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**Emily A. Boyle, Andrew David Thaler, Alixandra Jacobson, Sophie Plouviez & Cindy Lee Van Dover**

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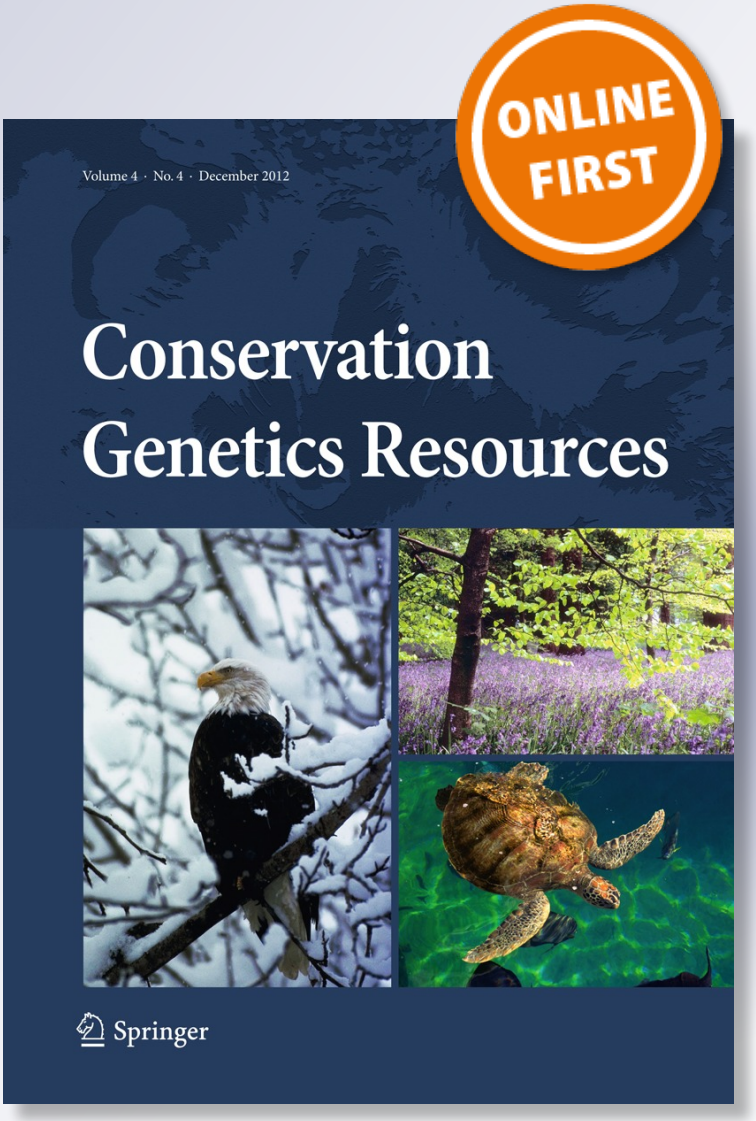


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TECHNICAL NOTE

# Characterization of 10 polymorphic microsatellite loci in *Munidopsis lauensis*, a squat-lobster from the southwestern Pacific

**Emily A. Boyle · Andrew David Thaler · Alixandra Jacobson · Sophie Plouviez · Cindy Lee Van Dover**

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**Abstract** *Munidopsis lauensis* is a deep-sea squat lobster commonly associated with hydrothermal vents at southwest Pacific back-arc spreading centers. Eight selectively neutral and unlinked polymorphic microsatellite loci were developed for this species and two additional loci from a related species were successfully cross-amplified. Eight of the ten total loci conformed to Hardy–Weinberg expectations. Average observed heterozygosity ranged from 0.23 to 0.64 (mean $H_O = 0.50$, SD = 0.15). Seven of ten loci cross-amplified in *Munidopsis antonii*, a closely related species. Microsatellites developed for *M. lauensis* are being deployed to study connectivity among populations of this species that occur at hydrothermal vents in Manus and Lau Basins.

**Keywords** *Munidopsis antonii* · Hydrothermal vent · Back-arc basin · Manus Basin

Deep-sea hydrothermal vents are patchy, ephemeral habitats that support biomass-rich communities. Assessing genetic structure among fauna commonly associated with these communities provides insight into patterns of dispersal, connectivity, and gene flow in deep-sea species (e.g., Vrijenhoek 1997, 2010). While there are numerous studies examining the population structure of vent-endemic species from the southwestern Pacific (see Vrijenhoek 2010), relatively few studies have focused on vent-associated vagrant species whose distribution is not exclusively limited to hydrothermal vents. Assessing genetic structure in vagrant deep-sea species can help distinguish between patterns of population structure specific to hydrothermal vent endemic populations and general patterns in the deep sea.

