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PERMANENT GENETIC RESOURCES

Microsatellite loci for the invasive colonial hydrozoan *Cordylophora caspia*

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Abstract

***Cordylophora caspia*, a colonial hydrozoan native to the Ponto-Caspian region, has become a common invader of both fresh and brackish water ecosystems of North America and Europe. We describe 11 polymorphic microsatellite loci for this species. Preliminary analyses indicate that population substructure may contribute to departures from Hardy–Weinberg equilibrium. In addition, new loci failed to consistently amplify *Cordylophora* samples known to be genetically distant from those utilized in this study, indicating the presence of cryptic diversity within the taxon.**

Keywords: *Cordylophora caspia*, cryptic diversity, hydrozoan, invasive species, microsatellite, primer development

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The Ponto-Caspian hydrozoan *Cordylophora caspia* recently has established with increasing frequency throughout North America and Western Europe (Folino 2000). In addition to potential impacts on native biological communities, *Cordylophora* frequently fouls intake pipes and filters of power plants, impinging on plant efficiency and production (Folino 2000). *Cordylophora* possesses broad environmental tolerance, can reproduce asexually through budding, and has the ability to form a dormant menont stage capable of regenerating under favourable conditions (Folino 2000). These characteristics make it ideally suited for translocation through ballast water or ship fouling, and potentially contribute to complex colonization scenarios. The high resolution markers described here will help to adequately assess invasion dynamics for this species.

For microsatellite development, *Cordylophora* DNA was isolated from fresh tissue of a single large Lake Michigan colony using DNAzol (Invitrogen), digested using *Bst*U I or *Eco*RV (New England Biolabs, NEB) and modified by linker ligation as originally described by Hamilton *et al*. (1999). SuperSNX linker-ligated fragments were enriched for microsatellites and cloned following procedures described by Glenn & Schable (2005). Specifically, fragments were hybridized with biotinylated microsatellite probes (TG_{12}, AG_{12}, AAG_8, ATC_8, AAC_8, AAT_{12}, ACT_{12}, $AAAC_6$, $AAAG_6$, $AATC_6$, $AATG_6$, $ACCT_6$, $ACAG_6$, $ACTC_6$, $ACTG_6$, $AAAT_8$, $AACT_8$, $AAGT_8$, $ACAT_8$ and $AGAT_8$), captured using streptavidin magnetic beads (NEB), recovered by amplification using SuperSNX24 forward primer, and cloned using the TOPO TA Cloning Kit (Invitrogen). Positive colonies were amplified using a 25-μL polymerase chain reaction (PCR) containing 0.5 U Jumpstart *Taq* DNA Polymerase (Sigma), 1× PCR buffer, 0.4 μM each M13 forward and reverse primers, 0.6 mM dNTPs, 2.0 mM $MgCl_2$, 25 μg/mL bovine serum albumin (BSA) and 1.5 μL *Escherichia coli* grown overnight in Luria-Bertani broth. PCR products were cleaned using Antarctic Phosphatase and Exonuclease I (NEB) and sequenced using BigDye Terminator version 3.1 Cycle Sequencing Reagent (Applied Biosystems, ABI). Sequence reactions were purified using DyeEx 96 (QIAGEN) and run on an ABI 3730xl DNA Analyser. Sequence data were assembled and edited using SEQUENCHER 4.6 (Gene Codes) and searched for microsatellite repeats using MSATCOMMANDER (Faircloth 2007). Thirty-two microsatellite primer pairs were designed with OLIGO 3.0 using a three-primer system (cf. Boutin-Ganache

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Table 1 Characterization of microsatellite loci for *Cordylophora caspia*

