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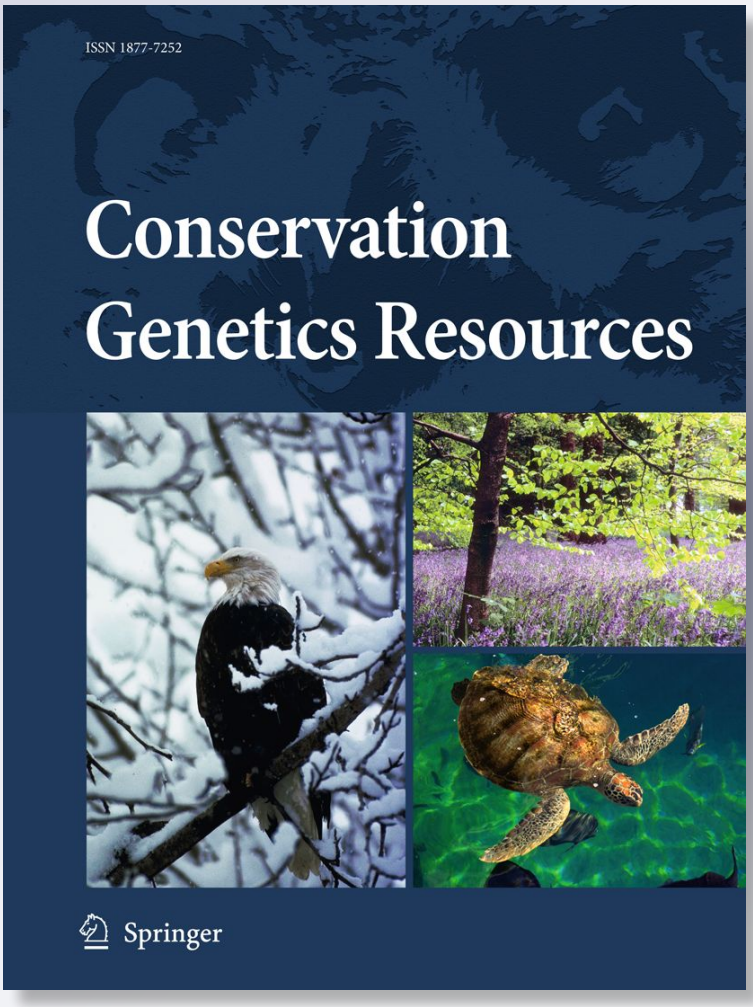

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TECHNICAL NOTE

Characterization of 15 polymorphic microsatellite loci in *Rimicaris exoculata*, and cross-amplification in other hydrothermal-vent shrimp

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Abstract *Rimicaris exoculata* is an alvinocarid shrimp endemic to the hydrothermal vents of the Mid-Atlantic Ridge. In order to study genetic variability and connectivity in this species, we developed fifteen polymorphic microsatellite markers. The markers were tested on one population and, except for one, all showed no departure from Hardy–Weinberg equilibrium, with an average overall observed heterozygosity of 0.63. Two primer pairs revealed possible linkage disequilibrium, and 14 cross-amplified at least one of the three co-occurring shrimp species tested (*Alvinocaris muricola*, *Alvinocaris markensis* and *Mirocaris fortunata*). These markers therefore open perspectives for population genetic studies of hydrothermal vent shrimp species in order to unravel connectivity and evolution of populations, and to add information on possible future impact studies.

Keywords Deep-sea hydrothermal vents · Alvinocarididae · *Rimicaris exoculata* · Shrimp · Microsatellites

Hydrothermal vents are particular ecosystems that support highly diverse communities which have developed in the absence of sunlight and photosynthesis, and in the presence of toxic chemicals such as hydrogen sulphide and methane (Van Dover 1995). These habitats are scattered worldwide along the global mid-ocean ridge system, on volcanic seamounts, and in back-arc spreading centres. Two characteristics of these specialized habitats, (1) spatially fragmented and (2) temporally ephemeral, pose a challenge to population persistence, and question the dispersal strategy that may better ensure subsistence of these vent communities. The remoteness of these habitats renders logistics associated to the study of these species very difficult, particularly the assessment of dispersal (Vrijenhoek 2010). To date most genetic studies of hydrothermal vent species focus on large scale phylogeographic patterns. Likely due to the high level of endemism of hydrothermal species, no studies have used highly variable markers such as microsatellites to infer the pattern of genetic structure and dispersal, despite recent efforts in developing markers for species with broader distributions, some in the framework of impact studies (Daguin and Jollivet 2005; Fusaro et al. 2008; Thaler et al. 2010; Zelnio et al. 2010).

