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Microsatellite markers for the threatened Bliss Rapids snail (*Taylorconcha serpenticola*) and cross-amplification in its congener, *T. insperata*

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Abstract

We developed and tested microsatellite markers to investigate population structure of a threatened North American freshwater gastropod, *Taylorconcha serpenticola*. Of the 21 primer pairs that were evaluated, 11 were readily optimized and scored, providing amplification of 12 loci that were screened for 820 specimens from 29 populations. The number of alleles across 11 of these polymorphic loci ranged from three to 20 and the observed heterozygosity varied from 0.0061 to 0.7561. All loci yielded suitable amplification products in the second species of *Taylorconcha* (*T. insperata*) and three proved to be diagnostic for these congeners, demonstrating that these markers are also useful for species identification studies.

Keywords: Bliss Rapids snail, cross-amplification, Hydrobiidae, microsatellites, *Taylorconcha insperata*, *Taylorconcha serpenticola*

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The Bliss Rapids snail, *Taylorconcha serpenticola*, is a small hydrobiid gastropod that lives in the Snake River and tributary springs in southern Idaho (Hershler *et al.* 1994). The snail was federally listed as threatened owing to its declining populations and threats posed by hydroelectric development, water withdrawal and diversions, water pollution and inadequate regulatory mechanisms (USFWS 1992). Here we describe the development of multiple microsatellite loci that may be used to investigate the population structure of this imperilled species.

DNA was isolated by homogenization of entire snails using a CTAB protocol (Bucklin 1992). Microsatellites were isolated by the Rocky Mountain Center of Conservation Genetics and Systematics (http://consgen.cr.usgs.gov) using the technique developed by St. John & Quinn (2005). About 10 μg of genomic DNA was digested with endonucleases *Csp*6I and *Xma*I to produce fragments ranging from 300 to 1200 base pairs. Double-stranded DNA linkers specifically designed for this application were added into the ends of the DNA fragments. The fragments were subsequently hybridized to an oligonucleotide probe that is bound to biotin containing the repeat sequence (CA) or a mixture of (AAC) (GCA) (CAT) and (GATA). The target fragments were captured using streptavidin magnetic beads. Captured fragments were amplified, cloned, and sequenced.

Eighteen clones were sequenced from the CA hybridization. Six contained recognizable microsatellites (33%), of which four had sufficient flanking sequence to design primers. Fifty-nine clones were sequenced from the mix probes (AAC, GCA, CAT, and GATA) with 26 of these containing repeats (44%) and 15 had adequate flanking sequence for primer design. Two clones contained two microsatellites each, thus a total of 21 primer pairs was designed using the software PRIMER 3 (Rozen & Skaletsky 2000).

Successful amplification and variability were assessed by typing seven individuals for each microsatellite locus. Polymerase chain reactions (PCRs) were performed using a M13-tailed forward primer as described by Boutin-Ganache *et al.* (2001). Each 12.5 μL reaction contained 2.5 μL of Invitrogen optimizer buffer D (17.5 mM $MgCl_2$, pH 8.5) (Invitrogen, Inc.), 1.25 μL of dNTPs (2.5 mM each), 0.042 μL M13-tailed forward primer (10 μM), 0.625 μL of M13 dye-labelled with Beckman Coulter dyes D2, D3, or D4 (Proligo) and reverse primer (10 μM), 0.0625 μL of *Taq* polymerase (0.31 U), 1.5 μL of template (*c.* 50–100 ng double-stranded

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Table 1 Characterization of microsatellite loci in the Bliss Rapids snail, *Taylorconcha serpenticola*. T_a, annealing temperature; N, number of individuals screened; H_O, H_E, observed and expected heterozygosities, respectively. M13 sequence 5′CACGACGTTGTAAAACGAC3′

