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PRIMER NOTE

Microsatellite loci for the New Zealand trematode *Maritrema novaezealandensis*

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Abstract

Seven polymorphic microsatellite loci were isolated and characterized from the coastal New Zealand trematode *Maritrema novaezealandensis*, an important driving force in the population dynamics of its snail and crustacean intermediate hosts. Observed heterozygosities ranged from 0.33 to 0.82, and three to 17 alleles were detected in 22 trematode sporocysts. These loci will be integral for identifying parasite genetic clones within hosts and addressing a range of questions concerning the molecular ecology of this species.

Keywords: enriched library, *Maritrema novaezealandensis*, microsatellite, trematode

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Digenetic trematodes are common inhabitants of marine, freshwater and terrestrial ecosystems. Despite their abundance and ecological importance, genetic markers appropriate for investigating the molecular ecology of trematodes have been developed for very few species. The marine trematode *Maritrema novaezealandensis* (Microphallidae) inhabits coastal New Zealand waters and is an intestinal parasite of the red-billed gull *Larus novaehollandiae scopulinus* (Martorelli *et al*. 2004). *Maritrema novaezealandensis* utilizes the snail *Zeacumantus subcarinatus* as a first intermediate host (sporocyst stage) and several crabs (*Hemigrapsus crenulatus*, *Macrophthalmus hirtipes* and *Halicarcinus whitei*) and amphipod (*Paracalliope novizealandiae*) species as second intermediate hosts (metacercaria stage). This trematode causes mortality in amphipod hosts (Fredensborg *et al*. 2004), and its castration of snail hosts is a key determinant of geographical variation in snail life-history traits (Fredensborg & Poulin 2006). Here we describe seven microsatellite loci developed for molecular ecological studies of *M. novaezealandensis*.

An initial *M. novaezealandensis* subgenomic library enriched for microsatellites was prepared and screened following Perrin & Roy (2000). All parasites and hosts were collected in Lower Portobello Bay, Otago Harbor, New Zealand. Total genomic *M. novaezealandensis* DNA was isolated from 150 sporocysts from a single *Z. subcarinatus* using the QIAGEN DNeasy Tissue kit. Genomic DNA was digested with the restriction enzyme *Mbo*I (Promega) and fragments spanning 400–1400 bp were ligated into a pUC19 cloning vector. DNA inserts were amplified with M13 primers, annealed to biotinylated $(GT)_{12}$ and $(GA)_{12}$ probes and microsatellite-containing DNA was selectively retained using streptavidin MagneSphere paramagnetic particles (Promega). Microsatellite-containing fragments were digested with *Mbo*I, ligated into a pUC 19 cloning vector and used to transform XL1-Blue competent cells (Stratagene). Microsatellite screening was performed as described in Perrin & Roy (2000). Positive clones were amplified and sequenced with M13 primers using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) on an ABI PRISM 3730 Genetic Analyser (Applied Biosystems). Polymerase chain reaction (PCR) primers were designed using the OLIGO software package (Molecular Biology Insights).

Few repeats longer than $(GA)_5$ or $(GT)_5$ were recovered with our first library and a subsequent subgenomic library was constructed using modified versions of Hamilton *et al*. (1999) and Glenn *et al*. (2000) as described in Heist *et al*. (2003) and Keeney & Heist (2003). Briefly, complementary linkers (linker-F: 5′-CTAAGGCCTTGATCGCAGAAGC-3′ and phosphorylated linker-R: 5′-p GCTTCTGCGATCAAGGCCTTAGAAAA-3′) were ligated to genomic DNA fragments, biotinylated $(GT)_{15}$ and $(GA)_{15}$ probes were hybridized to linker-ligated DNA and microsatellite-containing DNA was selectively retained using streptavidin MagneSphere paramagnetic particles (Promega). Retained fragments were amplified with PCR and digested with

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Table 1 Microsatellite loci developed for *Maritrema novaezealandensis*. Locus name is followed by repeat motif of cloned allele, primer sequences, primer annealing temperature (T_a), size range of alleles in base pairs (bp), number of alleles, sample size (n), observed (H_O) and expected (H_E) heterozygosities and GenBank Accession no. (Accession no.). *Significant deviation from Hardy–Weinberg equilibrium ($P < 0.001$)

