	Allele size range (bp)	Clone size (bp)	Primer sequence (5′–3′)
Dlu 21	AF314663	$(CA)_{12}(TC)_{12}$	0.67	0.67	4	220–300	253	TGTTTGTGCTCATCACCA
								TGACTTCTAAACTAAATCACTGTC
Dlu 22	AF314664	$(CA)_{12}$	0.33	0.28	2	130–160	152	CGTGTGCGGCGTGTCTTTC
								TATCCATAACGGTTGCCTTCTCCT
Dlu 25	AF314665	$(CA)_{30}$	1.00	0.50	2	130–175	189	CAATCCTGAGGCGGTGTG
								TTAAATAGTTGTGAAAGGTGAA
Dlu 26	AF314666	$(AG)_{24}$	0.67	0.44	2	160–175	193	ATATACTTATTTAATTCAACAC
								TTCTGACAGTCATTAATCTTAGAC
Dlu 27	AF314667	$(CA)_{13}$	1.00	0.61	3	180–210	204	AGCCAATGAATAAACAAGAGG
								TCATCCAAGAGCAGGTAAGC
Dlu 28	AF314668	$(CA)_{32}$	0.33	0.50	3	180–200	203	CCGCCATGAGGAAAGGAGAT
								GACATAATGAGTGATAGCCAGGTG
Dlu 203	AF314669	$(CA)_{16}$	0.67	0.67	4	180–200	186	GTGGTGGAGGGGGCACTGTAG
								AAACGTTCCAAATAACTGTCTTTA
Dlu 206	AF314670	$(CA)_{22}(CT)_6$	1.00	0.78	5	160–240	189	GGATTTCACACCAAAGGTCTCCTG
								TCTCTGCGGCTGTGCCAATCTC
Dlu 207	AF314671	$(CA)_{18}$	0.67	0.72	4	180–210	193	TACAGGAAGAGACTGACTGACTAA
								TCAAAGCTATCTGAAATCGCTAAT
Dlu 209	AF314672	$(CA)_{18}$	1.00	0.78	5	150–180	168	CCCCCAGTCTCTGACAAT
								CTCTAATCATGCGACTTTTACAAG
Dlu 210	AF314673	$(CA)_{27}$	0.67	0.61	3	150–220	185	TGTGTCCAGAAAGAGCAGAGAT
								TTTTAATTAGCCCCTGAAACAT
Dlu 213	AF314674	$(CA)_{15}$	0.67	0.44	2	150–180	171	ATCACCTCTTCAGGCTACTTTG
								ATCCCAGAAGCGATCCCTCTCAT
Dlu 216	AF314675	$(CA)_{15}$	1.00	0.83	6	140–220	157	GTAAACCCGCCCACAACTGCT
								CGCCACCGACACCTCCTATTTTAC
Dlu 229	AF314676	$(CA)_{42}$	1.00	0.78	6	130–200	188	TACCAGCCAGGTGTGCAAGCTATG
								CACACTGGAATGTTCACATGG
Dlu 230	AF314677	$(CA)_{40}$	1.00	0.72	4	120–180	168	CTATATTTTCGCCCAAGTGTTGAT
								AATTCCCATTTCCCATTG
Dlu 232	AF314678	$(CA)_{22}$	0.33	0.28	2	60–130	117	GCCCGGAGATTCCAGTTGAAGA
								AGCAGCCGCCACTGATAGACA
Dlu 233	AF314679	$(CT)_{14}(CA)_{37}$	1.00	0.78	5	150–200	180	GTAAAAGAACCTGCAGCCTTATGC
								CATCAGGGGTTTGAGTGTG
Dlu 235	AF314680	$(CA)_{23}$	1.00	0.61	3	140–160	148	GTGACGCCACCGCCACTTAGT
								AGTCCTCCACTGCTGTCTGTG
Dlu 243	AF314681	$(CA)_{14}$	0.67	0.44	2	160–180	181	CCGTTTGTTTGGGCACTT
								AGCAGCACAGAGCGAGAT
Dlu 245	AF314682	$(CA)_{12}$	0.67	0.50	3	180–200	194	ATTCAGAGCAGCACAGGTATTGG
								TGAAGTTTGTTTATCGCTCTGTGA
Dlu 246	AF314683	$(CA)_{11}$	1.00	0.78	5	170–220	197	ACTTCCTCTCACTCTCAACTG
								AAATCTCTGGGAACTTACAAAT
Dlu 257	AF314685	$(CA)_{19}$	0.33	0.61	3	160–190	181	TACACCTTGGCCGAAAATATGAAT
								GCTGTTCTGAGGGAAGGACAAATC
Dlu 259	AF314686	$(CA)_{34}$	1.00	0.78	5	140–220	184	ACCGCAACTCTTAACATACACAAG
								TGAGCTCAGTACAAAAACCATTAC
Dlu 266	AF314687	$(CA)_{52}$	1.00	0.78	5	170–200	181	GCCAACAGCCCCCTCTAGTGCT
								TTGCTGGATCAAGAGGAGGTGAG
Dlu 271	AF314688	$(CA)_{21}$	1.00	0.83	5	140–170	160	TCCCCCAAGCCATCAGTCTCC
								ATTGTTTATGCTCATTGTGTTAC
Dlu 272	AF314689	$(CA)_{47}$	0.33	0.72	4	100–160	160	AATCAGAATGAGCTTAATTGTCAA
								TTTAACATCGGCTTTGTATATTTC
Dlu 276	AF314690	$(CA)_{37}$	0.67	0.78	5	140–165	162	AACTTTTAGATTTTCAGCAGA
								ATAGAGATAAAAGGAGAGGAGATG