In order to assess the genetic variability of *Rimicaris exoculata*, an endemic species of hydrothermal shrimp from the Mid-Atlantic Ridge (MAR) (Copley et al. 1997), we have developed and characterized polymorphic microsatellite loci for this species.

Total genomic DNA was extracted from muscle tissue free from symbiont bacteria of five *R. exoculata* individuals using the CTAB protocol (Doyle and Doyle 1990). An enriched library was constructed by ecogenics GmbH (Zurich, Switzerland) from size selected genomic DNA ligated into SNX forward/SNX reverse linker (Hamilton et al. 1999) and enriched by magnetic bead selection with biotin-labelled $(CT)_{13}$, $(GT)_{13}$, $(AAC)_{10}$ and $(AAG)_{10}$ oligonucleotide repeats (Gautschi et al. 2000a, b). Of 528 recombinant colonies screened, 352 gave a positive signal after hybridization. Plasmids from 129 positive clones were sequenced and primers were designed for 34 microsatellite

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Table 1 Characterization of the polymorphic microsatellite loci identified in *Rimicaris exoculata* (Genbank accession numbers JF745100 to JF745114)

Locus	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	Allele size range (bp)	*n*	H_E	H_O	Cross amplification (# positive/# total)
Rim 7	$(AAC)_8$…$(AAC)_8$…$(AAC)_{14}$	F: CGATAGCACTAAAGACGACAGC	57	195–241	10	0.793	0.737	*A. muricola* (3/4)
		R: ACAAGGCGGTAAATGACTGG						*A. markensis* (3/4)
								M. fortunata (0/4)
Rim9	$(CAA)_{10}(CAG)_{19}(CAA)_{14}TAA(CAA)_7$	F: CGCCCATGTAAAATTACGTCAC	58	185–281	23	0.938	0.895	*A. muricola* (0/4)
		R: TGCCCTGCTTATAGCTGGATAC						*A. markensis* (0/4)
								M. fortunata (0/4)
Rim10	$(AAC)_{10}$	F: ATGAAACGCACGTGAGACAG	57	156–183	9	0.637	0.564	*A. muricola* (0/4)
		R: CTCGTGTGTATGTTGTCGATG						*A. markensis* (1/4)
								M. fortunata (1/4)
Rim11	$(GTT)_8$	F: ACTTGCGGCCAATAAAACAG	56	212–242	11	0.872	0.806	*A. muricola* (0/4)
		R: CATTGCGTTGATGAATGTCG						*A. markensis* (0/4)
								M. fortunata (1/4)
Rim 12	$(GTT)_9(GCT)_2(GTT)_6(GCT)_{23}$	F: GCAGGAGACTGACCAGATTG	56	214–241	11	0.883	0.80	*A. muricola* (4/4)
		R: TGCGCCTTCGTATGTTACAC						*A. markensis* (4/4)
								M. fortunata (4/4)
Rim19	$(AGAA)_{10}$	F: CCGGAATCAGAGATACCTTAACC	56	179–195	5	0.514	0.556	*A. muricola* (0/4)
		R: TCGCTGCCAGCATTTATTC						*A. markensis* (0/4)
								M. fortunata (1/4)
Rim23	$(CAA)_9$	F: ACAAATACGCCTCCCACTTC	57	164–211	9	0.576	0.611	*A. muricola* (0/4)
		R: ATGGGCTACGGAACAAAGG						*A. markensis* (0/4)
								M. fortunata (1/4)
Rim25	$(TCC)_8$	F: CCCAACGGTCACAGAATATAAAG	56	137–149	5	0.674	0.684	*A. muricola* (4/4)
		R: AGCACCCCTGTCCTACACC						*A. markensis* (4/4)
								M. fortunata (1/4)
Rim26	$(TTG)_{16}GTG(TTG)_9A(TGT)_{17}$	F: ACGTCAGCGTCTTACCAACC	56	202–318	22	0.934	0.806	*A. muricola* (0/4)
		R: TCCAATCCGAGATTAATGATACG						*A. markensis* (0/4)
								M. fortunata (1/4)
Rim 28	$(GTT)_7$	F: GGGATTCGGTGGTTGTGAC	56	124–133	2	0.304	0.364	*A. muricola* (1/4)
		R: CCACATTGGTCTCAATTTTCC						*A. markensis* (0/4)
								M. fortunata (1/4)
Rim30	$(AAC)_7$…$(AAC)_5$	F: ACATCGGCATTCAGAAGCAC	57	186–270	14	0.845	0.842	*A. muricola* (4/4)
		R: CGTGTTCACATACAAAGTAAATGG						*A. markensis* (4/4)
								M. fortunata (2/4)

