Conservation Genet Resour
DOI 10.1007/s12686-010-9243-0

TECHNICAL NOTE

Characterization of nine polymorphic microsatellite loci in *Chorocaris* sp. (Crustacea, Caridea, Alvinocarididae) from deep-sea hydrothermal vents

K. A. Zelnio · A. D. Thaler · R. E. Jones · W. Saleu · T. F. Schultz · C. L. Van Dover · J. Carlsson

Received: 21 April 2010 / Accepted: 24 April 2010
© Springer Science+Business Media B.V. 2010

Abstract Species in the genus Chorocaris are caridean shrimp found at deep-sea hydrothermal vents at mid-ocean spreading centers. Nine unlinked polymorphic microsatellite loci were developed for this Chorocaris sp. 2 from the Manus back-arc basin, southwest Pacific. Three loci deviated significantly from Hardy–Weinberg expectations. Average observed heterozygosity ranged from 0.17 to 0.74 (mean $H_O = 0.45$, SD = 0.21). Eight out of nine loci cross-amplified in one or two additional species of alvinocaridid shrimp that co-occur with Chorocaris sp. 2 at western Pacific vent habitats. The efficacy of our markers to detect genetic population structure is demonstrated using 362 individuals from 3 sites in Manus Basin. Microsatellites developed for Chorocaris sp. 2 are being deployed to study connectivity among populations of this species colonizing geographically discrete back-arc basin vent systems.

Keywords Alvinocarididae · Back-arc Basin · Chemosynthetic-based ecosystem · Chorocaris · Hydrothermal vent · Shrimp · Microsatellite

Deep-sea hydrothermal vents occur throughout the oceans at geologically active plate margins forming habitats for chemosynthetic-based ecosystems. Understanding population connectivity in these systems has been the focus of intense research (e.g., Hurtado et al. 2004; Shank and Halanych 2007; Vrijenhoek 1997). We are investigating connectivity of an alvinocaridid shrimp known as *Chorocaris* sp. 2 (currently under description by T. Komaii et al.) from vents in the western Pacific.

Genomic DNA was isolated from ethanol-preserved muscle tissue by Chelex-Proteinase K extraction. Tissue (10–30 mg) was digested with 240 µg Proteinase K (Bioline: Taunton, MA) in 600 µl 10% Chelex-100 resin (Bio-Rad: Hercules, CA) overnight at 60°C, heated to 100°C for 15 min, and centrifuged at 10,000 rpm for 5 min. Extracted DNA from *Chorocaris* sp. 2 was enriched for microsatellite-containing motifs following Glenn and Schable (2005), digested with *Rsa*I, ligated to SNX linkers, annealed with biotinylated oligonucleotides containing repetitive elements (Mix 2 and Mix 3, Glenn and Schable 2005), captured with streptavidin-coated magnetic beads, and PCR-amplified. PCR products were cloned using TOPO TA Kits (Invitrogen: Carlsbad, CA) and α-Select electrocompetent cells (Bioline: Taunton, MA) and sequenced using Big Dye Terminator v3.1 (Applied Biosystems). Repetitive elements were located using Msatfinder (Thurston and Field 2005) and flanking primers designed with Primer3 (Rozen and Skaletsky 2000). Forward primers were designed with a T3-tag (5′-ATTAACCCTCACTAAAGGGA-3′) to allow labeling PCR products with FAM-labeled (6-carboxy-fluorescine) T3- primer (Schuelke 2000). Clone sequences for markers developed here are deposited in GenBank (accession #HM067873-HM067881).

