

PRIMER NOTE

Characterization of microsatellite loci for five members of the minnow family Cyprinidae found in the Sacramento–San Joaquin Delta and its tributaries

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Two microsatellite-enriched libraries [(CAGA)_n, (TAGA)_n] were constructed using pooled DNA from three cyprinid species native to the Sacramento–San Joaquin Delta of California: Sacramento splittail (*Pogonichthys macrolepidotus*); Sacramento pikeminnow (*Ptychocheilus grandis*); and tui chub (*Siphateles bicolor*). Primers were designed for 105 loci and tested for levels of polymorphism in five cyprinid species found in the Delta: Sacramento splittail, Sacramento pikeminnow, tui chub, hitch (*Lavinia exilicauda*), and Sacramento blackfish (*Orthodon microlepidotus*). Fifty-one loci were polymorphic for at least one species and 31 loci were polymorphic for multiple species. The number of polymorphic loci per species ranged from 16 to 26.

Keywords: Cyprinidae, microsatellites, primers, Sacramento – San Joaquin Delta

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The freshwater fish of the family Cyprinidae are highly diverse, with over 2000 species world-wide and more than 280 species found in North America (Moyle & Cech 2000). In California, the Sacramento–San Joaquin Delta and associated tributaries provide important estuary and spawning habitats for eight native cyprinid species: Sacramento splittail (*Pogonichthys macrolepidotus*), Sacramento pikeminnow (*Ptychocheilus grandis*), tui chub (*Siphateles bicolor*), hitch (*Lavinia exilicauda*), Sacramento blackfish (*Orthodon microlepidotus*), California roach (*Lavinia symmetricus*), speckled dace (*Rhinichthys osculus*) and hardhead (*Mylopharodon conocephalus*). With the possible exceptions of the Sacramento blackfish and pikeminnow, these native cyprinid species, or some of their subspecies, are experiencing decreasing population sizes as a result of non-native fish introductions, dams, water diversions, contaminants, and other human activities (Moyle 2002).

A multiagency and academic collaboration is currently underway to increase our understanding of the Sacramento splittail's life history, susceptibility to environmental contaminants, population substructure and reproductive strategies. Microsatellite marker development for the Sacramento splittail enables a molecular genetic examination

of population substructure and reproductive strategies for this study. In addition, these markers will probably prove valuable in genetic studies and conservation management of other native Sacramento–San Joaquin cyprinids. Therefore, to maximize the overall utility of these markers, we created a multispecies library composed of three cyprinids: Sacramento splittail, Sacramento pikeminnow and tui chub.

Genomic DNA was extracted (Dneasy™ Tissue Kit, Qiagen Inc.) from dried fin tissue of Sacramento splittail, Sacramento pikeminnow and Mohave tui chub (*S. b. mohavensis*). The DNA was quantified and combined to create a final 1 : 1 : 1 ratio of DNA from all three species (final concentration 100 µg/100 µL in 25 mM Tris–HCl plus 2.5 mM ethylenediaminetetraacetic acid, pH 8.0; 100 µL total). Two libraries enriched for tetranucleotide repeat motifs (CAGA)_n and (TAGA)_n were constructed from this pooled DNA (Genomic Identification Services) and screened for 300–1000 base pair inserts according to the protocol described in Meredith & May (2002). A total of 159 and 155 clones containing inserts of an adequate size from the (CAGA)_n and (TAGA)_n libraries, respectively, were purified (Qiaprep Spin Miniprep Kit, Qiagen Inc.) and sequenced (ABI BigDye™ Terminator Cycle Sequencing Ready Reaction kit version 1.0, Applied Biosystems) using 20 µM of pUC19 forward and/or reverse sequencing primers. Sequencing products were purified using magnetic bead

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separation (RapXtract™ II, ProLinx), suspended 2 : 1 in a formamide/loading dye mix, and denatured at 65 °C for 3 min. Sequencing results were visualized on an M.J. Research BaseStation™. Consensus Sequences were created to integrate the sequences of duplicate inserts (SEQMAN software, LASERGENE 5.1, DNASTAR Inc.) and primer pairs flanking repeat motifs were designed (PRIMERSELECT software, LASERGENE 5.1, DNASTAR Inc.).