Table 1 *Continued*

Locus names	GenBank accession no.	Repeat motif	H_O	H_E	No. of alleles	Allele size range (bp)	Clone size (bp)	Primer sequence (5′–3′)
Dlu 282	AF314692	$(CA)_{15}$	1.00	0.61	3	140–170	151	CACACTCCACAGGTAGGTTT GTTTCACTGGTGGTTTCTTAG
Dlu 284	AF314693	$(CA)_{17}$	0.67	0.44	2	160–180	160	ACCCAGCGGCTCGGAACTAAG TTCCAAGGATCTGAATTTTACAGG
Dlu 45	AF314694	$(GATA)_{29}$	1.00	0.61	3	300–500	458	TGGGCCTTAGTGCAGAGGA TGGTTAGGCAGAATTCTCCAG
Dlu 403	AF314695	$(GACA)_9(GATA)_{23}$	0.33	0.50	2	290–350	325	AGATGGACAGATGGACAAATAGAC CTATCGTCCTATCGTCTTTCTCC
Dlu 405	AF314696	$(GATA)_{21}$	0.33	0.28	2	200–220	207	CAGCCCTCCGCGTGAAAACAAT ACCGTAAGGGGGCAGCAGAAGG
Dlu 408	AF314697	$(GATA)_{10}$	0.67	0.44	2	200–250	243	TGCAAATGTTGCCCCAAGAGATGT ACCCCCTTCGCCTGAGTTTAACC
Dlu 409	AF314698	$(GATA)_{20}$	1.00	0.78	5	160–250	206	TGCGATCCTAGAAGGAGTAAAACA ATTCCATTTGCTGTCAACTTCAAA
Dlu 416	AF314699	$(GATA)_{26}$	1.00	0.78	6	150–260	214	TATTAATCAACATAACTGACAAAG TTCTGAAATGATGAAAAAGTC
Dlu 420	AF314700	$(GATA)_{26}$	1.00	0.78	5	120–280	186	TGATCGTGCCTTTCATAGTCAATG CAGACAACCAGGGATCGTAACAGT
Dlu 433	AF314701	$(CAGA)_{12}(GATA)_{18}$	1.00	0.78	5	220–350	287	AACAGCGATAAAATGGGGCTAATA CCTGACATAGTGGATGAATAAACC
Dlu 434	AF314702	$(GATA)_{19}$	1.00	0.72	4	230–250	252	ACGAGATGCCATTGATGTGAGTGC ATGGGTGGATGGATGAATGGAATG
Dlu 439	AF314703	$(ACAG)_7(GATA)_{25}$	1.00	0.83	6	200–360	240	GAGACAGTCCACACTTCACATTGT TTCCATAATACACTCTTGGCATAG
Dlu 451	AF314704	$(GATA)_{13}$	0.67	0.78	5	230–280	246	GGCGGACGGACGGATGG GTCCCGGCGGAACCACAG
Dlu 454	AF314705	$(GATA)_{28}$	1.00	0.67	3	200–300	247	CAGAACAATTTTCCTAAGCAAGTG GCTCACAAATCAATTTATGTCAGC
Dlu 455	AF314706	$(GATA)_{23}$	1.00	0.78	5	170–240	216	AAATTAAAATCCTGAATTCAATAT TATATGCAGGTAAAAATTAACAAC
Dlu 456	AF314707	$(GACA)_{10}(GATA)_{19}$	1.00	0.72	4	225–285	251	TTGCAACTGCTGAGAAAATACACA CACACGAGGAGACAGAAACACAG
Dlu 461	AF314708	$(GACA)_{26}(GATA)_{13}$	1.00	0.78	5	120–200	242	ATAGCTTTTGTGCAAATAAGTCTG TAAAAGGTGAGTCAAGCAGAAGTG
Dlu 463	AF314709	$(GA)_{25}(GATA)_{17}$	0.67	0.78	5	170–300	217	TACAAGCACTCCAGTAATCAATCA GGGGGTCAAACACAGTATTAGTAG
Dlu 466	AF314710	$(GATA)_{14}$	1.00	0.72	4	220–280	253	CTGCGCATGCATTTTTCATTCCTG TTTGTTTACGCTGCACGCTTGAGC
Dlu 467	AF314711	$(GATA)_{17}$	1.00	0.78	5	270–344	334	ACGTGGGTACCTTGCTCGGAGTAT ACGCGCTAATAGACAGTGGACCAA
Dlu 476	AF314712	$(GATA)_{35}$	1.00	0.83	6	160–380	263	ATGGTTGGCTACTTTAACAATCAA TACACCTCCAATCTCGTTTCATAA
Dlu 482	AF314713	$(GATA)_{21}$	1.00	0.72	4	170–250	209	GAAGAGGGCAGTAGGGTCAGATG GGCGTGTCAGGGGAGGAA
Dlu 488	AF314714	$(GATA)_{22}(GATG)_7$	1.00	0.83	5	130–220	232	ACCCATCAATAACAGAGGTGAGG ATCAAATACTCCCGGATACCACAC
Dlu 498	AF314716	$(GATG)_{10}(GATA)_{45}$	1.00	0.78	6	130–520	254	TTTGAGGACCGGAACTAACTGTAG TTCATGCAGCCTTTAATGCTTAA
Dlu 4105	AF314717	$(GATA)_{33}$	1.00	0.83	6	65–165	214	AAGCAAGTGTAATGCTCAAA GGCTTTAAATTTTATTCTGTT
Dlu 4123	AF314719	$(GATA)_{20}$	1.00	0.78	5	130–200	177	ACGAGATGCCATTGATGTGAGT AGACGGCGGCAGACAGATA
Dlu 4126	AF314720	$(GATA)_{28}$	1.00	0.83	6	130–170	172	CACAGTCCTAACAGTCGAACTAAA AATATTTTTGCTATGTAGGCTAAA
Dlu 4128	AF314721	$(GATA)_{24}$	0.00	0.00	1	180	210	TCGTAAATCTATCTTTCTATCTAC GCTGGAAAGACAAGTAGAGTT