Genomic DNA templates were amplified with 20 µl polymerase chain reactions (PCRs) prepared 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),

K. A. Zelnio (✉) · A. D. Thaler · R. E. Jones · W. Saleu · T. F. Schultz · C. L. Van Dover · J. Carlsson
Marine Conservation Molecular Facility, Marine Laboratory, Nicholas School of the Environment, Duke University, Beaufort, NC 28516, USA
e-mail: kzelnio@gmail.com

J. Carlsson
Department of Zoology, Ecology and Plant Science, University College Cork, Cork, Ireland

2 μl $MgCl_2$ (final concentrations indicated in Table 1), 1 μl 2.5 mM dNTP's, 0.2 μl 10 μM forward primer, 0.8 μl 10 μM reverse primer, 0.8 μl 10 μM 5′-FAM-labeled T3 primer (Eurofins: Huntsville, AL), and 0.2 μl Taq polymerase (1 unit, Bioline: Taunton, MA). Reactions were run at: 95°C 4 min; 25 cycles of 94°C 45 s, T_A^oC 30 s (indicated in Table 1), 72°C 45 s; 8 cycles of 94°C 45 s, 53°C 30 s, and 72°C 45 s; final extension 72°C for 10 min.

PCR products were diluted 1:4 and 1 μl was added to 0.05 μl Orange DNA size standard (MCLab, San Francisco CA) and 8.95 μl water and denatured at 95°C for 10 min. Size-fragment analysis was conducted on an ABI 3730xl DNA analyzer. Chromatograms were scored using Genemarker v1.8 (SoftGenetics LLC: State College, PA).

Deviation from Hardy–Weinberg Equilibrium (HWE), heterozygote excess and deficiency, and linkage disequilibrium were tested with Genepop version 4.0.10 (Rousset 2008) and corrected for multiple comparisons using the sequential Bonferroni approach (Rice 1989). Presence of null alleles, stutter, and large allele dropout were assessed using MicroChecker (1000 randomizations; van Oosterhout et al. 2004). LOSITAN (50,000 simulations; Antao et al. 2008) was used to detect loci potentially under selection.

Approximately 2,400 clones were screened and 114 primer pairs developed. Nine loci were polymorphic, reproducible, and not prone to stuttering. These loci were assessed on 45 individuals of *Chorocaris* sp. 2. There was no evidence for linkage disequilibrium among loci nor did LOSITAN indicate selection at any locus. Three loci (Cho46, Cho63, and Cho76) deviated significantly from HWE (Table 1), showed significant homozygote excess ($P < 0.05$), and remained significant after sequential Bonferroni corrections ($k = 9$). MicroChecker indicated the presence of null alleles at these three loci. Carlsson (2008) modeled the effect of null alleles on assignment testing and F_{ST} estimations and determined that power of assignment tests was slightly reduced (0.2–2.4%) and a slight overestimation of F_{ST}. While caution should be exercised, using loci with null alleles sparingly would not likely alter outcomes of assignment testing and still retain utility where unaffected markers are unavailable (Carlsson 2008).

The efficacy of the six loci marker system unaffected by null alleles is demonstrated by estimating the probabilities that full siblings share identical genotypes (P_{SIB}) and that two individuals have identical genotypes (P_{ID}) (Woods et al. 1999; Waits et al. 2001), which constrains the number

Table 1 Polymorphic loci (GenBank accessions HM067873-HM067881) from 45 individuals of *Chorocaris* sp. 2