To identify polymorphic loci, DNA was amplified with the newly designed primers from individuals of the three species used to make the library along with hitch and Sacramento blackfish. For all samples screened, 5 ng of genomic DNA was amplified in 10 µL total volume containing: 20 mM Tris-HCl, pH 8.4; 50 mM KCl; 1.5 mM MgCl₂; 0.8 mM dNTPs; 1 µM forward and reverse primers; and 0.4 units *Taq* polymerase (Promega). Amplification was conducted using a touchdown protocol with the following parameters: 95 °C for 1 min followed by 30 cycles of 95 °C for 1 min, 67 °C for 45 s with a 0.5 °C decrease each cycle, and 72 °C for 2 min. The amplified products were diluted 1 : 1 with 98% formamide loading buffer, denatured at 95 °C for 3 min, and chilled on ice for 2–3 min. Samples were separated on a 5% denaturing polyacrylamide gel at 50 W for 70 min. Amplified products were visualized using the Sybr-Green™-agarose overlay protocol described

by Rodzen *et al.* (1998) and scanned with a Molecular Dynamics FluorImager 595.

Primer sequences and characteristic data for all polymorphic microsatellite loci are provided in Table 1. Note that repeat motifs and clone sizes are specific to the one sequenced clone for each locus and could originate from any one of the three species included in the library. Observed and expected heterozygosities were calculated using CERVUS 2.0 (Marshall *et al.* 1998) and are presented exclusively for the 26 polymorphic loci of the Sacramento splittail, the species of current research interest to our laboratory. Deviations from Hardy–Weinberg equilibrium and linkage disequilibrium were calculated using FSTAT (Goudet 2001). After the Bonferroni adjustment, none of the loci displayed significant linkage disequilibrium and only *CypG50* significantly deviated from Hardy–Weinberg equilibrium with a deficiency in observed heterozygosity. All splittail individuals were collected from the same river location so this deviation is most likely the result of the presence of a null allele. Table 2 provides cross-species amplification results for all five species. Of the 105 primer pairs developed, 37 (35.2%) resulted in poor or no amplification and 17 (16.2%) displayed only a monomorphic pattern for all tested species. Thirty-one (60.8%) of the 51 primer pairs that successfully amplified polymorphic loci

Table 1 Characterization of 51 polymorphic microsatellite loci for the fish family Cyprinidae based on libraries constructed from *Pogonichthys macrolepidotus* (*P. mac.*), *Ptychocheilus grandis* and *Siphateles bicolor* DNA

Locus	GenBank access. no.	Primer sequence (5'–3')	Repeat motif (cloned allele)	Clone size (bp)	<i>P. mac.</i>		
					<i>n</i>	<i>H_E</i>	<i>H_O</i>
<i>CypG1</i>	AY439120	F: ACTACAGCCGTGCAAAGTATG R: CTTAAATAGCCAAGTTGATTGTA	(CAGA) ₃ (TAGA) ₆	227	20	0.69	0.85
<i>CypG2</i>	AY439121	F: GCAAAAATCAGCAATAGATCACTAA R: TTCTGCCATGCATTTGAACC	(CAGA) ₇ (TAGA) ₄	153	20	0.53	0.50
<i>CypG3</i>	AY439122	F: AGTAGGTTTCCAGCATCATTGT R: GACTGGACGCCTCTACTTTTCATA	(CAGA) ₂ (TAGA) ₁₁	201	21	0.68	0.67
<i>CypG4</i>	AY439123	F: GTTGCATCTGCTTTATACCTCATT R: CCAGTGCCTCCCTTTTGTPTTA	(TAGA) ₁₂	162	21	0.68	0.71
<i>CypG5</i>	AY439124	F: AGTTGATGCCTGTTTTGTTTTGTAT R: AGTTGTGTCAGGGTCTGTAGAA	(TAGA) ₁₂	166			
<i>CypG6</i>	AY439125	F: CCACCTGTCCACCCACTA R: TTTTTATAAAGCCTGAGAAACAT	(TAGA) ₇	182			
<i>CypG8</i>	AY439126	F: AGACAAAACAATGGGACTGAC R: GACAGACAGATAGACAAAAGATACA	(CAGA) ₆	159	20	0.66	0.70
<i>CypG9</i>	AY439127	F: GCAGTCACGTATTAAGGCGAGCAG R: GAGCGACTCTCAGGCACCTACC	(CAGA) ₅	113			
<i>CypG10</i>	AY439128	F: GCCCGTAGCAAGCCCTGTG R: AACCGCCCACTGTGCTTTTG	*1.	160			
<i>CypG11</i>	AY439129	F: CTGGCAAATAAACGTGTCTGGA R: TTGTGCGTTTGTCTAGGAGTGAAG	(CA) ₈	117	20	0.74	0.60