Table 1 *Continued*

Locus names	GenBank accession no.	Repeat motif	H_O	H_E	No. of alleles	Allele size range (bp)	Clone size (bp)	Primer sequence (5′–3′)
Dlu 4131	AF314722	$(GATA)_{23}$	1.00	0.78	6	150–220	188	AACCCTATATTTATAACCTCTAAT
								CACCACAATGACATACAAGTA
Dlu 4139	AF314723	$(GATA)_{24}$	0.67	0.67	4	170–230	199	TAATTAAAAGCTATTGTTGAACTT
								GACATTGAATCTATTAAAACATAC
Dlu 4146	AF314724	$(GATA)_{21}$	0.67	0.78	5	160–520	169	TTTTATGTGATTATATGGGTGTAT
								TTAATTGAAGCTCCTGACCTGTAT
Dlu 4153	AF314726	$(GATA)_{32}$	0.67	0.50	3	60–120	205	GGTCATGACACTAGAAGGTGTTAA
								CAGTGAGCCAATTGATAGACAGAC
Dlu 4166	AF314728	$(GATA)_{25}$	1.00	0.72	4	275–400	323	CTCTCGCTGAACTCTACCAATC
								AAAAGCAGGACTGAGAATGAGAG
Dlu 4171	AF314729	$(GATA)_{23}$	1.00	0.83	6	75–160	191	TGGCATCTGCTTCCAGTTTAT
								GGGCCAAATCGCACTCTT
Dlu 4183	AF314730	$(GATA)_{27}$	1.00	0.83	5	65–220	209	CTGAAAGCACCTCCTCCATTAG
								GTTCTCTTCTCCTGTTTCGCTTAT
Dlu 4184	AF314731	$(GATA)_{15}$	1.00	0.83	6	75–165	166	CCATGCATGCACCAATGTAGAAAT
								CAGCAGTGCCCATATGATTACACA
Dlu 4201	AF314732	$(GATA)_{21}$	1.00	0.83	6	85–170	189	CCAACCTTCTGAACAACTGTAAAT
								GTGGTAAAGAGGTCTGCCTGTAT
Dlu 4211	AF314733	$(GATA)_{13}(GACA)_{17}$	0.67	0.78	5	60–200	198	TGTCTGAACGCCACAACCTG
								CACCCATACAGATGAGGGGGAGAG
Dlu 4217	AF314734	$(GATA)_{13}(GACA)_{7}$	0.33	0.72	4	200–275	230	TGGGATGGCATGAGGATGAGTAA
								CACATGCCTGCAAGATTGACTGAT
Dlu 4219	AF314735	$(GATA)_{23}$	0.67	0.50	3	200–296	208	CGCCCCACATCCTCTACACTCAA
								TCAACAAAGGGACATAGATAAGAT
Dlu 4235	AF314736	$(GATA)_{12}$	1.00	0.78	5	175–240	171	TGGTATTAACCGTTTACTTCCACA
								TAAACTCCGCTTTTGTTATCAGC
Dlu 4237	AF314737	$(GATA)_{31}$	1.00	0.72	4	210–280	229	CATTTCCCTCCCTTTATACATTTC
								TGCAGCATTAAACAGCATTTGAAC
Dlu 4243	AF314738	$(GATA)_{24}$	1.00	0.78	5	50–200	160	TGGTTGGATGCTGAAATAAAGTAA