Locus	Motif of original clone	Primer sequence (5′–3′)	[$MgCl_2$] (mM)	T_A (°C)	Allele size range (bp)	N_A	H_O	H_E	P	Cross amplification (# positive/# total)
Cho30	$(AC)_7$	F: GTCATCCCACGACGTGTATC	2.5	49	182–196	4	0.17	0.17	0.820	*Chorocaris* sp. 1 (3/3)
		R: ACTTGACCTTTGCCTTGGTG								*Alvinocaris* sp. (2/3)
Cho36	$(TACA)_6$	F: GCATCAATCCTGTGCACACGC	2.0	60	149–189	8	0.50	0.52	0.450	*Chorocaris* sp. 1 (2/3)
		R: GTAAAGGAGCCMCCCTYGAATG								*Alvinocaris* sp. (2/3)
Cho46	$(CTTA)_4$	F: AGACCGAGCTAGCAAACGAG	1.5	51	241–245	2	0.17	0.51	0.001*	*Chorocaris* sp. 1 (2/3)
		R: CAATTCGCCTAACAGCAATG								*Alvinocaris* sp. (3/3)
Cho63	$(ATAG)_8$	F: TATGCAAGGAGTGCATGTCG	2.0	62	157–179	6	0.43	0.69	0.001*	*Chorocaris* sp. 1 (0/3)
		R: GCTAAGCTCAACTTCTAAATGG								*Alvinocaris* sp. (3/3)
Cho76	$(GT)_5$	F: TAAACGTGAGCGCAATTTCC	2.0	47	204–231	16	0.64	0.82	0.002*	*Chorocaris* sp. 1 (3/3)
		R: CACAAACGCGTGTGAGCGC								*Alvinocaris* sp. (3/3)
Cho83	$(TAAA)_3$	F: GGCTACATGCAACCCAAGGG	1.5	46	154–173	9	0.74	0.77	0.720	*Chorocaris* sp. 1 (3/3)
		R: GGTATCATTTATATGGACGCC								*Alvinocaris* sp. (3/3)
Cho91	$(ATTT)_3$	F: CCAGTTTGAACCATCTTGC	1.0	46	203–212	9	0.60	0.64	0.600	*Chorocaris* sp. 1 (0/3)
		R: GACGAATGAAAGTCTGGAACC								*Alvinocaris* sp. (0/3)
Cho99	$(GTAT)_3$	F: CCTGTGGTGGCGAAGTGG	2.0	63	222–232	4	0.57	0.51	0.460	*Chorocaris* sp. 1 (3/3)
		R: AAGACTACAAGGGGCAGC								*Alvinocaris* sp. (0/3)
Cho100	$(ACAT)_4$	F: CCAACGTTTAGCCGAGTGG	1.5	58	190–201	7	0.26	0.30	0.240	*Chorocaris* sp. 1 (3/3)
		R: GCTAATTTTGGTTCACTAC								*Alvinocaris* sp. (0/3)

Concentration of $MgCl_2$ ([$MgCl_2$]), 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. Significant deviation from Hardy–Weinberg Equilibrium after sequential Bonferroni correction is indicated with an asterisk. Cross-amplification with *Alvinocaris* sp. and *Chorocaris* sp. 1 phylotypes are reported from 3 individuals tested for each species

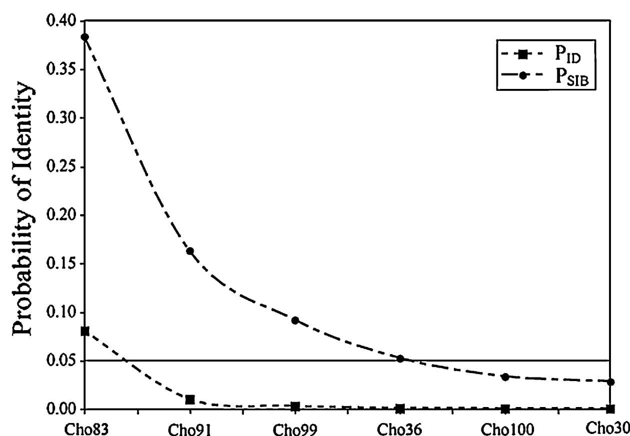

Fig. 1 Cumulative rank-order probability of identity of two individuals pulled from a random pool (P_{ID}) and probability that a sibling pair has identical genotypes (P_{SIB}) for the six microsatellite loci system not affected by null alleles developed for *Chorocaris* sp. 2. A cutoff level for sufficient power is denoted at the 0.05 level (Woods et al. 1999)

of markers required to attain enough resolving power for assignment testing (Paetkau 2003). Gimlet v1.3.3 (Valière 2002) was used to estimate P_{SIB} and P_{ID} on 362 individuals of *Chorocaris* sp. 2 from three sites in Manus Basin. At least five out of the six markers was needed (Fig. 1) to detect identical genotypes in our sample pool below a 5% threshold (Woods et al. 1999). This brief example demonstrates the utility of our markers for this species, which are being deployed to ascertain population structure at multiple spatial scales.

All markers were tested for cross amplification in two alvinocarid shrimp from Manus Basin, *Alvinocaris* sp. and *Chorocaris* sp. 1. Eight out of nine loci showed positive cross-amplification (Table 1). Three of these loci (Cho63, Cho99, Cho100) showed positive cross-amplification only on *Chorocaris* sp. 1 (Table 1).