Table 1 continued

Locus	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	Allele size range (bp)	*n*	H_E	H_O	Cross amplification (# positive/# total)
Rim31	$(GAA)_8$	F: CATCACAGAGTCAAACATGACC	56	166–187	7	0.627	0.589	*A. muricola* (0/4)
		R: TGTCTGATCATCACTGCAACG						*A. markensis* (0/4)
								M. fortunata (1/4)
Rim32	$(GTT)_7$	F: TGGTAGTCAATGGTGGTGGAC	56	152–164	4	0.228	0.250	*A. muricola* (4/4)
		R: GCATTTGCTAACCGTGATCG						*A. markensis* (4/4)
								M. fortunata (4/4)
Rim 37	$(GTT)_9ATT(GTT)_4ATT(GTT)_4ATT(GTT)_{12}$	F: ATCTGCTCTGCAACCGTATG	58	177–273	13	0.914	0.529	*A. muricola* (0/4)
		R: CTTCCCACTGGTTGGCTAAG						*A. markensis* (0/4)
								M. fortunata (1/4)
Rim 49	$(CAA)_{14}$	F: AACAATAACATCATATTCAATAGCAAC	54	91–139	10	0.889	0.812	*A. muricola* (0/4)
		R: GTTTGGTCTTTGCGACATGC						*A. markensis* (0/4)
								M. fortunata (1/4)

The following details are reported: name, motif, primer sequence, annealing temperature (Ta°C) and cross-amplification on three other shrimp species, *Alvinocaris muricola*, *Alvinocaris markensis* and *Mirocaris fortunata*, based on four individuals from each species. Also descriptive statistics are presented, based on one population analysed, number of alleles *n*, and expected and observed heterozygosities, H_E and H_O

inserts, of which 19 were tested for polymorphism using 15 individuals from several locations sampled. Of these, 15 were polymorphic (Table 1). Sequences have been deposited in GenBank (accession no. JF745100 through JF745114).

Amplification reactions were prepared in a 10 μL volume containing 10 ng of genomic DNA, 1×Qiagen HotStart *Taq* buffer, 200 μM of dNTP's, 0.3 μM of each primer and 0.5 U of HotStart *Taq* polymerase (Qiagen). The PCR amplification was conducted in Perkin-Elmer Gene Amp System 7200 (Waltham, MA, USA) with the following program: 15 min at 95°C; 30 cycles composed of 30 s at the annealing temperature (Table 1), 30 s elongation at 72°C and 30 s of denaturation at 95°C, followed by 1 min at the annealing temperature and a final 30 min elongation step at 72°C. The PCR products were amplified with one primer of each primer pair end-labelled with a fluorescent dye, FAM, NED or HEX. Fragments were separated on an ABI 3130 XL automatic sequencer (Applied Biosystems, Foster City, CA, USA) with the internal size standard Rox 350. Alleles were scored using Peak Scanner version 1.0 (Applied Biosystems).

Variability of these markers was tested on 31 individuals from a Mid-Atlantic Ridge population (TAG). The number of alleles per locus (*n*), and the observed (H_O) and expected (H_E) heterozygosities were calculated with the program GENETIX, 4.05 (Belkhir et al. 1996–2004; Table 1). Most loci were highly polymorphic, with the number of alleles ranging from 2 (Rim28) to 23 (Rim9); H_O ranged from 0.25 to 0.90 and H_E from 0.23 to 0.94. No significant heterozygote deficiency was observed, except for the marker Rim 37. High frequency of null alleles is likely at this locus, as confirmed by further analysis using the MICRO-CHECKER software (van Oosterhout et al. 2004). We tested for linkage disequilibrium between all pairs of loci according to the Black and Krafsur (1985) procedure, the significance of the results were tested with 1,000 permutations, at the 5% level, two pairs were significant, Rim28–Rim12 ($P = 0.01$), and Rim 28–Rim49 ($P = 0.00$). These microsatellite loci should be useful for assessing genetic structure at different geographical scales, and due to their high variability they should also be useful for fine-scale genetics and paternity analyses of the hydrothermal shrimp *R. exoculata*.

All markers were tested for cross-amplification in three other hydrothermal shrimp species, *Alvinocaris muricola*, *Alvinocaris markensis*, and *Mirocaris fortunata* from the Mid-Atlantic Ridge (MAR), using four individuals from each species, of which several amplifications were positive (Table 1). These markers will be used to study genetic variability of hydrothermal shrimp on several populations along the MAR.

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