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TECHNICAL NOTE

Microsatellite markers isolated from the Mexican banded spring snail *Mexipyrgus churinceanus*

Lyndon M. Coghill · Johel Chaves-Campos · Flor E. Espinoza · Stacey L. Lance · Travis C. Glenn · Steven G. Johnson

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Abstract The first twelve novel unlinked polymorphic microsatellite DNA loci were isolated from the Mexican banded spring snail (*Mexipyrgus churinceanus*). Genetic variability of each locus was assessed using 25 individuals from a single population from Cuatro Ciénegas, Mexico. Mean observed heterozygosity varied from 0.14 to 0.92. The relatively high levels of detected polymorphism indicate that these markers will be useful for future population genetic studies of this Cuatro Ciénegas endemic species.

Keywords Cuatro Ciénegas · Mexipyrgus churinceanus · Microsatellites · PCR · Primers

The banded spring snail (*Mexipyrgus churinceanus*: Hydrobiidae) is an aquatic snail endemic to the pools and springs of the 200 square-km valley of Cuatro Ciénegas in Coahuila, México. This is one of the richest and most diverse ecosystems in North America but water development has substantially reduced groundwater-dependent ecosystem size and is placing at risk the species dependent upon this aquatic environment (Wolaver et al. 2008). To understand the effects of water loss on this and other endemic species it is critical to investigate the current population structure and genetic diversity of these organisms.

We collected banded spring snails from several pools and springs in Cuatro Ciénegas, Mexico (see Johnson 2005 for localities). Tissue samples were preserved in 100% ethanol and DNA was extracted using Qiagen DNEASY Plant kit (Qiagen). After extraction, DNA from a single individual collected in the Mojarral Este pool (26 °55.48 N, 102 °07.28 W) was serially enriched twice for microsatellites using 3 probe mixes (mix 2 = $(AG)_{12}$, $(TG)_{12}$, $(AAC)_6$, $(AAG)_8$, $(AAT)_{12}$, $(ACT)_{12}$, $(ATC)_8$; mix 3 = $(AAAC)_6$, $(AAAG)_6$, $(AATC)_6$, $(AATG)_6$, $(ACAG)_6$, $(ACCT)_6$, $(ACTC)_6$, $(ACTG)_6$; mix 4 = $(AAAT)_8$, $(AACT)_8$, $(AAGT)_8$, $(ACAT)_8$, $(AGAT)_8$) following Glenn and Schable (2005). Briefly, the DNA was digested with restriction enzyme *Rsa*I (New England Biolabs) and simultaneously ligated to double-stranded SuperSNX linkers (SuperSNX24 Forward 5′-GTTTAAGGCCTAGCTAGCAGCAGAATC and SuperSNX24 Reverse 5′-GATTCTGCTAGCTAGGCCTTAAACAAAA). Linker-ligated DNA was denatured and hybridized to biotinylated microsatellite oligonucleotide mixes, which were then captured on magnetic streptavidin beads (Dynal). Unhybridized DNA was washed away and the remaining DNA was eluted from the beads, amplified in polymerase chain reactions (PCR) using the forward SuperSNX24 as a primer, and cloned with TOPO-TA Cloning Kits (Invitrogen). Positive white clones with inserts were detected using the α-galactosidase gene. A total of 192 positive clones were isolated and the inserts were amplified with M13 forward and reverse primers. The plasmid inserts were sequenced using BigDye Terminators v3.1 (Applied Biosystems; AB) and an AB 3130*xl* capillary sequencer. Sequences from both strands were assembled and edited in Sequencher 4.1 (Genecodes) and exported to msatcommander 0.8.1 for microsatellite searching (Faircloth 2008). About 96 clones provided

L. M. Coghill · J. Chaves-Campos · F. E. Espinoza · S. G. Johnson (✉)
Department of Biological Sciences, University of New Orleans, 2000 Lakeshore Dr, New Orleans, LA 70148, USA
e-mail: sgjohnso@uno.edu

T. C. Glenn
Department of Environmental Health Science, University of Georgia, Athens, GA 30602, USA

S. L. Lance
Savannah River Ecology Laboratory, University of Georgia, Aiken, SC 29802, USA

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readable sequences and 82 contained unique microsatellite loci. Fifty-six PCR primers were designed using Oligo 6.67 (Molecular Biology Insights).

PCR conditions were optimized using a subset of individuals from a single pool located in the Pozas Azules area (26°49.83′N, 102°01.76′W). PCR amplifications were conducted in a volume of 25.0 μL using 40 ng whole genomic DNA, 2.5 μL of 10× PCR buffer (Qiagen), 0.2 mM dNTPs, 0.43 μM of each primer, and 1.0 units of *Taq* DNA polymerase (Qiagen). The thermal profile included an initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing temperature (Table 1) for 45 s, and extension at 72°C for 30 s, followed by a final extension at 72°C for 5 min. All loci were tested for polymorphism using high resolution Metaphor Agarose (Lonza). Twelve loci revealed polymorphism. We genotyped 22–25 individuals collected from the pool mentioned above using the same PCR conditions described previously; one of each of the primer pairs was labeled with a fluorescent dye. PCR products were resolved on an AB 3100 Genetic Analyzer and scored using the software GeneMapper version 3.7 (Applied Biosystems). More than half of the individuals were genotyped twice at every locus as a quality control measure.