To our knowledge, this is the first report of microsatellite markers developed for a deep-sea hydrothermal vent shrimp. Microsatellite markers have been developed for four other invertebrates from deep-sea chemosynthetic ecosystems (*Branchipolynoe seepensis*: Daguin and Jollivet 2005; *Bathymodiolus childressi*: Carney et al. 2006; *Riftia pachyptila*: Fusaro et al. 2008; *Ifremeria nautilei*: Thaler et al. 2010). *Chorocaris* sp. 2 microsatellite markers will be deployed on populations in the western Pacific to explore connectivity within Manus Basin. The data generated will establish a baseline of genetic diversity to assess changes caused by volcanic eruptions, mining, or other disruptive events.

Acknowledgments This research was supported by a research contract from Nautilus Minerals Niugini Limited to CLVD, JC, and TS and by Duke University. JC acknowledges support from the Beaufort Marine Research Award in Fish Population Genetics funded by the Irish Government under the Sea Change Programme. Samples used in this study were collected on behalf of the people of Papua New Guinea.

References

Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: a workbench to detect molecular adaptation based on a Fst-outlier method. BMC Bioinformatics 9:323

Carlsson J (2008) Effects of microsatellite null alleles on assignment testing. J Hered 99:616–623

Carney SL, Formica MI, Divatia H, Nelson K, Fisher CR, Schaeffer SW (2006) Population structure of the mussel “*Bathymodiolus*” *childressi* from Gulf of Mexico hydrocarbon seeps. Deep Sea Res I 53:1061–1072

Daguin C, Jollivet D (2005) Development and cross-amplification of nine polymorphic microsatellite markers in the deep-sea hydrothermal vent polychaete *Branchipolynoe seepensis*. Mol Ecol Notes 5:780–783

Fusaro AJ, Baco AR, Gerlach G, Shank TM (2008) Development and characterization of 12 microsatellite markers from the deep-sea hydrothermal vent siboglinid *Riftia pachyptila*. Mol Ecol Resour 8:132–134

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. In: Zimmer EA, Roalson EH (eds) Methods in enzymology 395, molecular evolution: producing the biochemical data, Part B. Academic Press, San Diego, pp 202–222

Hurtado LA, Lutz RA, Vrijenhoek RC (2004) Distinct patterns of genetic differentiation among annelids of eastern Pacific hydrothermal vents. Mol Ecol 13:2603–2615

Paetkau D (2003) An empirical exploration of data quality in DNA-based population inventories. Mol Ecol 12:1375–1387

Rice WR (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 HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp 365–386

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotech 18:233–234

Shank TM, Halanych KM (2007) Toward a mechanistic understanding of larval dispersal: insights from genomic fingerprinting of the deep-sea hydrothermal vent tubeworm *Riftia pachyptila*. Mar Ecol 28:25–35

Thaler AD, Zelnio KA, Jones RE, Carlsson J, Van Dover CL, Schultz TF (2010) Characterization of 12 polymorphic microsatellite loci in *Ifremeria nautilei*, a chemoautotrophic gastropod from deep-sea hydrothermal vents. Cons Gen Resour. DOI: 10.1007/s12686-010-9174-9

Thurston MI, Field D (2005) Msatfinder: detection and characterisation of microsatellites. Distributed by the authors at http://www.genomics.ceh.ac.uk/msatfinder/. CEH Oxford, Mansfield Road, Oxford OX1 3SR

Valière N (2002) GIMLET: a computer program for analysing genetic individual identification data. Mol Ecol Notes 2:377–379

van Oosterhout C, Hutchinson W, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Vrijenhoek RC (1997) Gene flow and genetic diversity in naturally fragmented metapopulations of deep-sea hydrothermal vent animals. J Hered 88:285–293

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Mol Ecol 10:249–256

Woods JG, Paetkau D, Lewis D, McLellan BN, Proctor M, Strobeck C (1999) Genetic tagging of free-ranging black and brown bears. Wildl Soc Bull 27:616–627