								TGAGCCTCATCATAGATGGATAGA
Dlu 4259	AF314739	$(GATA)_{24}$	1.00	0.78	5	70–220	193	GGGTGCAGAAACGTATCCAAAAAC
								AAGCATCATTCAACACCACATTCA
Dlu 4276	AF314740	$(GATA)_{15}$	1.00	0.78	5	145–200	181	CGAAGCTGCACTGACTGC
								GGAAATGGACACCTTTGACA
Dlu 4283	AF314741	$(GATA)_{8}$	0.67	0.72	4	100–150	107	CAGAAAAACGGTATAAAAGATGAA
								AAATATTAAACAGTTGGATGGATG
Dlu 4287	AF314742	$(GTTA)_{8}(GATA)_{27}$	0.67	0.72	4	230–260	255	TTGACATTTTTAAAACGCCAAACC
								TGCAAGCTTCCATTAGAAAGGAGT
Dlu 4296	AF314743	$(GATA)_{27}$	1.00	0.83	6	165–245	210	AAGAACAATTTAAAACAGTGAGTG
								TACCCTTATGTTTAATGTGTTAGG
Dlu 4300	AF314744	$(GATA)_{22}$	1.00	0.83	6	290–375	234	CACACCTGTTAGTGAGCTCCTCTC
								AAACCAATAAAGCAATAGATAGAA
Dlu 4303	AF314745	$(GATA)_{19}$	0.00	0.00	1	200	188	AGATAGGCAGACGGACGGACAGAC
								AACGAGATGCCATTGATGTGAGTG
Dlu 4307	AF314746	$(GATA)_{28}$	1.00	0.72	4	200–275	218	AAGAACAATTTAAAACAGTGAGTG
								TACCCTTATGTTTAATGTGTTAGG
Dlu 4314	AF314747	$(GATA)_{25}$	0.67	0.67	4	220–290	253	GAGGGTCTGTGGAGAACA
								TTTCACTTCAATGACAAAAATA
Dlu 4315	AF314748	$(GATA)_{21}$	0.67	0.61	3	250–300	257	TGGCCAGTCTTTCCATCCT
								ATGCAAAGCTTATCTTTGAGGTGTT
Dlu 4338	AF314750	$(CT)_{18}$	0.67	0.72	4	150–185	172	CTTCCTGACAATGTTCATCTTAT
								CACAATGCCATGGCAACAAG
Dlu 4339	AF314751	$(GATA)_{18}$	1.00	0.83	6	210–300	253	TGTTCCTCGGTCAGCTCTTCATCA
								GGCCAAAGGGGCAGCACATAC

Table 2 Cross-species amplification of 82 microsatellite loci designed for the Lost River sucker (*Deltistes luxatus*). Three individuals each of *Chasmistes brevirostris*, *Catostomus snyderi*, *Catostomus rimiculus*, and *Catostomus occidentalis* were surveyed. P, indicates that the amplified locus was polymorphic; M, indicates that the amplified locus was monomorphic; na, indicates no amplification