Locus	Repeat motif	Primer sequences (5′–3′)	T_a (°C)	Range (bp)	# Alleles	n	H_O (H_E)	Accession no.
Mno-1	$(GTGA)_{30}$	F: CCGGTATACAAGAACACACC R: CCCTCTCTAACTGATTGATTC	58	124–240	14	22	0.82 (0.79)	DQ414412
Mno-2	$(GA)_{13}(GT)_4(TG)_5$	F: GGTCAGTTGCCCACTAATCG R: CCATGCTCAATGATAGTCGC	65	154–190	17	21	0.80 (0.93)	DQ414413
Mno-6	$(GTGA)_5$	F: TCAATGCCTTATGTGCCACCG R: GCCCAACCCAACTCTCAACG	66	73–109	5	15	0.33* (0.74)	DQ414414
Mno-28	$(GT)_9$	F: GATGGGTGCGAATATCACAG R: ATTACTCTGAACGGTACTCCG	58	99–113	8	22	0.77 (0.75)	DQ414415
Mno-30	$(GT)_9$	F: TGTTCTATTGCGTACAAGAC R: GTGGTTACAAGATGATACCC	62	54–62	5	22	0.68 (0.72)	DQ414416
Mno-45	$(AG)_7$	F: GATACTCTACGTGAGCCTCGC R: CGGATGAGGTGATAGAACGG	62	91–95	3	22	0.50 (0.51)	DQ414417
Mno-47	$(TG)_{10}$	F: CGTGGAACGAGCAGACATC R: GGGAGCATTCTAAGGGGAA	62	84–114	7	22	0.64 (0.66)	DQ414418

*Mbo*I prior to ligation and transformation (described above). Microsatellite screening was performed as described in Heist *et al*. (2003).

Successful primer amplification and optimal annealing temperatures were determined with DNA from individual *M. novaezealandensis* sporocysts isolated from 12 *Z. subcarinatus*. Individual sporocyst DNA was extracted in 400 μL of 5% chelex containing 0.1 mg/mL proteinase K, incubated at 60 °C for 2 h and boiled at 100 °C for 8 min. PCRs (10 μL) consisted of approximately 10 ng genomic DNA, 0.6 μM of each primer, 0.5 U *Taq* DNA polymerase (Bioline), 200 μM of each dNTP, 3 mM $MgCl_2$ and 1× *Taq* buffer [16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl, 0.01% Tween 20]. PCR amplification was performed on an Eppendorf Mastercycler gradient thermal cycler and consisted of 4 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at gradient of 46–66 °C and 45 s at 72 °C and a final extension for 4 min at 72 °C. PCR products were resolved on 9% nondenaturing polyacrylamide gels with a 10-bp DNA ladder. Successfully amplified loci were tested for polymorphism using optimal annealing temperatures with individual sporocyst DNA from 22 *Z. subcarinatus*. DNA extractions, PCR and allele resolution were performed as described for primer screening. Polymorphic loci were tested for deviations from Hardy–Weinberg equilibrium allele frequencies and all pairs of loci were tested for linkage disequilibrium using GDA 1.1 (Lewis & Zaykin 2001). Alpha significance levels were adjusted using the sequential Bonferroni approach (Rice 1989).

Two hundred and eighty-seven colonies were screened for microsatellites from the initial library and 58 colonies (20%) were positive for GA/GT repeat DNA. All 58 clones were sequenced, six primer pairs were designed and three polymorphic loci (Mno-1, Mno-2 and Mno-6) were developed. The majority of positive clones (> 90%) contained repeat motifs shorter than $(GA)_6$ or $(GT)_6$. Seven hundred and thirty colonies were screened for microsatellites from the second library and 254 colonies (35%) were positive for GA/GT repeat DNA. One hundred and twenty-one clones were sequenced, 26 primer pairs were designed and four polymorphic loci (Mno-28, Mno-30, Mno-45 and Mno-47) were developed. Loci possessed three to 17 alleles and observed heterozygosities of 0.33–0.82 (Table 1). One locus (Mno-6) deviated significantly from Hardy–Weinberg expectations with fewer heterozygotes than expected. Several sporocysts failed to amplify for this locus, possibly due to mutations in flanking sequence (null alleles). No locus pairs were in significant disequilibrium with or without Bonferroni adjustment of alpha. These markers are currently being employed to investigate transmission strategies and within-host interactions among parasite clones.

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