Conservation Genet Resour
DOI 10.1007/s12686-012-9656-z

TECHNICAL NOTE

Rapid isolation of 14 microsatellite markers for Van Diemen's siphon limpet *Siphonaria diemenensis*

Jonathan Sandoval-Castillo · Michael G. Gardner · Luciano B. Beheregaray

Received: 1 April 2012 / Accepted: 14 April 2012

Abstract Siphon limpets are an ecologically important component of the intertidal zone of Australia's temperate coast. Using 1/16 of a shotgun pyrosequencing plate, a set of 14 microsatellites was developed for the Van Diemen's siphon limpet (*Siphonaria diemenensis*). From 2,744 sequences that contained putative microsatellite motifs, 18 were selected for primer design and tested for amplification. Fourteen microsatellite loci were successfully genotyped in 48 specimens of *S. diemenensis* from two localities. The number of alleles per locus varied from 5 to 35 (mean = 14.6) and the observed heterozygosity ranged from 0.53 to 0.90 (mean = 0.77). Only one locus showed significant deviation from Hardy–Weinberg equilibrium, probably due to null alleles. No linkage disequilibrium between pairs of loci was detected. Connectivity and seascape genetic studies on siphon limpets using the markers reported here should provide important information for management of temperate Australian marine biodiversity.

Keywords Seascape genetics · Marine connectivity · Population genetics · MPA · Mollusc · Phylogeography

J. Sandoval-Castillo · M. G. Gardner · L. B. Beheregaray (✉)
Molecular Ecology Laboratory, School of Biological Sciences, Flinders University, Adelaide, SA 5001, Australia
e-mail: Luciano.Beheregaray@flinders.edu.au

M. G. Gardner
School of Biological Sciences, Flinders University, Adelaide, SA 5001, Australia

M. G. Gardner
South Australian Regional Facility for Molecular Ecology and Evolution, North Tce, Adelaide, SA 5000, Australia

Marine invertebrates provide useful systems for study gene flow since they allow comparisons between co-distributed species with different dispersal capabilities (Hart and Marko 2010). Van Diemen's siphon limpet (*Siphonaria diemenensis*) is a marine intertidal mollusc distributed from central New South Wales to southern Western Australia, including Tasmania (Shepherd and Thomas 1989). *Siphonaria diemenensis* has a planktonic larvae period that has not been directly determined, however circumstantial evidence suggests a large potential planktonic dispersal (Mapstone 1978; Johnson and Black 1984). Van Diemen's limpet's wide distribution and potential long dispersal capability make it an attractive model to evaluate factors that could affect connectivity among populations along the temperate coast of Australia. This information is potentially relevant for the design of an effective system of marine protected areas (MPAs) in the region.

Microsatellite loci for Van Diemen's siphon limpet were obtained using a next generation DNA sequencing (NGS) technology following Gardner et al. (2011). A total of 10 μg of genomic DNA was extracted from muscle tissue of a siphon limpet specimen and sent to the Australian Genome Research Facility (www.agrf.com.au) for DNA sequencing. Sequencing was conducted on 1/16 of a PicoTiter plate using the Roche GS FLX (454) system (Margulies et al. 2005). Using default settings in MSATCOMMANDER v. 0.8.1 (Faircloth 2008) all contigs were screen for microsatellites. MICROFAMILY v. 1.2 (Meglécz 2007) and PRIMER 3 (Rozen and Skaletsky 1999) were used to select unique contigs and design primer sets as described in Carvalho and Beheregaray (2011).

A total of 58,395 reads were obtained, from which 2,744 sequences contained putative microsatellite motifs. MICROFAMILY recovered 220 unique loci, from which the best 18 loci were chosen for polymerase chain reaction

Published online: 28 April 2012

(PCR) trials. Amplifications and PCR conditions followed Beheregaray et al. (2004) using a 63–55 °C touchdown. From initial PCR optimizations with the 18 markers, 14 amplified consistently and were selected for further analyses. For the microsatellite loci characterization, 24 individuals from Peak Bay, South Australia (34°29′12″S, 136°04′02″E) and 24 individuals from Bridport, Tasmania (40°59′09″S, 147°23′32″) were genotyped.