Locus	*Ch. brevirostris*	*C. snyderi*	*C. rimiculus*	*C. occidentalis*	Locus	*Ch. brevirostris*	*C. snyderi*	*C. rimiculus*	*C. occidentalis*
Dlu 21	P	P	P	P	Dlu 455	P	P	P	M
Dlu 22	P	P	P	P	Dlu 456	P	P	P	P
Dlu 25	P	P	P	P	Dlu 461	P	P	P	P
Dlu 26	P	P	P	P	Dlu 463	P	P	P	P
Dlu 27	P	P	P	P	Dlu 466	P	P	P	P
Dlu 28	P	P	P	na	Dlu 467	na	na	P	P
Dlu 203	P	P	P	P	Dlu 476	P	P	P	P
Dlu 206	P	P	P	P	Dlu 482	P	P	P	P
Dlu 207	P	P	P	P	Dlu 488	P	P	P	P
Dlu 209	P	P	P	P	Dlu 498	P	P	P	P
Dlu 210	P	P	P	P	Dlu 4105	P	P	P	P
Dlu 213	P	P	P	P	Dlu 4123	P	P	P	P
Dlu 216	P	P	P	P	Dlu 4126	na	na	P	P
Dlu 229	P	P	P	P	Dlu 4128	M	P	P	M
Dlu 230	P	P	P	P	Dlu 4131	P	P	P	P
Dlu 232	P	P	P	P	Dlu 4139	P	P	P	P
Dlu 233	P	P	P	P	Dlu 4146	P	P	P	P
Dlu 235	P	P	P	P	Dlu 4153	P	P	P	P
Dlu 243	P	P	P	P	Dlu 4166	P	P	P	P
Dlu 245	P	P	P	P	Dlu 4171	P	P	P	P
Dlu 246	P	P	P	P	Dlu 4183	P	P	P	P
Dlu 257	P	P	P	P	Dlu 4184	P	P	P	P
Dlu 259	P	P	P	P	Dlu 4201	P	P	P	P
Dlu 266	P	P	P	M	Dlu 4211	P	P	P	P
Dlu 271	P	P	P	P	Dlu 4217	P	P	P	P
Dlu 272	P	P	P	P	Dlu 4219	P	P	P	P
Dlu 276	P	P	P	P	Dlu 4235	P	P	P	P
Dlu 282	P	P	P	P	Dlu 4237	P	P	P	P
Dlu 284	P	P	P	P	Dlu 4243	P	P	P	P
Dlu 45	P	P	P	P	Dlu 4259	P	P	P	P
Dlu 403	P	P	P	P	Dlu 4276	P	P	P	P
Dlu 405	P	P	P	P	Dlu 4283	P	P	P	P
Dlu 408	P	P	P	P	Dlu 4287	P	P	P	na
Dlu 409	P	P	P	P	Dlu 4296	P	P	P	P
Dlu 416	P	P	P	P	Dlu 4300	P	P	P	P
Dlu 420	P	P	P	P	Dlu 4303	P	P	P	P
Dlu 433	P	P	P	P	Dlu 4307	P	P	P	P
Dlu 434	P	P	P	P	Dlu 4314	P	P	P	P
Dlu 439	P	P	P	P	Dlu 4315	P	P	P	P
Dlu 451	P	P	P	P	Dlu 4338	P	P	P	P
Dlu 454	P	P	P	P	Dlu 4339	P	P	P	P

DNA polymerase (GIBCO). Reaction mixtures were amplified using the following procedure: 94 °C for 2 min, 30 cycles of (94 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min), and 72 °C for 5 min. Amplification products were mixed 1 : 1 with 98% formamide loading dye, denatured for 3 min at 95 °C and cooled on ice before running on 5% denaturing acrylamide gels at 60 W for 30 min. Products were fluorescently detected using the agarose and ™Vistra Green overlay procedure of Rodzen *et al*. (1998) and scanned with a Molecular Dynamics 595 fluorimager.

A total of 31 dinucleotide and 58 tetranucleotide primer pairs amplified unambiguous PCR products in *D. luxatus*, *Ch. brevirostris*, *C. snyderi*, *C. rimiculus*, and *C. occidentalis* using identical reagent and thermal cycling conditions. With the exception of Dlu 4339, all loci exhibited patterns consistent with disomic inheritance. Of the 89 primers tested across five species, seven were monomorphic. Amplification product details for *D. luxatus* are reported in Table 1 and cross-amplification results are reported in Table 2. As indicated by a high amplification success across

three Catostomid genera, the microsatellite markers described herein will be useful in future studies involving suckers.

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