Locus	Primer sequence	T_a (°C)	Repeat motif	Size range (bp)	N_A	n	H_E	H_O	N_p	GenBank
CC02	F: 5′-ACCCTTAGCACCTTACATA-3′ R: 5′-PET-CGCAACAACACCTAAAT-3′	64–54 TD	$(TACA)_4(ACAT)_{10}$	218–310	21	122	0.91972	0.68033*	2	EU196241
CC08	F: 5′-NED-ATCTGCCAATAAATAAGAAT-3′ R: 5′-TATCGACGCCATCTACT-3′	59–49 TD	$(AAC)_{19}(ACG)_{10}$	205–250	12	121	0.75488	0.7438	1	EU196246
CC10	F: 5′-VIC-ATTATCTCAAGCGAACATC-3′ R: 5′-TGTCTTTCAATACCCAATG-3′	64–54 TD	$(ACAG)_{21}$	203–285	18	127	0.59923	0.55906	0	EU196247
CC11	F: 5′-PET-ACCGAAATCACTCTACACA-3′ R: 5′-GGTATTTCTTTCGTTCCTTA-3′	56	$(AAC)_9$	188–208	5	115	0.55508	0.49565	0	EU196248
CC12	F: 5′-PET-GTGTGCCATTGAGAAGTT-3′ R: 5′-TGTATGTACTGAAACGACTATC-3′	59–49 TD	$(AAAT)_5$	286–347	5	110	0.58813	0.53636	0	EU196249
CC16	F: 5′-AATCGTTATCCTTATACATATT-3′ R: 5′-VIC-CTTTAATTTGCGATCTCT-3′	59–49 TD	$(ACT)_{21}$	263–350	17	122	0.72509	0.70492	0	EU196250
CC22	F: 5′-FAM-CTCTTTTGGCTTCTTCTAC-3′ R: 5′-TGAAACCGCATTACTAAA-3′	64–54 TD	$(ACAT)_8$	151–236	24	119	0.9093	0.77311	1	EU196251
CC29	F: 5′-NED-CATCGTCTGGTAGAGAGAA-3′ R: 5′-ACCAAACCCGTATTCTAAT-3′	59–49 TD	$(ACTC)_6$	170–182	3	125	0.55595	0.44*	1	EU196242
CC30	F: 5′-FAM-GTTTTGCAGAATTGGTACA-3′ R: 5′-GTCGTCTCGTCGTTCA-3′	59–49 TD	$(TTG)_{19}$	311–346	14	118	0.78485	0.72034	0	EU196243
CC31	F: 5′-CCATACGGCCTTACA-3′ R: 5′-FAM-TTTCTCTTTCTCCCCATA-3′	64–54 TD	$(AATC)_{23}$	189–269	22	123	0.86872	0.87805	0	EU196244
CC32	F: 5′-NED-TAGACGTAACGCTTGC-3′ R: 5′-CACTTTTCTATGATACCTGC-3′	64–54 TD	$(ACTC)_{17}$	255–393	39	117	0.89645	0.64103*	2	EU196245

T_a, annealing temperature; TD, touchdown PCR programme; N_A, number of alleles; n, number of individuals tested; H_E, expected heterozygosity; H_O, observed heterozygosity; * denotes loci that deviate significantly from HWE after Bonferroni correction for multiple tests; N_p, number of subpopulations (out of three total) exhibiting significant deviation from HWE (after correction) when populations are tested independently. Fluorescent labels (ABI) are shown in bold.

et al. 2001). New primer pairs were initially examined for amplification success and polymorphism using 11 individuals. Each 15-µL PCR contained 0.375 U Jumpstart *Taq* DNA polymerase (Sigma), 1× PCR buffer, 1 µм untagged primer, 0.1 µм tagged primer, 0.9 µм FAM-labelled CAG or M13 universal primer, 1 mм dNTPs, 1.5 mм $MgCl_2$, 25 µg/mL BSA and 10–100 ng of DNA template. All primers were tested using a 60 °C touchdown thermal cycling programme (Don *et al*. 1991). Cycling parameters were: 95 °C for 2 : 30; 20 cycles of 95 °C for 20 s, 60 °C (–0.5 °C per cycle) for 20 s, 72 °C for 30 s; 15 cycles of 95 °C for 20 s, 50 °C for 20 s, 72 °C for 30 s; 72 °C for 10 min. Samples were sized on an ABI 3730xl DNA Analyser using GeneScan-500 LIZ size standard (ABI) and analysed using GENEMARKER 1.60. Primer pairs that failed to amplify, were difficult to score, or that appeared monomorphic were not used for additional polymorphism testing.

Eleven polymorphic primer pairs were direct-labelled for additional polymorphism testing. Most loci were amplified with one of two touchdown PCR programmes. Touchdown cycling parameters were: 95 °C for 1 min; 12 cycles of 95 °C for 30 s, 64 °C (or 59 °C) for 30 s (–0.8 °C per cycle), 72 °C for 45 s; 23 cycles of 95 °C for 30 s, 54 °C (or 49 °C) for 30 s, 72 °C for 45 s; 72 °C for 15 min. Each 15-µL PCR contained 0.5 U *Taq* DNA polymerase (QIAGEN), 1× PCR buffer, 1 µм each forward and reverse primer, 1 mм dNTPs, 1.6 mм $MgCl_2$, and 10–100 ng of DNA template. Locus CC11 was amplified using a single annealing temperature with cycling parameters: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 56 °C for 1 min, 72 °C for 1 min; 72 °C for 15 min.

