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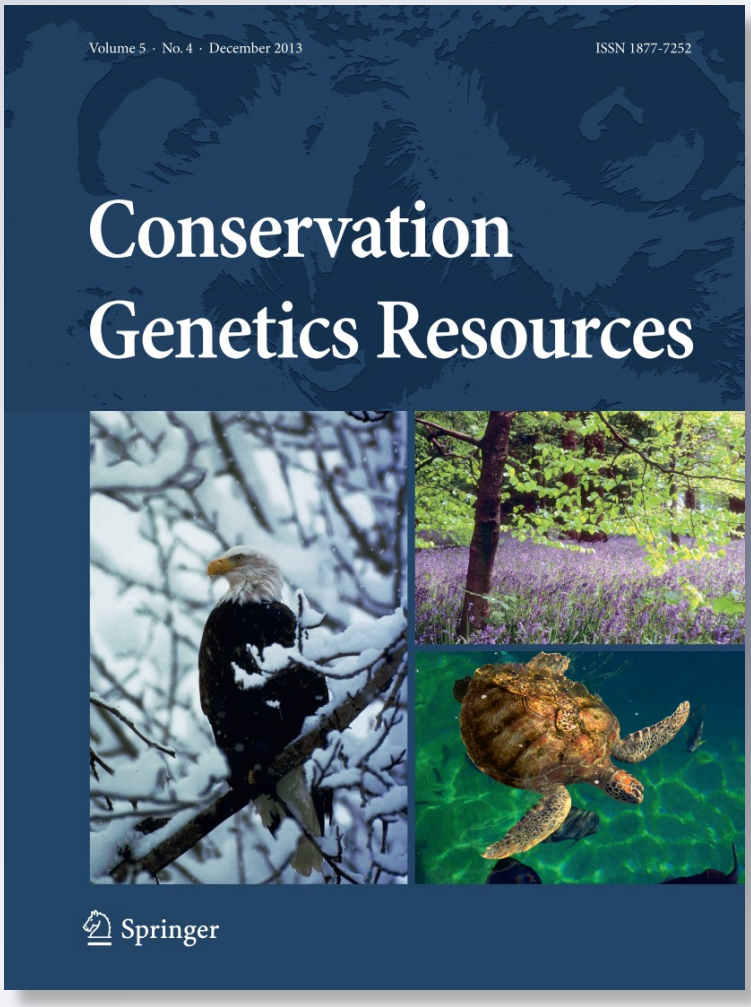
Conservation Genetics Resources

ISSN 1877-7252
Volume 5
Number 4

Conservation Genet Resour (2013)
5:1005-1007
DOI 10.1007/s12686-013-9955-z



Springer

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DOI 10.1007/s12686-013-9955-z

TECHNICAL NOTE

Characterization of 9 polymorphic microsatellite loci in *Lamellibrachia* sp. 2, a tubeworm found at deep-sea hydrothermal vents and cold seeps

Alixandra Jacobson · Sophie Plouviez · Andrew David Thaler · Cindy Lee Van Dover

Received: 25 April 2013 / Accepted: 6 May 2013 / Published online: 14 May 2013

Abstract *Lamellibrachia* sp. 2 is a deep-sea vestimentiferan tubeworm found at hydrothermal vents on the Mid-Cayman Spreading Center and at hydrocarbon seeps in the Gulf of Mexico and off the coast of Barbados. Nine selectively neutral and unlinked polymorphic microsatellite loci were developed for this species. Eight of these loci conformed to Hardy–Weinberg expectations. Average observed heterozygosity ranged from 0.14 to 0.92. Microsatellites developed for *Lamellibrachia* sp. 2 are being deployed to study connectivity and gene flow among populations of this species.