Table 1 Continued

Locus	GenBank access. no.	Primer sequence (5'-3')	Repeat motif (cloned allele)	Clone size (bp)	<i>P. mac.</i>		
					<i>n</i>	H_E	H_O
<i>CypG12</i>	AY439130	F: GAAATTGAATGTAAGGAAGCAGAA R: TGGAATTGGCAACATAAGGA	(CAGA) ₆	165			
<i>CypG13</i>	AY439131	F: ACACCCAGTTCTCTGATGGA R: CATTTATTTTCATACTGCACTACACA	(TAGA) ₁₀	172			
<i>CypG14</i>	AY439132	F: CTGTGTGACAGAAGACCTG R: ATCTGTGGGATTTGAGGGTTTAT	(CAGA) ₁₀	161	17	0.71	0.71
<i>CypG15</i>	AY439133	F: CTTTGTTTTATCGAATGGATTTATCA R: TCCTGCCCTGCTTGTPTTTC	*2.	217			
<i>CypG16</i>	AY439134	F: GCTGCCACTTGCCTCTACCT R: TCATTTGGGACCCTGTGTTC	(CT) ₁₁	96	20	0.67	0.60
<i>CypG17</i>	AY439135	F: CTTTCCTTTCTTCCCTGTCTCA R: GGATAGCGCAGTGTAAATAAAATAA	(TTCTG) ₅ (TCCTG) ₄	135			
<i>CypG18</i>	AY439136	F: TATCTGAGAGTATCGAAGCGTAAT R: TCTGTTTTTGACAACCGTCTTATT	(GT) ₂ AT(GT) ₅	136			
<i>CypG19</i>	AY439137	F: GCGGATGTGTTGGCTTGAA R: GTGTGCAGCGGTGAGTCC	(CT) ₄ CAGA(CA) ₃	151			
<i>CypG21</i>	AY439139	F: ATTTGAGGGTTTATAGACCTTACA R: CAAGCCTGTTGTGATGAGAAG	(CAGA) ₈ (TAGA) ₂₄	241			
<i>CypG22</i>	AY439140	F: AGTCAGTCAGTCCACAAGGTATCT R: TTGGTGGGAATTATTAACAGAGTTC	*3.	252			
<i>CypG23</i>	AY439141	F: AACAGACGGGTAGACGG R: TGTTGCATGCCCTACAGATTG	(CAGA) ₇	113	20	0.90	0.87
<i>CypG24</i>	AY439142	F: CTGCCGCATCAGAGATAAACACTT R: TGGCGGTAAGGGTAGACCAC	(CAGA) ₁₉	196	21	0.32	0.38
<i>CypG25</i>	AY439143	F: AAACCTTCAGTCCCTCCTT R: AGCTTAATGCCCTGGTATGG	(CA) ₄ (CAGA) ₆	122			—
<i>CypG26</i>	AY439144	F: TTGGTTAGACACGATTGATGT R: TCCAAGTCTAAAGTATACAACAGTC	*4.	244			
<i>CypG27</i>	AY439145	F: AAGGTATTCTCCAGCATTTTAT R: GAGCCACCTGGAGACATTACT	(TAGA) ₈	225			
<i>CypG28</i>	AY439146	F: AGGAACTAGTCATTTATCTCAC R: CACACCTATGCGTTCCTTACTAC	(TAGA) ₈	182	21	0.53	0.38
<i>CypG29</i>	AY439147	F: CCTAACCTCAGGCTGCTACTG R: CAAAGACTTGATTGAACAAAGACTC	(TAGA) ₂₃ (CAGA) ₉	321			
<i>CypG30</i>	AY439148	F: GAAAAACCCTGAGAAATTCAAAAGA R: GGACAGGTAAATGGATGAGGAGATA	(TAGA) ₇	210			
<i>CypG31</i>	AY439149	F: GACATGGTGTTCGACAAGATT R: GGGTTACCCTATTTGATTACAGC	(TAGA) ₇	116			
<i>CypG32</i>	AY439150	F: CAAATACGATCTGCTCTGTCT R: TGTCTAAAGTGTACCATTGAAG	(TAGA) ₁₆	164			
<i>CypG33</i>	AY439151	F: TATGAGCTTTGGAAAGAGACACTG R: AATAGCCGGGAAATTTATCAATAGA	(CTGT) ₆	102	21	0.61	0.71
<i>CypG34</i>	AY439152	F: GCCCGTTGCAAGACACCT R: AACAAATAAATGGCAAGTAACACA	(CAGA) ₇	164	19	0.61	0.58