The PCRs were performed separately, products were combined in three runs namely PlexA, PlexB and PlexC (Table 1), and amplified peaks were detected on an ABI 3130 Sequencer (Applied Biosystems). The resulting profiles were examined to determine alleles using GENEMAPPER 4.0 (Applied Biosystems). Scoring errors and null alleles in the genotypes were assessed using MICROCHECKER (Van Oosterhout et al. 2004). Number of alleles (N_A), expected (H_E) and observed (H_O) heterozygosity, linkage disequilibrium and Hardy–Weinberg proportions were estimated using GENEPOP V4 (Rousset 2008). Sequential Bonferroni corrections were applied when conducting multiple statistical tests (Rice 1989).

Table 1 Characteristics of the 14 microsatellite loci isolated from *Siphonaria diemenensis*

Locus	Primer sequences	Motif	Size (bp)	M13 label	NA	H_E/H_O	H–W (*P*)	F_{IS}
PlexA								
Side16	F: AGGGGAATACCATTATCAC	(TTTC)15	268–472	6FAM	35	0.967/0.917	0.553	0.053
	R: ATAGGCATAGGAACAAAAAG							
Side15	F: GTGTTTGTTTTTACACTTGC	(TA)9	183–207	VIC	11	0.892/0.833	0.368	0.067
	R: ACAGTTAATAGTGATGCCC							
Side07	F: AAATAAGAAACAACAGGACG	(AG)10	296–320	VIC	11	0.778/0.684	0.046[b]	0.124
	R: CAGATCAACCGATAAAATG							
Side13	F: GAACATGGTACAGATTATGG	(CAA)10	165–192	PET	9	0.769/0.708	0.168	0.08
	R: TACAAGCTGAAGTAAGATGG							
Side19	F: GGTCAATGTCTGTAAAAAGG	(TAA)13	266–293	PET	10	0.658/0.542	0.099	0.079
	R: GTGAGGTAAAAACTATGTGG							
PlexB								
Side12	F: TAGTCAAAACCAATAACAGG	(AT)9	268–276	6FAM	5	0.645/0.625	0.339	0.152
	R: GATGTCTGTACCAAAAATGC							
Side05	F: GAATTGAAGGTGTTAGTTCC	(GA)11	319–341	VIC	10	0.881/0.750	0.182	0.043
	R: GATAAGAGTAGGCCAGAATG							
Side17	F: CTTTGTTCCACTCACTCC	(CAAA)10	208–264	NED	14	0.867/0.583	**0.002**[a]	0.332
	R: CTTCATGTCTGTGTATTTGC							
Side03	F: ATACTTCGAGTCCACAAAAG	(GTTT)14	245–313	PET	15	0.896/0.917	0.478	0.023
	R: TTTCGGTCAGTACCTTTATC							
PlexC								
Side20	F: AATATACACACGGGACCTAC	(GA)14	204–256	6FAM	26	0.947/0.870	0.098	0.083
	R: TTGTCCAAAATCACGAAC							
Side01	F: CAATATGCTGAGTGTGACG	(TGTT)11	385–441	6FAM	14	0.821/0.739	0.181	0.102
	R: CTTCTCTTCCTACATAAAAACC							
Side10	F: TCTGAGGCGTAATCTTTAAC	(AAAC)15	200–244	VIC	12	0.900/0.870	0.053	0.034
	R: TCGTATTTATTGTTTCTCCC							
Side04	F: TTTGATTTCAGATACACGTC	(GTT)11	284–335	PET	16	0.914/0.917	0.526	0.003
	R: GCAGACAAAATAAGAAAGG							
Side09	F: ATCTCGTTGGTTTATATTGG	(GACA)17	313–401	NED	17	0.934/0.917	0.482	0.019
	R: GGCTGATGATTTTTATTGAC							