We estimated observed and expected heterozygosity, and tested for deviations from Hardy–Weinberg equilibrium (H–W) using Arlequin 3.1 (Excoffier et al. 2005) after controlling for Type I error following Wigginton et al. (2005). We also calculated gametic phase disequilibria for all possible microsatellite pairs using Arlequin and GenePop 4.0.10 (Rousset 2008). We estimated potential occurrence of null alleles using the program Microchecker 2.2.3 (Brookfield 1996).

Characteristics of the 12 working primer pairs are given in Table 1. No significant linkage was detected among the 12 loci ($P > 0.16$, for each pair of loci). Allele numbers detected at each locus ranged from 4 to 23, with an average of 12 alleles per locus. Mean observed heterozygosity

Table 1 Characteristics of twelve polymorphic microsatellite loci isolated from *Mexipyrgus churinceanus*

Locus	PRIMERS 5′–3′	Repeat	Alleles (*n*)	bp	Tm	$MgCl_2$	*Ho*	*He*
Mc01	ACGCTTCAGGTCAGTGGTG ACACACCCACAAAGCACAG	$(CTTT)_5$	4 (24)	198–206	58	1	0.62	0.58
Mc05	GTGCTTCTTGTTTGTCGGTTG ACACATCATCGTAACGTTTCTCC	$(GAT)_{20}$	13 (25)	153–240	56	5	0.83	0.87
Mc06	AGCTCGGCCAAGGAAAGTC TGTGTGCTGCAGAATGTTG	$(GTTT)_7$	9 (25)	239–267	56	3	0.44*	0.77
Mc09	AACTTGTGTGTGTTGCGTG TGTATTGCTCCAATGAAGGGG	$(GTT)_{17}$	23 (25)	303–420	58	1	0.64*	0.92
Mc30	CAGGCTGAACTAAGAAGGCG GTGCACGGGATTCCAACTC	$(AAG)_{17}$	23 (25)	209–243	58	1	0.72*	0.96
Mc32	ACTGTTGACCTGTCATATTGATTG AGGCGGCATACAACTAAACTC	$(GAT)_9$	15 (22)	229–354	56	1	0.14*	0.88
Mc34	AAGCGGCAGAACTTCACAG ACCAACTAACCAGCCAACC	$(GTTT)_{12}$	4 (25)	208–250	58	1	0.76	0.63
Mc36	AAATGAGCGAGCGGTTTTG TCTTAACAGTCCTTGATTCTTGAC	$(ACG)_{12}$	12 (25)	294–331	56	1	0.88	0.88
Mc45	ACCGAGAACCACTGTAAGGG AGGCCACACTCCTACACAC	$(CTGT)_8$	6 (25)	174–184	58	1	0.84	0.76
Mc51	TGCAATTGGAATCTCTACCTGTC ACCACAGCGGGTGTAATAG	$(ATCT)_{17}$	13 (25)	282–347	56	1	0.92	0.92
Mc55	TCTTCCTGGCTTTCCCTCG TTGTCGTTTTGTGCGGTTG	$(GT)_{25}$	12 (25)	166–203	56	5	0.96	0.89
Mc60	TGAGGCTTCCGGTGTATCG AGGACGGAAGAAAGGACGG	$(CTTT)_{18}$	15 (25)	393–545	57	1	0.96	0.90

Number of alleles, number of genotyped individuals (*n*), allele range (bp), annealing temperature (Tm), mM of $MgCl_2$, observed (*Ho*) and expected heterozygosity (*He*) are provided for each locus. Sequences were deposited in GenBank under Accession nos GU292206–GU292214 and HM564392–HM564394

* Significant deviations from Hardy–Weinberg proportions

varied from 0.14 to 0.92. Four loci deviated significantly from H–W due to homozygote excess. Microchecker suggested the possible presence of null alleles at these four loci. Null allele frequencies (Mc06: 0.18, Mc09: 0.16, Mc30: 0.11 and Mc32: 0.39) were calculated using Brookfield Estimator 1 because all samples amplified at least one band (Brookfield 1996).

Overall, the high numbers of alleles per locus and high heterozygosity indicate the utility of these microsatellite loci to estimate genetic diversity, population genetic structure and gene flow, relatedness and parentage analysis. Even loci with null alleles can be used to estimate relatedness and relationship, as long as the presence of null alleles is accounted for by adjusting genotype frequencies according to the probability of the limited number of possible true genotypes (Wagner et al. 2006).

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