Locus	GenBank Accession no.	Primer sequence	Repeat motif	T_a	N	No. of alleles	Size range	H_O	H_E	P
BRSCA4	EF660862	F: M13-GGGTGTGGTAAGCCAAGAAA R: TGAAAGAAACCATTACAGAAATGAC	$(CA)_8$	63	820	4	175–183	0.0268	0.0278	1.0000
BRSM3	EF660863	F: M13-ATGACCACTGCACTGGAACC R: CAATGGCTTTAGCCTTGGAA	$(GATA)_7(GA)_4(GATA)_{18}$	63	820	20	294–374	0.6902	0.8875	0.0000
BRSM4-1	EF660864	F: M13-GGTCAGGAGGACAACACTGC	$(TAG)_5$	64	820	3	262–268	0.0207	0.0706	0.0000
BRSM4-2		R: AACCTTCGGCATAGGAGACC				4	275–284	0.0314	0.0396	0.0018
BRSM6	EF660865	F: M13-AATCGCGACCTTTTGAACAC R: CACTACTACCACAACTACCACTACTGC	$(TAG)_6CAG(TAG)_4$	68	820	4	205–214	0.0061	0.0073	1.0000
BRSM16	EF660866	F: M13-ACGGGATTGATGGGAAATTA R: TTCCGACAACCCCACACTAT	$(AGAT)_4(CAT)_8$	63	820	5	277–289	0.2220	0.0341	0.0000
BRSM18	EF660867	F: M13-TCGGAATAGGCTGGACTACG R: GGCTGGCCTAGTCTGAAGTG	$(AGT)_{12}AT(AGT)_{10}$	66	820	13	270–318	0.4866	0.6590	0.0000
BRSM30	EF660868	F: M13-GCTGAGTGAACTGGGAGAGG R: GTCTTGCCACACGAGAAACA	$(AGT)_{12}$	66	820	8	367–385	0.2720	0.2775	0.6289
BRSM37	EF660869	F: M13-AGGGAGCCAGTGAAAGGACT R: GGGAGCTACTACCACCACCA	$(AGT)_{11}AAT(AGT)_5$ $AAT(AGT)_{15}$	68	820	16	104–193	0.6537	0.7472	0.0000
BRSM52	EF660870	F: M13-CCGAAATCCAATTCCTCCTT R: GCTGCTAATGATGACGACGA	$(CAT)_6$	66	820	1	104	—	—	—
BRSM56	EF660871	F: M13-CCCTCTTTCGGACTGCATTA R: GCACAGACAGAAACAACAGACC	$(GTCT)_6CTCTATGT(ATCT)_3$ $GGCT(GTCT)_4(ATCT)_{13}$	66	820	19	197–286	0.7561	0.8404	0.0003
BRSM57	EF660872	F: M13-TGTCCCTGGTCACGTCATAA R: CACCCATCCCAGAACAAGAA	$(CAT)_7TAT(CAT)_5$	66	820	7	260–287	0.0134	0.0146	1.0000

DNA) and 5.9 μL of sterile water. The thermal profile for the PCR consisted of an initial 2-min denaturation step at 94 °C, followed by 35 cycles of denature 1 min at 94 °C, anneal 1 min, extend 1 min at 72 °C, and a final extension step at 72 °C for 5 min. The PCR products were run on the CEQ8000 XL DNA Analysis System (Beckman Coulter). All loci were run with the S400 size standard (Beckman Coulter) and analysed using the Frag3 default method.

Eleven primer pairs were easily optimized and scored. Three or four alleles were observed for primer pair BRSM4 in some individuals, which suggests that BRSM4 can amplify two loci. These 12 loci were used to screen a larger data set of 820 individuals from 29 populations of the Bliss Rapids snail (Table 1). Observed and expected heterozygosities and deviations from Hardy–Weinberg equilibrium (HWE) were calculated using ARLEQUIN 3.01 (Excoffier *et al.* 2005). Linkage disequilibrium was tested using GENEPOP (Raymond & Rousset 1995). There were 22 significant departures from HWE ($P < 0.05$) among the 319 possible combinations of population and loci. Given the large number of combinations, it is possible that some departures were caused by chance. Following correction for the multiple simultaneous test (Bonferroni correction), six population/locus comparisons were significant, all of which were at the M3 locus. The test for linkage disequilibrium examined each pair of loci in each population for a total of 1585 possible comparisons. Following application of the Bonferroni correction, none were found to be significant.

Table 2 Characteristics of amplification products in *Taylorconcha insperata* using primers developed in *T. serpenticola*

Locus	T_a	N	No. of alleles	Size range
BRSCA4	63	38	2	181–183
BRSM3	63	38	26	319–443
BRSM4-1	64	38	1	268
BRSM4-2			2	275–278
BRSM6	68	38	1	205
BRSM16	63	38	2	277–283
BRSM18	66	38	2	395–398
BRSM30	66	38	7	356–374
BRSM37	68	38	8	182–203
BRSM52	66	38	1	107
BRSM56	66	38	4	185–193
BRSM57	63	38	2	266–269

The second species of *Taylorconcha*, *T. insperata*, which was recently described from populations in the Owyhee River and lower Snake River (Hershler *et al.* 2006), was also tested with samples treated and genotyped as for *T. serpenticola*. Results are presented in Table 2. All 12 loci could be amplified in this species. Three loci (M18, M52 and M56) are diagnostic for *T. serpenticola* and *T. insperata*. Our results show these microsatellite markers can be used for species identification and for population genetic studies.

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