Keywords Mid-Cayman Spreading Center · Von Damm vent field · Barbados seeps · Gulf of Mexico seeps · Vestimentifera · Gene flow · Connectivity

Deep-sea hydrothermal vents and cold seeps are chemosynthetic ecosystems that support high-biomass communities and endemic taxa, but there are often few shared species between vents and seeps (Van Dover et al. 2002). *Lamellibrachia* sp. 2, is a deep-sea vestimentiferan tubeworm known from Gulf of Mexico (depth: 1,175–2,320 m; Miglietta et al. 2010) and Barbados seeps (1,325 m; Van Dover laboratory, unpublished) as well as from the recently discovered Von Damm vent field (2,300 m), Mid-Cayman Spreading Center (MCSC; Van Dover laboratory, unpublished). The ability of the tubeworm to live at both seeps and vents may favor connectivity among populations: the MCSC vents may act as stepping-stones for dispersal between Caribbean and Gulf of Mexico seeps.

Lamellibrachia sp. 2 has not been formally described, but it is morphologically (lacks a ventral vestimental fold; Miglietta et al. 2010) and genetically (COI, 16S) distinct from other *Lamellibrachia* species (Miglietta et al. 2010; Southward et al. 2011). Studies using microsatellite markers can reveal the genetic structure of populations, as well as estimate connectivity and gene flow patterns (Balloux and Lugon-Moulin 2002). Microsatellite markers will be used to assess connectivity among *Lamellibrachia* sp. 2 populations at the MCSC hydrothermal vent field and hydrocarbon seeps in the Gulf of Mexico and Barbados.

Lamellibrachia sp. 2 individuals were collected from the Von Damm vent field along the MCSC in January 2012 (R/V Atlantis cruise AT18-16) using a multi-chamber suction sampler mounted on the Remote Operated Vehicle Jason II. On board, tissue from the vestimentum of *Lamellibrachia* sp. 2 individuals was preserved in 95 % ethanol for shore-based DNA extraction.

Genomic DNA was isolated using a CTAB extraction procedure (Doyle and Dickson 1987; Plouviez et al. 2009). Tissues were digested in 600 µL of a 2 % CTAB buffer solution (1.4 M NaCl, 0.2 % 2-mercaptoethanol, 20 mM EDTA, 100 mM Tris–HCl pH 8 and 0.1 mg/mL proteinase K) for 2 h at 60 °C. DNA was then purified using chloroform-isoamyl alcohol (24:1) and precipitated together with 1 mL of 100 % isopropanol at −20 °C for 2 h. DNA pellets were washed with 70 % ethanol and re-suspended in 50 µL of nanopure H_2O. 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 tri-, tetra-, penta- and hexanucleotide repeat motifs in sequences that had significant flanking regions for primer

A. Jacobson (✉) · S. Plouviez · A. D. Thaler · C. L. Van Dover
Division of Marine Science and Conservation, Nicholas School of the Environment, Duke University, 135 Duke Marine Lab Road, Beaufort 28516, NC, USA
e-mail: aj58@duke.edu

Table 1 Polymorphic microsatellite loci from individuals of *Lamellibrachia* sp. 2 collected from the Von Damm vent field (MCSC)

