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PRIMER NOTE

Twenty-seven new microsatellites for the migratory Asian catfish family Pangasiidae

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Abstract

In this paper, we describe primers for 27 new microsatellite loci tested on five species of migratory Asian catfish: *Pangasius krempfi*, *P. bocourti*, *P. conchophilus*, *P. pleurotaenia*, and *Helicophagus waandersii*. These primers were developed from a $(GATA)_n$ library created from the pooled DNA of three species of pangasiid catfish. All 27 loci are polymorphic in at least one species. Fifteen loci are polymorphic in at least three species. The primers described in this paper are thus shown to be useful in several species within the catfish family Pangasiidae, and may prove useful in additional species in future tests.

Keywords: catfish, Mekong River, microsatellites, Pangasiidae, *Pangasius*, *Helicophagus*

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The Asian catfish family Pangasiidae comprises more than 20 species, including at least 12 species found in the Mekong River Basin, Southeast Asia (Pouyard *et al*. 2000). Within this region, pangasiid catfish are one of the dominant food fish, popular in aquaculture and frequently the basis of local wildcapture fisheries. Well known for large and sometimes spectacular migrations, pangasiid catfish move extensively between Vietnam, Cambodia, Laos, and Thailand (Lieng *et al*. 1995; Baird *et al*. 2001). Pangasiid catfish fisheries are probably the largest and most complex catfish fisheries in the world. Today, overfishing, dam building, and habitat degradation threaten several of these species (Dudgeon 1992; Hogan 1998).

Although DNA technology is an important tool in the management of many fisheries, the application of population genetic data to management in the developing countries of Southeast Asia is only now beginning. For this reason, almost no information exists about the genetic population structure or current levels of gene flow occurring within Mekong species. Here, we describe 27 new primer pairs designed to amplify microsatellite loci. These primers were developed for population genetic studies of several species of pangasiid catfish of the Mekong River Basin.

Two subgenomic libraries were constructed by Genomic Identification Services (Chatsworth, California, USA) using a mixture of equal masses of DNA of three species: *Pangasius larnaudei*, *P. conchophilus*, and *P. pleurotaenia*. DNA was extracted from dried fin using a standard TNES-urea procedure of Belfiore & May (2000), quantified and diluted, then mixed 1:1:1 in a buffer solution (final concentration 100 μg/100 μL in 25 mM Tris plus 2.5 mM EDTA, pH 8.0; 125 μL total). We mixed DNA of three species to minimize the cost of library construction per species and thus ultimately created a single multispecies library. We also suspected that pooling DNA would decrease the chance of sequencing identical clones during the screening process. Over 90% (115 of a total 126) of the clones from this multispecies library contained unique sequences, whereas single species libraries in our laboratory typically average about 60–70% unique sequences. Aside from the use of pooled DNA, the library construction followed the procedure described in (Belfiore & May 2000). Of the clones initially screened, 100% ($n = 5$) $(GATA)_n$ and 100% ($n = 7$) $(CA)_n$ contained microsatellites. In addition, approximately 300 recombinant clones from the $(GATA)_n$ library were sampled and amplified in 15 μL polymerase chain reactions (PCR) containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 5 mM $MgCl_2$, 2.5 mM dNTPs, 0.3 μL pUC forward and reverse sequencing primers, and 0.5 U *Taq* DNA polymerase (Gibco). The PCR reactions were amplified using an MJ Research PTC-100 96 V thermocycler according to the following themocycler profile: 94 °C for 4 min 30 s, 25 cycles of 94 °C for 30 s, 57 °C for 30 s, 72 °C for 30 s, and 72 °C for

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Table 1 Summary of data for 27 microsatellite loci for the Asian catfish family Pangasiidae based on the analysis of 2–3 individuals of five species; *Pangasius krempfi*, *P. conchophilus*, *P. bocourti*, *P. pleurotaenia*, and *Helicophagus waandersii*. The locus identification number is listed with the associated GenBank accession number, primer sequence, repeat motif, clone size, and product size range