*Munidopsis lauensis* is a squat lobster commonly associated with, but not endemic to, deep-sea hydrothermal vents of southwestern Pacific back-arc spreading centers (Baba and de Saint Laurent 1992). This paper describes 10 microsatellite markers developed to assess genetic structure among populations of *M. lauensis* in the southwest Pacific.

*Munidopsis lauensis* genomic DNA was extracted from ethanol preserved abdominal muscle tissue using Wizard SV Genomic DNA Extraction Kit following protocols provided by the manufacturer (Promega, Madison, WI, USA). Eight microsatellite loci developed for *Munidopsis polymorpha* (Cabezas et al. 2009) and three loci developed for *Munida rugosa* and *Munida sarsi* (Bailie et al. 2011) were tested for cross-amplification in *M. lauensis*. Two of these loci cross-amplified in *M. lauensis* (*Mp1* and *Mp8*). To develop additional microsatellite loci, *M. lauensis* genomic DNA was sent to the Institute for Genome Science and Policy at Duke University and sequenced on a Roche 454 GS-FLX Titanium sequencer. Twenty-two tri-, tetra-, penta-, or hexa-nucleotide repeat motifs were detected using the program msatcommander (v 0.8.2; Faircloth 2008). Primers were developed for well-conserved flanking regions surrounding repeat motifs using Primer 3 (v 0.4.0; Rozen and Skaletsky 2000). A T3′-tail (5′-ATTA ACCCTCACTAAAGG GA-3′) was incorporated into forward primers to bind fluorescently labeled T3 dyes (FAM, NED, VIC, PET; Schuelke 2000).

Genomic DNA was amplified in a 20 µl PCR reaction under the following conditions: 2 µl of template DNA, 2 µl

E. A. Boyle · A. D. Thaler (✉) · A. Jacobson · S. Plouviez · C. L. Van Dover
Marine Laboratory, Nicholas School of the Environment, Duke University, 135 Duke Marine Lab Road, Beaufort, NC 28516, USA
e-mail: andrew.thaler@duke.edu; andrew.david.thaler@gmail.com

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of 10× PCR Buffer (200 mM Tris, pH 8.8; 500 mM KCl; 0.1 % Triton X-100, 0.2 mg/ml BSA), 1.6 µl of $MgCl_2$ (25 µM), 1.6 µl dNTPs (2.5 µM), 1.5 µl reverse primer (10 µM), 0.3 µl forward primer (10 µM), 1.5 µl of fluorescently-labeled T3 primer (FAM, NED, VIC, or PET; 10 µM; Eurofins: Huntsville, AL, USA; Table 1), 0.2 µl of Taq (1 unit, Bioline: Taunton, MA, USA), and 9.3 µl of nanopure $H_2O$. Reactions were run under the following conditions: 4 min at 94 °C; 35 cycles of 94 °C for 30 s, $T_A$ °C (indicated in Table 1) for 30 s, 72 °C for 45 s; final extension of 72 °C for 10 min.

PCR products were diluted 1:4 with nanopure $H_2O$ and 1 µl of product was added to 0.05 µl LIZ500 size standard (MCLab, San Francisco, CA, USA) and 8.95 µl nanopure water for a total volume of 10 µl. 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 and chromatograms scored using the computer program GeneMarker (v1.8, SoftGenetics LLC: State College, PA, USA). HW-QuickCheck (Kalinowski 2006) was used to measure deviation from Hardy–Weinberg equilibrium. Sequential Bonferroni was used to correct for multiple tests (Rice 1989). Presence of null alleles, stutter, and large allele dropout were assessed with MicroChecker (1,000 randomizations; van Oosterhout et al. 2004). LOSITAN (default settings; Beaumont and Nicholas 1996, Antao et al. 2008) was used to detect loci under selection.

Thirty-three primer pairs were screened for robust amplification on genomic DNA from four individuals of *M. lauensis*. Of these 33 loci, ten were polymorphic, variable, and reproducible. These ten loci were deployed on a minimum of 28 *M. lauensis* individuals from Manus Basin, Papua New Guinea. Three of these 10 loci deviated significantly from Hardy–Weinberg equilibrium (*Mp1*, *Mp8* and *Mp21*); two loci remained significantly out of Hardy–Weinberg equilibrium after correction for multiple tests (*Mp8*, *Mp21*; Table 1). Micro-Checker found no evidence of stutter or large allele dropout at any of the loci. There were indications of the presence of null alleles at two loci (*Mp1*, *Mp21*). There was no indication that any of the loci tested are under selection using LOSITAN.