Locus	Repeat Motif	Primer Sequence (5′–3′)	T_a (°C)	Allele size range (bp)	N	N_a	H_o	H_e	P
Lam1	$(TCGG)_8$	F: CAACCAGGTAGCTGTCGATG	48.5	142–352	36	7	0.83	0.62	0.00[a,b]
		R: GGCCACCAAACGATGTAACT							
Lam4	$(GGT)_{12}$	F: TCCTTATGGAGGACCAGTGC	50.3	180–300	37	4	0.57	0.43	0.26[a]
		R: GGAAGGAATTTCGTCAAACG							
Lam7	$(TAACCC)_{10}$	F: AATGTGGGCACAGCTTTTTC	45.9	111–345	33	5	0.30	0.27	0.90
		R: TGTCAAAATCGCCCAATATG							
Lam8	$(TGGG)_7$	F: TCCTTTCATCCTCCAGTCGT	59.0	121–205	37	5	0.43	0.59	0.25[a]
		R: GGAGGAGGAACGAACATTGA							
Lam10	$(GTAT)_{33}$	F: CGTACTATTGGGGGACAAATG	59.0	189–277	36	21	0.92	0.95	0.58
		R: TCACCGCGAACAATATGCTA							
Lam12	$(GCGT)_9$	F: AGGATGTCGTGAAATCGTGA	58.1	150–206	35	5	0.14	0.14	1.00
		R: ATATACCACGCACGACAGCA							
Lam16	$(TGCG)_9$	F: TGCATTGAAATATGGCAGGA	59.0	242–266	30	7	0.73	0.79	0.60
		R: GAGTGGGACAGGAAGCAATC							
Lam17	$(CGCA)_7$	F: AAGAAACACTCAGGGGCGTA	59.0	220–240	34	6	0.82	0.75	0.42
		R: GCGATCACCAATTCAGGACT							
Lam19	$(TGTC)_{12}$	F: CGTTATTGCGTGACGATTTG	59.0	220–252	31	9	0.90	0.88	0.94
		R: TTGACCCGTCATTGTTGCTA							

T_a annealing temperature, *bp* base pairs, N number of individuals that successfully amplified (out of 38), N_a number of observed alleles, H_o observed heterozygosity, H_e expected heterozygosity, P probability of deviation from Hardy–Weinberg Equilibrium

[a] Significant deviation from Hardy–Weinberg Equilibrium before Bonferroni correction ($P < 0.05$)

[b] Significant deviation from Hardy–Weinberg Equilibrium after Bonferroni correction ($P < 0.05$)

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 (5′-ATT AAC CCT CAC TAA AGG GA-3′; Schuelke 2000).

Genomic DNA was amplified in 20 µl polymerase chain reactions (PCR) as follows: 2 µl template, 2 µl 10× PCR Buffer (200 mM Tris, pH 8.8; 500 mM 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-, VIC-, or NED-labeled T3 primer (10 µM; Eurofins: Huntsville, AL), 0.2 µl Taq polymerase (1 unit, Bioline: Taunton, MA), and 11.5 µl nanopure water (Jacobson et al. 2013). 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 was 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 BayeScan (v2.1, Foll and Gaggliotti 2008) was used to detect loci potentially under selection.

Twenty-four primer pairs were screened for robust amplification using DNA from four *Lamellibrachia* sp. 2 individuals. Nine loci were polymorphic and reproducible from the screened primers. These nine loci were then assessed on 38 *Lamellibrachia* sp. 2 individuals from the Von Damm site (MCSC). Three loci (*Lam1*, *Lam4*, and *Lam8*) deviated significantly from Hardy–Weinberg Equilibrium before Bonferroni correction (Table 1). Only one of these three loci (*Lam1)* remained significant after Bonferroni correction ($k = 9$). Micro-Checker found evidence of possible stutter and null alleles at only one locus (*Lam8*), but there was no evidence for large allele dropout at any of the nine loci. BayeScan did not indicate selection at any locus.

These nine markers developed for *Lamellibrachia* sp. 2 will be used to test whether the MCSC vent population is genetically different from the seep populations (related to

environmental differences between seep and vent habitats). They will also be used to help determine if the MCSC site acts as a stepping-stone along the northward flowing hydrographic currents between seeps off Barbados and in the Gulf of Mexico, thus contributing to gene flow over large distances.

Acknowledgments This research was supported by NSF award OCE-1031050 and NASA ASTEP subcontract through WHOI (NNX09AB75G) to CLVD. We are thankful to the Chief Scientist, C. German, the crew of the R/V Atlantis (AT18-16) and the crew of the ROV Jason. We also acknowledge Mengyou Wu for help with DNA extractions. SP, ADT, CLVD conceived the study; SP, CLVD collected samples, SP and AJ undertook molecular benchwork; AJ, SP, ADT analyzed the data; AJ, SP, ADT, and CLVD drafted the manuscript.

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