Locus ID	GenBank accession no.	Primer sequence (5′–3′)	Repeat (cloned allele)	Product length (bp)	Product size range (bp)
PSP-G 404	AF378261	F: TTTTTAGAGGATCAACTTACACA R: TGTTCGTCTGCTTTATCACTC	$(GATA)_{14}$	148	100–154
PSP-G 412	AF378262	F: CTGCTTCAGAGTCTGCTCCTT R: CGATACACCTGATTTACCAAACC	$(GATA)_{10}$	140	170–320
PSP-G 415	AF378263	F: TCTAGTGGTAAGCAAGCAAGATTA R: CCCACATGTAACTGACAGAAACTC	$(GATA)_{20}$	160	150–220
PSP-G 427	AF378264	F: CATTGGATATCTATGTTTT R: TAATGTGGAGTATTTATGC	$(GATA)_{18}$	150	120–190
PSP-G 430	AF378265	F: TCCAAAAATCCCTTGAGG R: CTTTTCAGTCCATAGGAAGATAG	$(CAGA)_{13}(GATA)_{17}$	194	170–224
PSP-G 432	AF378266	F: TTCTATTATGTTCACTCGCTCGTT R: AGTGTGACCAGCCATGTTGTTATG	$(GATA)_{13}$	145	142–200
PSP-G 433	AF378267	F: AACTCTACAAGTATTGGCACTCA R: TGATGTTATAATATCTCGTCACTT	$(GATA)_{14}$	160	100–220
PSP-G 436	AF378268	F: GTCAAGAATCTACAGGTCAACAGA R: TGGGTTCCAATACTTGTAGAGTT	$(GATA)_{16}$	124	110–162
PSP-G 438	AF378269	F: AGTGTGATTTAATTGCAGTCAGAG R: AAATATTGCTCAGTTTACACA	$(GATA)_{17}$	132	110–150
PSP-G 456	AF378270	F: CTATCTGAGAGTTTTGACCTTGTG R: AGAAACCTTTTGGCTCTTTTA	$(GATA)_{28}(ATGG)_{9}$	202	90–130
PSP-G 475	AF378271	F: GATAACATTCCCTGGCTTAGC R: CGTTGTAGAAATGCAATGACA	$(GATA)_{10}$	97	80–140
PSP-G 502	AF378272	F: AGGGGCAGGAAAGATGGAGATAAG R: ACGCTTTGCAGTTTCTGAGTAACA	$(GATA)_{16}$	242	148–310
PSP-G 505	AF378273	F: AGGGGATGTTAAATGGCACGAGAG R: CAGCCCAGGAATGTGTCAACTACC	$(GATA)_{1}(TA)_{1}(GATA)_{10}$	162	150–212
PSP-G 507	AF378274	F: GTGGGAACACAAATGAGAATGTAA R: TGAAAAGCCTTTAATGTTGACACA	$(GATA)_{22}$	185	130–370
PSP-G 509	AF378275	F: TATGCTGAGGCAGTGGGAAACTCT R: CCTCGTACGGTAGGGTTGCTCATA	$(GATA)_{15}$	197	204–250
PSP-G 513	AF378276	F: CACTCTTTCTTTTCCCTCCGTTAT R: GTGAGTCGCCTTAAAAAGTGATGG	$(GATA)_{22}$	238	232–300
PSP-G 520	AF378277	F: AGCCATGGTGACATCATCATCATA R: AACAGACAACCAGATAGCCAGTCA	$(GATA)_{9}$	163	176–200
PSP-G 521	AF378278	F: CAGCGAGGATCTGCAAACATCAAC R: GCTGGACGCCATCTACTATGAAGG	$(GATA)_{14}$	166	120–180
PSP-G 546	AF378279	F: CAAAAACAAAAATGTCTGAACACT R: TAAAGGGACAAACAGGATGACAGA	$(GATA)_{17}$	145	90–224
PSP-G 550	AF378280	F: CAATGCCATTAATATTTCAGAGAA R: TTTTTACATGTGCCTGCCTATC	$(GATA)_{16}$	127	110–146
PSP-G 565	AF378281	F: ACCGGGTTTTAGAGTGACG R: GTGGGACAAAGGGAAAAGTG	$(GATA)_{15}(CTGT)_{2}(CCAT)_{1}(GATA)_{26}$	341	300–700
PSP-G 570	AF378282	F: TCCGCCACCCACAGTCAAATGGAT R: CCCGGGCTCCCACTGACAAGACA	$(GATA)_{7}$	209	212–260
PSP-G 573	AF378283	F: AACCCGTTAACTGCTAATCAACAG R: TTCCTTCAGTGTGGCAGTCGC	$(GATA)_{20}$	193	160–220
PSP-G 575	AF378284	F: TTGTGCAAAGTTCAAATA R: TATGTAAATACTGATGTAAATCTA	$(GATA)_{10}$	94	90–120
PSP-G 576	AF378285	F: AACTCCAAAATCCTCCCAGACTTT R: GACAATTTCATGAACACCCCTCTC	$(GATA)_{12}$	174	160–200
PSP-G 579	AF378286	F: GAGAGGGGGTGAAATAATGATAGG R: ATGGTTCTCCTGCAAGCAATGTCT	$(GATA)_{11}$	202	180–230
PSP-G 593	AF378287	F: ATTGTCTATTGCTGCTGGATACCA R: GATTTTTGCCTTTGTTCTCCTGAG	$(GATA)_{18}$	165	146–210