All ten markers were tested for cross amplification (CA) using the reported reaction conditions on three *M. antonii* individuals from Lau Basin. Seven loci (*Mp1*, *Mp12*, *Mp14*, *Mp16*, *Mp21*, *Mp27*, and *Mp29*) showed positive cross-amplification, three of which successfully cross-amplified in all three *M. antonii* individuals (Table 1).

**Table 1** Polymorphic microsatellite loci from *M. lauensis*

| Locus | Repeat motif | Primer sequence (5′–3′) | $T_A$ (°C) | Allele size range (bp) | N | $N_A$ | $H_O$ | $H_E$ | *P* | CA |
|---|---|---|---|---|---|---|---|---|---|---|
| *Mpl* | $(TAA)_{33}$ | F: TTCCACAATGAGCACTGGAG | 56.0 | 112–249 | 35 | 11 | 0.54 | 0.74 | 0.003 | 1/3 |
| | | R: GCATATGTGGAGCCTGGATT | | | | | | | | |
| *Mp8* | $(TAAA)_3$ | F: AGCATCAATTCTGCCCTTTC | 56.0 | 187–228 | 49 | 2 | 0.63 | 0.44 | 0.002* | 0/3 |
| | | R: GATCACCCCACTTGAAGGAA | | | | | | | | |
| *Mpl2* | $(TAA)_{33}$ | F: TCCAATTTCCATGTGTTCCA | 55.0 | 103–149 | 35 | 9 | 0.49 | 0.42 | 0.140 | 2/3 |
| | | R: GGACAGGGTGGATATGAGTGA | | | | | | | | |
| *Mp14* | $(TAAA)_4$ | F: TCGACTACGAAATCGAGCAA | 46.3 | 155–242 | 33 | 4 | 0.64 | 0.53 | 0.120 | 3/3 |
| | | R: CATCTCGCCCTTAACACAGG | | | | | | | | |
| *Mp15* | $(GGA)_{13}$ | F: AGAAGGGGAAAAGGGAGACA | 53.5 | 110–190 | 30 | 4 | 0.27 | 0.35 | 0.140 | 0/3 |
| | | R: CCTCCGCCTTCTTCTTCTCT | | | | | | | | |
| *Mpl6* | $(CAT)_7$ | F: AGCAGGACGAATGAAAGTGC | 55.0 | 173–447 | 30 | 5 | 0.23 | 0.28 | 0.360 | 2/3 |
| | | R: AAGAAAGACATGGCGTGAGG | | | | | | | | |
| *Mp21* | $(CAT)_{15}$ | F: TGGAGGTGGGGTCAATAAAG | 46.0 | 155–310 | 28 | 17 | 0.57 | 0.87 | 0.000* | 2/3 |
| | | R: CGTGTTAAGACAACCGTGGA | | | | | | | | |
| *Mp24* | $(TAGA)_{13}$ | F: CCAATTGCAAATTGTTTAAAAGG | 55.0 | 219–278 | 32 | 9 | 0.44 | 0.43 | 0.990 | 0/3 |
| | | R: CCTCCCTCTCTCCCTCTCAC | | | | | | | | |
| *Mp27* | $(TCA)_{10}$ | F: AAGTTGGCTCGCACTTTGTT | 55.0 | 197–209 | 33 | 5 | 0.52 | 0.47 | 0.620 | 3/3 |
| | | R: GGTGTGCCGAGCATTTTTAT | | | | | | | | |
| *Mp29* | $(TTG)_6$ | F: GTTTTTGCGACGGAAATGTT | 55.0 | 203–246 | 33 | 8 | 0.64 | 0.66 | 0.900 | 3/3 |
| | | R: CGGTTTTGGCGAAAAACTTA | | | | | | | | |

Annealing temperature ($T_A$), size range of alleles (base pairs), number of alleles ($N_A$), observed heterozygosity ($H_O$), expected heterozygosity ($H_E$), and probability of deviation from Hardy–Weinberg equilibrium (*P*) are reported for ten microsatellites tested on *M. lauensis*. Significant deviation from Hardy–Weinberg equilibrium after Bonferroni correction for multiple tests ($P < 0.05$) is indicated with an asterisk. Successful cross-amplification (CA) with a total of three individuals *M. antonii* are reported

*Munidopsis lauensis* microsatellite markers will be deployed on individuals from sites throughout Manus Basin and neighboring southwest Pacific back-arc basins to assess patterns of population structure, connectivity, and gene flow. Population studies of *M. lauensis* will provide a useful comparison against vent endemic species from the same sampling locations for which microsatellite markers have also been developed (*Ifremeria nautilei*: Thaler et al. 2010, 2011; *Bathymodiolus manusensis*: Schultz et al. 2010; *Chorocaris* spp.: Zelnio et al. 2010).

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