Characterization of 13 polymorphic microsatellite loci in *Rimicaris hybisae*, a shrimp from deep-sea hydrothermal vents

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Abstract

Rimicaris hybisae is a deep-sea alvinocaridid shrimp found at hydrothermal vents along the Mid-Cayman Spreading Center. Eleven selectively neutral and unlinked polymorphic microsatellite loci were developed for this species and two additional loci were found to cross-amplify from a related species. Nine loci conformed to Hardy-Weinberg expectations. Seven loci cross-amplified with *Chorocaris* sp. 2, an alvinocaridid shrimp found at vents in the Southwestern Pacific. Microsatellite loci developed for *R. hybisae* are being deployed to study connectivity of populations along the Mid-Cayman Spreading Center.

Keywords

Mid-Cayman Spreading Center, hydrothermal vent, *Rimicaris hybisae*, *Chorocaris*, Alvinocarididae

Deep-sea hydrothermal vents are patchily distributed habitats that support large, endemic communities. Studying the fauna of these ecosystems is crucial to understanding how dispersal, isolation, connectivity and evolution function in deep-sea vent environments (e.g., Vrijenhoek et al. 1997). Vents located along the Mid-Cayman Spreading Center (MCSC) are geographically isolated from well-studied vents on the Mid-Atlantic Ridge (Connelly et al. 2012). Two vent fields were confirmed by exploration along the MCSC (Connelly et al. 2012): Von Damm (2300 m) and Beebe (4960 m). Population genetic studies using microsatellite markers can reveal recent connectivity among populations (e.g., Thaler et al. 2011, Teixeira et al. 2012a) and genetic relationships down to the kinship level between individuals of a targeted species (Plouviez et al. 2008) for populations associated with these relatively isolated vent systems.

Rimicaris hybisae (Nye et al. 2012), an alvinocaridid shrimp, is known only from the MCSC. *R. hybisae* occur in dense aggregations at both Von Damm and Beebe. This paper describes 13 microsatellite markers developed to detect *R. hybisae* population structure at MCSC sites.

Rimicaris hybisae genomic DNA was isolated from ethanol-preserved abdomen tissue by Chelex-Proteinase K extraction. Tissue was digested with 120 μg Proteinase K (Bioline: Taunton, MA) in 600 μl 10% Chelex-100 resin (Bio-Rad: Hercules, CA) overnight at 60^{o}C, heated to 100^{o}C for 15 min, and centrifuged at 10 000 rpm for 5 min. Genomic DNA was sent to the Institute for Genome Science and Policy at Duke University to be sequenced on a Roche 454 GS-FLX Titanium sequencer. The program msatcommander (v 0.8.2; Faircloth 2008) was used to locate di-, tri-, tetra-, penta- and

hexanucleotide repeat motifs in sequences that had significant flanking regions for primer development. Primer 3 (v 0.4.0; Rozen and Skaletsky 2000) was used to design primers around repeat motifs. A T3-tail was added to the end of all forward primers to allow fluorescent labeling of PCR products (Schuelke 2000).

Genomic DNA was amplified in 20 µl polymerase chain reactions (PCR) as follows: 2 µl template, 2 µ 10x PCR Buffer (200mM Tris, pH 8.8; 500mM KCl; 0.1% Triton X-100, 0.2 mg/ml BSA), 1.5 µl $MgCl_2$ (25 µM), 1 µl dNTPs (2.5 µM), 0.2 µl forward primer (10 µM), 0.8 µl reverse primer (10 µM), 0.8 µl 5'-FAM-labeled T3 primer (10 µM; Eurofins: Huntsville, AL), 0.2 µl Taq polymerase (1 unit, Bioline: Taunton, MA), and 11.5 µl nanopure water. Reactions were run under the following conditions: 94°C for 4 min; 25 cycles of 94°C for 30 s, T_A°C for 45 s (indicated in Table 1), 72°C for 45 s; 8 cycles of 94°C for 30 s, 53°C for 45 s, 72°C for 45 s; final extension of 72°C for 10 min.

PCR products were diluted 1:4 and 1 µl added to 0.05 µl LIZ500 size standard and 8.95 µl nanopure water for a total volume of 10 µl (MCLab, San Francisco, CA). Samples were denatured at 94°C for 10 min, then placed on ice for 5 min. Size-fragment analysis was performed on an ABI 3730xl DNA analyzer. Chromatograms were scored using the program Genemarker (v1.8, SoftGenetics LLC: State College, PA). Deviation from Hardy-Weinberg Equilibrium was calculated using HW-QuickCheck (Kalinowski 2006) and corrected for multiple tests using the sequential Bonferroni method (Rice 1989). Presence of null alleles, stutter, and large allele dropout were assessed using Micro-Checker (1,000 randomizations; van Oosterhout et al. 2004). The software

LOSITAN (default settings; Antao et al. 2008; Beaumont and Nichols 1996) was used to detect loci potentially under selection.

Primers developed for other alvinocaridid species were tested for cross-amplification (Zelnio et al. 2010, Teixeira et al. 2012b) following the PCR conditions indicated by the authors.