Table 1 Continued

Locus	GenBank access. no.	Primer sequence (5'–3')	Repeat motif (cloned allele)	Clone size (bp)	<i>P. mac.</i>		
					<i>n</i>	H_E	H_O
<i>CypG35</i>	AY439153	F: CTCGGCACTGCACTTACTCG R: TTAGCTTTGTGCATGTTCTTTCTC	(CAGA) ₅	205	21	0.38	0.38
<i>CypG36</i>	AY439154	F: GCATGTTGCTAAGGTTTCAAAATACA R: TCACGCTCTATCCTTATCAGGTAGG	(CAGA) ₆ (TAGA) ₁₃	271			
<i>CypG37</i>	AY439155	F: AAGGGTGTCTGACTAAAAGAG R: AGCAACATCATACACCACCATA	(TAGA) ₂₈	199			
<i>CypG38</i>	AY439156	F: ATCGGACTAACGGTATGC R: AGGCTCGAAATTGGTCTAT	(CA) ₂ (CAGA) ₈	99			
<i>CypG39</i>	AY439157	F: TTACTCGTGCCCGTAGCA R: TCAAATGCACATGATTGTCTT	(CAGA) ₈	166	19	0.56	0.63
<i>CypG40</i>	AY439158	F: TGCCAGACAATTCATAACAGT R: TGAGAGGGGAAGAGACACATTT	(TCTA) ₄ (TCTG) ₅	154	20	0.85	0.85
<i>CypG41</i>	AY439159	F: GGAAGTGAAGGAGGGGAGGATG R: TTGTCCATATTGCACAGCCCTAAT	(TAGA) ₈	166			
<i>CypG42</i>	AY439160	F: GGATGGGTGGATGGATGGATTAT R: TAGTTAACTACTCCCCAACACTGA	(CTGT) ₇	115	20	0.68	1.00
<i>CypG43</i>	AY439161	F: CATTGACACATTGGCTTTGA R: AATTTTGTAGTTGTATCCCTTTAT	(TAGA) ₉	151	17	0.89	0.77
<i>CypG44</i>	AY439162	F: TGCCCGTAACAAGACACC R: TTTTACATCATCGAAGGACAT	(CAGA) ₁₀	174	20	0.14	0.15
<i>CypG45</i>	AY439163	F: GCGGATAAGCCCAGAGT R: CATGATGACCACAGGAAGTAA	(CAGA) ₆	129	22	0.63	0.59
<i>CypG46</i>	AY439164	F: CTATAGAAAGAAGTCGTCAGAAAC R: AGGGAAGAAATTAACCACAG	*5.	184	17	0.79	0.53
<i>CypG47</i>	AY439165	F: GCTATAATCGCCGCTTCC R: FATGTGCCATATGCTAAATCTCT	(CAGA) ₈	160			—
<i>CypG48</i>	AY439166	F: GTGCTCATGGACAAACTGTA R: CGCACGAGGCACCCACTA	*6.	190	21	0.85	0.86
<i>CypG49</i>	AY439167	F: ATGGAACAACGCTCTAATGACAAA R: CTGCTACTGGAGAATGACTACTG	(CAGA) ₆ (TAGA) ₉	122	20	0.47	0.45
<i>CypG50</i>	AY439168	F: CATTTTGTAGGTGATAATTGACTGT R: AAAAAAGTGCTAAACAAAATGTCT	*7.	153	16	0.84	0.31
<i>CypG51</i>	AY439169	F: TCGGTAAAATCTACCTCTGA R: AATGGGTGGTTTCTGGTTTCAT	(CAGA) ₂ (TAGA) ₁₃	160			
<i>CypG52</i>	AY439170	F: CTAGTAGTGTACAGGAAGTTG R: ATCGCATCATTACCACAT	(TAGA) ₅ (CAGA) ₆	118	20	0.23	0.15
<i>CypG53</i>	AY439171	F: CTGTTATAGCCTAGAAAGTCTGAC R: ACATAGGCTACAAAATATCTGTTT	(TAGA) ₁₈	170	20	0.90	1.00