Number of alleles (N_A) and range of allelic size were based on 48 specimens

Hardy–Weinberg *P* values (H–W), observed (H_O) and expected (H_E) heterozygosity, and inbreeding index (F_{IS}) are based on 24 specimens from Bridport, Tasmania

[a] Significant after Bonferroni correction

[b] Not significant after Bonferroni correction. Forward primers were tagged with a 5′M13 universal sequence (5′TGTAAAACGACGGCC) and labelled using four different fluorescence labels

All 14 loci were polymorphic, with an average of 14.6 alleles per locus (ranging from 5 to 35 alleles) and H_O ranging from 0.46 to 0.92 (mean = 0.77). After sequential Bonferroni correction, only Side17 showed a possibility of null alleles. No deviations from Hardy–Weinberg equilibrium were detected, except for Side17, and there was no evidence of linkage disequilibrium between pairs of loci. We tested cross-amplification for all the loci in the congeneric *S. denticulata* using eight samples from New South Wales, Australia. Amplification succeeds for nine loci and polymorphism was detected on all of them. Connectivity and seascape genetic studies on Australian siphon limpets using the microsatellite markers reported here would provide valuable information for the design of MPAs in temperate waters of Australia.

Acknowledgments Funding for this study was provided by the Australian Research Council (DP110101275 to Beheregaray, Möller & Waters). We thank Minami Sasaki and Seraina Bracamonte for laboratory assistance. This article is publication #46 of the Molecular Ecology Group for Marine Research (MEGMAR).

References

Beheregaray LB, Moller LM, Schwartz T, Chao NL, Caccone A (2004) Microsatellite markers for the cardinal tetra *Paracheirodon axelrodi*, a commercially important fish from central Amazonia. Mol Ecol 4:330–332

Carvalho D, Beheregaray L (2011) Rapid development of microsatellites for the endangered Neotropical catfish *Conorhynchus conirostris* using a modest amount of 454 shot-gun pyrosequencing. Conserv Genet Resour 3:373–375

Faircloth BC (2008) msatcommander: detection of microsatellite repeat arrays and automated, locus-specific primer design. Mol Ecol Resour 8:92–94

Gardner MG, Fitch AJ, Bertozzi T, Lowe AJ (2011) Rise of the machines—recommendations for ecologists when using next generation sequencing for microsatellite development. Mol Ecol Resour 11:1093–1101

Hart MW, Marko PB (2010) It's about time: divergence, demography, and the evolution of developmental modes in marine invertebrates. Integr Comp Biol 50:643–661

Johnson MS, Black R (1984) The Wahlund effect and the geographical scale of variation in the intertidal limpet *Siphonaria* sp. Mar Biol 79:295–302

Mapstone GM (1978) Egg capsules and early development in *Siphonaria diemenensts* (Quoy and Gaimard, 1833) and *Siphonaria baconi* (Reeve, 1856). J Malacologic Soc Australia 4

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen Y-J, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Irzyk GP, Jando SC, Alenquer MLI, Jarvie TP, Jirage KB, Kim J-B, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, Wang SH, Wang Y, Weiner MP, Yu P, Begley RF, Rothberg JM (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380

Meglécz E (2007) MICROFAMILY (version 1): a computer program for detecting flanking-region similarities among different microsatellite loci. Mol Ecol Notes 7:18–20

Rice W (1989) Analyzing tables of statistical tests. Evolution 43: 223–225

Rousset F (2008) Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour 8:103–106

Rozen S, Skaletsky H (1999) Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 132:365–386

Shepherd SA, Thomas IM (1989) Marine invertebrates of Southern Australia. Part II (Mollusca). South Australian Government Printing Division, Adelaide

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538