Thirty-one primer pairs were screened for robust amplification using DNA from ten *Rimicaris hybisae*. Eleven loci were polymorphic and reproducible from the species-specific primers, and two loci developed for *Chorocaris* spp. (*Cho36, Cho83*) were polymorphic and reproducible when cross-amplified with *R. hybisae*. Six primers designed for *Chorocaris* spp. from Zelnio et al. (2010) and six primers designed for *Rimicaris exoculata* (Teixeira et al. 2012b) did not cross-amplify with *Rimicaris hybisae*.

Thirteen loci were assessed on a minimum of 26 *Rimicaris hybisae* from the MCSC. Six loci (*Rim2*, *Rim11, Rim13, Rim15, Rim18,* and *Rim23*) deviated significantly from Hardy-Weinberg Equilibrium (Table 1) but only four remained significant after Bonferroni correction (*Rim13, Rim15, Rim18, Rim23*; $k = 13$). Micro-Checker indicated the presence of null alleles at four loci (*Rim13, Rim15, Rim18,* and *Rim23*), and one locus showed evidence of stutter (*Rim13*). There was no evidence for linkage disequilibrium among loci, nor did LOSITAN indicate selection at any locus. The 11 *Rimicaris* primers were tested for cross-amplification on four *Chorocaris* sp. 2 individuals from the Southwestern Pacific. Seven loci cross-amplified in at least one *Chorocaris* individual and six of these loci were polymorphic (Table 1).

This is the first report of microsatellite markers developed for *Rimicaris hybisae*. These microsatellite markers will be deployed on MCSC populations to assess patterns of

91 population connectivity and gene flow at ecological and evolutionary scales; testing for
92 depth and for cohort differences as potential factors of genetic structure among and
93 within sites.
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Table 1 Polymorphic microsatellite loci from *Rimicaris hybisae* individuals

Locus	Repeat Motif	Primer Sequence (5'-3')	T_A (°C)	Allele Size Range (bp)	N	N_A	H_O	H_E	P	Cross Amplification with *Chorocaris*
Cho36	$(TACA)_6$	F: GCATCAATCCTGTGCACACGC R: GTAAAGGAGCCMCCCTYGAATC	60	109-175	76	4	0.45	0.52	0.16	n/a
Cho83	$(TAAA)_3$	F: GGCTACATGCAACCCAAGGG R: GGTATCATTTATATGGACGCC	46	148-266	58	6	0.48	0.50	0.39	n/a
Rim2	$(GTT)_{15}$	F: ATTAACCCTCACTAAAGGGAA R: ACAGACCAACATGCACAAGC	53.5	208-245	32	6	0.81	0.62	0.02	1/4
Rim6	$(TAAA)_{13}$	F: CCCCATTTTCCGGATTACTT R: CCAATGTTTGGGAGAAGTTCA	58.3	201-229	33	7	0.76	0.63	0.08	2/4
Rim9	$(TAA)_7$	F: ATTAACCCTCACTAAAGGGAT R: TTTAATTTGTATATGAGCGCTA	56	166-169	38	3	0.16	0.15	0.81	4/4
Rim11	$(TAA)_{25}$	F: ATTAACCCTCACTAAAGGGAT R: GTATCACTGCATGGCCTGTG	53.5	173-217	35	23	0.83	0.94	0.02	0/4
Rim13	$(CA)_8$	F: TCGAAACCATCATCCAATCA R: ATAATGGTGTCGTCCGTCCT	55	211-223	32	6	0.44	0.71	0.00*	4/4
Rim14	$(GA)_7$	F: GCCCGCGTTACTATGACAAC R: GGGGAAGGAGGAGGTAAGGT	46.3	306-399	33	3	0.39	0.33	0.44	4/4
Rim15	$(CA)_{22}$	F: AGGGAAGCGGTTGAATGTTA R: GCAGAACAACAAGGTCATCG	56	207-237	32	11	0.53	0.79	0.00*	2/4
Rim18	$(AC)_{18}$	F: GGACCCATCACCAAACAAAC R: GGGGAGCTAAGTGGGAGTGT	60.5	218-266	30	19	0.67	0.89	0.00*	0/4
Rim23	$(CA)_{12}$	F: AGGGGTAAGAAAAGGCGAAC R: GAAAGGAGTAAGGAAGAGAGG	55	162-220	26	11	0.46	0.74	0.00*	0/4
Rim25	$(GA)_9$	F: GCTGTGGAAGGGATGAAGAA R: GCTCACCCTCTCCCTTTACC	47.4	213-333	30	3	0.40	0.35	0.50	0/4
Rim26	$(TG)_9$	F: TGAGCTTTTACGTAATTCTCTTGG R: GCTCACTCCTCGCAACTACA	55	234-250	32	4	0.44	0.39	0.62	4/4

Report includes annealing temperature (T_A), size range of alleles (base pair length), number of observed alleles (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E), and probability of deviation from Hardy-Weinberg equilibrium (P). Number of *Chorocaris* sp. 2 phylotypes (out of 4) that showed successful cross-amplification for each primer set are reported (n/a indicates that the results are not applicable because the primers were designed specifically for *Chorocaris* sp. 2). Asterisk indicates significant deviation from Hardy-Weinberg Equilibrium after Bonferroni correction ($P < 0.05$). A star indicates evidence of polymorphism in cross-amplifications.

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