*1. (CAGA)₂(CA)₂(CAGA)₃(CA)₂(CAGA)₃; *2. (TAGA)₃(CAGA)₂CA(CAGA)₆; *3. (CAGA)₆(TAGA)₂(CAGA)₆(TAGA)₃(CAGA)₇(TAGA)₂; *4. (CAGA)₂(TAGA)₂TGA(CAGA)₂TAGA(CAGA)₂CAAA(CAGA)₅(TAGA)₃; *5. (TAGA)₆(CAGA)₆(TAGA)₁₀; *6. (TAGA)₈TACGG(TAGA)₁₀; *7. (CAGA)₅(TAGA)₅CAGA(TAGA)₈.

P. mac. *n* refers to the number of splittail individuals genotyped for calculation of expected and observed heterozygosities (H_E and H_O , respectively). The H_E and H_O values of *CypG50* are offset in bold and only this locus significantly diverged from Hardy–Weinberg expectations in splittail (P -value < 0.002).

Table 2 Cross-species amplification results (number of alleles per locus with size range (base pairs) in parentheses) of 51* microsatellite loci for the fish family Cyprinidae based on the analysis of five species: *Pogonichthys macrolepidotus* ($n = 16$ – 22); *Ptychocheilus grandis* ($n = 6$); *Siphateles bicolor* spp. ($n = 4$); *Lavinia exilicauda* ($n = 6$); *Orthodon macrolepidotus* ($n = 6$)