A total of 128 individual *Cordylophora* colonies from three sites in Lake Michigan were tested for microsatellite polymorphism. Loci were tested for linkage and Hardy–Weinberg equilibrium (HWE) using GENEPOP 3.4 (Raymond & Rousset 1995); observed and expected heterozygosities were determined using Arlequin (Schneider *et al*. 2000) (Table 1). No significant linkage disequilibrium was observed, but five loci deviated significantly from HWE when data were analysed as a single population, and three of these results remained significant after Bonferroni correction. When subpopulations were analysed individually, no single locus consistently deviated from HWE for all populations. While this suggests the likelihood of underlying population structure, the possibility of null alleles cannot be ruled out. Although the presence of clonal genotypes was generally rare, several clones were observed in neighbouring colonies from the same sampling site (not shown). Clonal genotypes were removed from the data set for equilibrium analyses.

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Table 2 Allele sizes (in base pairs) observed in *Cordylophora* samples genetically distant from those used for polymorphism testing. Genetic distance (*p*) is based on uncorrected *p*-distance (*percentage*) from the Lake Michigan sample at the mitochondrial COI locus. GenBank accession numbers for COI sequences are indicated; five samples from Petaluma River with the same COI haplotype were all tested for polymorphism. '–' denotes amplification failure

Sample location	GenBank	*p*	CC02	CC08	CC10	CC11	CC12	CC16	CC22	CC29	CC30	CC31	CC32
Lake Balaton, Hungary	EF540787	8.02	—	274/319	—	—	339/339	—	169/229	—	—	—	—
Petaluma River, California	EF540792	12.6	—	229/229	—	—	—	—	—	—	—	—	—
Petaluma River, California	EF540792	12.6	—	229/229	—	—	—	—	—	—	—	—	—
Petaluma River, California	EF540792	12.6	—	229/236	—	—	—	—	—	—	—	—	—
Petaluma River, California	EF540792	12.6	—	—	—	—	—	—	—	—	—	—	—
Petaluma River, California	EF540792	12.6	—	—	—	—	—	—	—	—	—	—	—
Napa River, California	EF540790	12.6	—	—	—	—	—	—	—	—	—	—	—
Exeter, New Hampshire	EF540782	14.6	—	—	—	—	—	—	—	—	—	—	—
Ryck River, Germany	EF540784	14.6	—	—	—	—	—	—	—	—	—	—	—

Given the taxonomic uncertainty surrounding the genus *Cordylophora* (Folino 2000), primers were tested on samples that, although nominally conspecific, are genetically distinct enough to represent cryptic sister species to the Lake Michigan samples (N.C. Folino-Rorem, C. b'Ausilio, and J.A. Darling, unpublished data). Failure to consistently amplify these samples (Table 2) indicates that these primers are not useful for all specimens currently recognized as *Cordylophora caspia* and further indicates the need for taxonomic re-evaluation of the genus.

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References

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *BioTechniques*, **31**, 24–28.

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS (1991) 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Research*, **19**, 4008.

Faircloth BC (2007) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Notes*. doi: 10.1111/j.1471–8286.2007.01884.x.

Folino NC (2000) The freshwater expansion and classification of the colonial hydroid *Cordylophora* (Phylum Cnidaria, Class Hydrozoa). In: *Marine Bioinvasions: Proceedings of the First National Conference January 24–27, 1999* (ed. Pederson J), pp. 139–144. Massachusetts Institute of Technology Sea Grant College Program, Cambridge, Massachusetts.

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. In: *Methods in Enzymology 395, Molecular Evolution: Producing the Biochemical Data, Part B* (eds Zimmer EA, Roalson EH), pp. 202–222. Academic Press, San Diego, California.

Hamilton MB, Pincus EL, Di Fiore A, Flesher RC (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. *BioTechniques*, **27**, 500–507.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN ver. 2.000: A Software for Population Genetics Data Analysis*. Genetics and Biometry Laboratory. University of Geneva, Switzerland.

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