Table 2 Cross-species amplification of 27 microsatellite loci for the Asian catfish family Pangasiidae based on the analysis of five species; *Pangasius krempfi*, *P. bocourti*, *P. conchophilus*, *P. pleurotaenia*, and *Helicophagus waandersii*: species, sample size (*n*), and alleles per locus. The numbers in each cell indicates the number of observed alleles; '—' indicates amplification but unclear; '0' indicates no amplification or smear only

	Species				
Locus ID	*P. krempfi* (*n* = 3)	*P. bocourti* (*n* = 2)	*P. conchophilus* (*n* = 3)	*P. pleurotaenia* (*n* = 3)	*H. waandersii* (*n* = 3)
PSP-G 404	1	2	5	5	—
PSP-G 412	—	4	—	—	—
PSP-G 415	0	0	2	0	—
PSP-G 427	4	0	5	3	—
PSP-G 430	5	0	5	4	4
PSP-G 432	5	3	6	5	—
PSP-G 433	0	0	4	1	3
PSP-G 436	5	2	5	0	—
PSP-G 438	3	3	4	3	5
PSP-G 456	1	2	—	—	—
PSP-G 475	0	0	3	0	0
PSP-G 502	—	—	6	4	1
PSP-G 505	1	4	5	3	0
PSP-G 507	—	3	4	—	—
PSP-G 509	4	3	4	1	3
PSP-G 513	0	2	4	3	0
PSP-G 520	0	0	0	4	0
PSP-G 521	—	—	4	—	—
PSP-G 546	—	3	3	4	4
PSP-G 550	3	3	6	0	2
PSP-G 565	4	3	6	3	3
PSP-G 570	—	2	4	3	2
PSP-G 573	4	0	—	2	5
PSP-G 575	0	0	0	3	0
PSP-G 576	3	4	0	4	5
PSP-G 579	1	4	6	5	4
PSP-G 593	0	0	6	0	0
Total number of polymorphic loci	10	15	21	15	10

2 min. To identify inserts of 300–700 bp, 2 μL of PCR product was run on a 3% agarose gel with 0.03× GelStar nucleic acid stain. Colonies containing 300–700 bp inserts were used to inoculate 3 mL LB solution and incubated overnight at 37 °C. Plasmids were purified using a Qiagen Plasmid Miniprep Kit (#27106). Approximately 130 clones from the $(GATA)_n$ library were sequenced using the ABI Big Dye™ Terminator cycle sequencing protocol and visualized on an ABI 377 DNA sequencer (Applied Biosystems). No primers were developed for the $(CA)_n$ library.

Forty primer pairs were designed from approximately 100 distinct sequences using PRIMERSELECT software (DNAStar, Inc.). To identify polymorphic loci, genomic DNA of 2–3 individuals of each of five species was combined with 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.2 mM dNTPs, 1.25 mM $MgCl_2$, 0.5 μL primer, and 0.5 U *Taq* DNA polymerase (Gibco) in a 10-μL reaction. The microsatellite region was amplified using the following PCR conditions: 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 (MJ Research PTC-100 96 V thermocycler). PCR products were mixed 1:1 with 98% formamide loading dye, denatured for 3 min at 95 °C, cooled on ice, and run on a 5% denaturing acrylamide gel. Gels were stained using an agarose overlay containing 1.0 μL SYBR GreenI nucleic acid stain (BioWhittaker Molecular Application) as described in Belfiore & May (2000). Amplified products were visualized on a Molecular Dynamics Fluorimager 595. Primer details and summary data for new loci are reported in Table 1. Note that the data represent the sequenced clone only, and that each clone was derived from only one of three possible pangasiid species (whose identity is unknown). All primer pairs were tested on 2–3 individuals of five species of catfish; *P. krempfi*, *P. conchophilus*, *P. bocourti*, *P. pleurotaenia*, and *Helicophagus waandersii* (Table 2). Every marker is polymorphic in at least one species. This demonstrates the utility of these loci in population genetic studies. In addition, 15

loci are polymorphic in three or more species. Thus, the primers described in this paper appear broadly applicable to many species of the catfish family Pangasiidae.

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