Locus	Sacramento splittail (<i>Pogonichthys macrolepidotus</i>)	Sacramento pikeminnow (<i>Ptychocheilus grandis</i>)	Tui chub (<i>Siphateles bicolor</i> spp.)	Hitch (<i>Orthodon macrolepidotus</i>)	Sacramento blackfish (<i>Lavinia exilicauda</i>)
<i>CypG1</i>	2 (215–230)	0	0	0	0
<i>CypG2</i>	3 (135–155)	3 (215–220)	3 (180–195)	—	Mono (220)
<i>CypG3</i>	7 (175–220)	5 (225–300)	5 (175–255)	10 (170–330)	4 (180–210)
<i>CypG4</i>	8 (130–165)	0	0	0	0
<i>CypG5</i>	0	3 (135–160)	4 (165–240)	9 (185–240)	5 (150–220)
<i>CypG6</i>	0	4 (180–200)	—	9 (200–240)	0
<i>CypG8</i>	3 (150–160)	0	Mono (135)	4 (210–250)	—
<i>CypG9</i>	Mono (105)	Mono (110)	2 (110–120)	3 (100–110)	2 (105–110)
<i>CypG10</i>	0	4 (125–150)	4 (140–160)	—	Mono (120)
<i>CypG11</i>	7 (115–130)	0	0	Mono (80)	0
<i>CypG12</i>	Mono (170)	Mono (130)	Mono (130)	5 (130–160)	Mono (160)
<i>CypG13</i>	0	4 (175–215)	3 (150–220)	8 (180–310)	0
<i>CypG14</i>	6 (145–165)	0	0	—	9 (200–300)
<i>CypG15</i>	Mono (220)	3 (160–215)	0	3 (250–265)	3 (500–540)
<i>CypG16</i>	6 (90–120)	Mono (80)	Mono (85)	—	Mono (85)
<i>CypG17</i>	0	4 (120–140)	0	0	0
<i>CypG18</i>	—	2 (135–140)	—	7 (130–155)	0
<i>CypG19</i>	Mono (150)	Mono (150)	2 (145–155)	Mono (160)	Mono (155)
<i>CypG21</i>	0	5 (210–295)	0	4 (235–285)	9 (190–285)
<i>CypG22</i>	0	3 (240–255)	3 (220–250)	5 (225–310)	Mono (170)
<i>CypG23</i>	13 (150–240)	Mono (110)	—	2 (100–105)	0
<i>CypG24</i>	3 (195–215)	3 (170–230)	0	8 (170–320)	5 (220–330)
<i>CypG25</i>	—	0	3 (155–200)	—	0
<i>CypG26</i>	0	0	3 (255–310)	7 (230–380)	Mono (85)
<i>CypG27</i>	Mono (220)	3 (240–260)	0	8 (240–325)	5 (315–365)
<i>CypG28</i>	4 (170–185)	0	0	0	0
<i>CypG29</i>	0	5 (255–340)	—	5 (245–400)	Mono (80)
<i>CypG30</i>	0	5 (180–205)	2 (200–210)	7 (170–220)	3 (210–220)
<i>CypG31</i>	Mono (75)	3 (115–130)	Mono (130)	4 (120–155)	—
<i>CypG32</i>	Mono (180)	—	3 (160–210)	4 (260–340)	—
<i>CypG33</i>	4 (90–100)	Mono (110)	Mono (110)	Mono (220)	2 (170–210)
<i>CypG34</i>	4 (145–175)	0	0	—	0
<i>CypG35</i>	4 (190–215)	Mono (130)	Mono (130)	Mono (130)	Mono (130)
<i>CypG36</i>	—	7 (235–320)	0	5 (190–300)	9 (175–250)
<i>CypG37</i>	0	6 (140–175)	0	0	3 (190–335)
<i>CypG38</i>	0	2 (90–95)	2 (90–95)	0	0
<i>CypG39</i>	3 (155–165)	0	0	0	0
<i>CypG40</i>	8 (220–260)	3 (500–550)	—	0	Mono (130)
<i>CypG41</i>	0	Mono (155)	2 (150–160)	0	0
<i>CypG42</i>	4 (100–115)	0	0	0	0
<i>CypG43</i>	8 (165–200)	0	0	0	0
<i>CypG44</i>	2 (160–175)	0	0	0	0
<i>CypG45</i>	3 (120–130)	Mono (120)	4 (125–140)	0	Mono (120)
<i>CypG46</i>	9 (185–255)	0	0	0	0
<i>CypG47</i>	Mono (160)	Mono (155)	4 (145–160)	Mono (220)	Mono (160)
<i>CypG48</i>	8 (160–190)	3 (160–175)	5 (130–190)	7 (200–280)	3 (210–235)
<i>CypG49</i>	3 (105–120)	2 (115–125)	0	6 (115–210)	6 (155–210)
<i>CypG50</i>	5 (145–165)	0	0	0	0
<i>CypG51</i>	0	5 (150–215)	3 (105–115)	8 (120–220)	3 (150–165)
<i>CypG52</i>	3 (115–125)	0	0	0	0
<i>CypG53</i>	12 (150–205)	0	0	0	3 (155–210)
Total no. of polymorphic loci	26	23	18	23	16

*Loci *CypG7* and *CypG20* were removed in the later stages of this study after loci were named. Mono = monomorphic; '—' indicates amplification but unclear; '0' indicates no amplification.

were polymorphic for two or more of the species, resulting in 16–28 polymorphic loci per species. The microsatellite loci presented here should prove useful for future genetic studies of the mentioned species in addition to other untested cyprinid species.

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