Conservation Genet Resour (2012) 4:695–698
DOI 10.1007/s12686-012-9624-7

TECHNICAL NOTE

Characterization of 10 novel microsatellite loci and cross-amplification of two loci in the snapping turtle (*Chelydra serpentina*)

Christina M. Davy · Ashley E. Leifso · Ida M. Conflitti · Robert W. Murphy

Received: 19 February 2012 / Accepted: 20 February 2012 / Published online: 6 March 2012

Abstract We use 454 ("shotgun") sequencing to obtain a partial genomic library for the snapping turtle (*Chelydra serpentina*). We characterize ten microsatellite loci from these sequences and test cross-amplification of loci originally developed for the alligator snapping turtle (*Macrochelys temminckii*). We genotype 127 individuals from Ontario at twelve loci. The number of alleles per locus ranges from 1 to 14; heterozygosity ranges from 0.157 to 0.850. These loci will be used to study population genetic structure in this long-lived reptile and may cross-amplify in two closely related species.

Keywords Microsatellite · *Chelydra serpentina* · *Macrochelys temminckii*

The snapping turtle (*Chelydra serpentina*) is a long-lived species found from southern Canada east of the Rocky Mountains southwards to the Gulf Coast and Florida (Ernst and Lovich 2009). The International Union for the Conservation of Nature (IUCN) lists the species as Least Concern. However, its life history makes it vulnerable to over-harvesting (van Dijk 2011). Reliable microsatellite markers for this species may provide valuable information for potential conservation initiatives. We use 454 sequencing to develop novel microsatellite markers, and cross-amplify nine microsatellite markers previously developed for the alligator snapping turtle (*Macrochelys temminckii*; Hackler et al. 2006).

For microsatellite development, we isolated genomic DNA from snapping turtle blood using phenol–chloroform procedure (Sambrook et al. 1989) and cleaned the DNA using EtOH precipitation. We obtained a partial genomic library by sequencing on a Roche GS Junior (Roche, Branford, CT) at Trent University's Natural Resources DNA Profiling and Forensics Centre. The run produced 127,778 sequences averaging 423 base pairs, which we searched for tri- and tetra-nucleotide microsatellites using the program MSTACOMMANDER (Faircloth 2008). We examined potential target loci by eye to identify loci with appropriate flanking regions for primer design and designed 40 primer pairs using Primer 3 (Rozen and Skaletsky 2000). We labelled forward primers with a fluorescent 5′ M13 tail and labelled reverse primers with a 5′ pigtail (GTTTCTT; Brownstein et al. 1996) to facilitate adenylation.

We collected blood samples from 127 *C. serpentina* from across southern Ontario by caudal venipuncture and stored the blood on FTA cards (Whatman, Inc.). We extracted genomic DNA from each card following the method suggested by Smith and Burgoyne (2004) for samples with nucleated erythrocytes.

We ran a temperature gradient with two DNA samples at each locus and used the optimal temperature for all subsequent PCR reactions. Amplification followed the methods of Schuelke (2000), using 4.0 μL of M13-labelled

C. M. Davy (✉) · I. M. Conflitti · R. W. Murphy
Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks St., Toronto, ON M5S 3B2, Canada
e-mail: christina.davy@utoronto.ca

C. M. Davy · I. M. Conflitti · R. W. Murphy
Department of Natural History, Royal Ontario Museum, 100 Queen's Park, Toronto, ON M5S 2C6, Canada

A. E. Leifso
Wildlife Preservation Canada, RR #5, 5420 Highway 6 North, Guelph, ON N1H 6J2, Canada

Table 1 Characteristics of ten novel and two cross-amplified microsatellite loci for 127 *Chelydra serpentina* sampled from across southern Ontario

Locus	Primer sequence (5′–3′)*	Repeat motif	Annealing temperature (°C)	Size (bp)	N	*k*	H_o	H_e	PI
Cs08	F: TGTAAAACGACGGCCAGTGCTGGACATGTACGTGCAAA	AGAT	54	157–198	127	11	0.850	0.859	0.0356
	R: GTTTCTTTGTATATGTCCTTTAGGCATTTATGTG								
Cs12	F: TGTAAAACGACGGCCAGTGGCATTTCTAGCCAACAGGA	AGAT	58	197–250	126	12	0.841	0.808	0.0536
	R: GTTTCTTAGCGGTGTGCTTTTCTCAGT								
Cs14	F: TGTAAAACGACGGCCAGTCAGACAGGGGCTGTTGAGTC	AGG	60	231	57	1	–	–	1.000
	R: GTTTCTTGGCAGCTCTGTGTGTCAGTC								
Cs16	F: TGTAAAACGACGGCCAGTTCCAAATGCAGCTCTCTTCA	AAT	58	193–198	127	4	0.701	0.678	0.1645
	R: GTTTCTTCCTTGCCATCTCGAACAAAT								
Cs17	F: TGTAAAACGACGGCCAGTTGGAAACTCTCCTTGTCTGTCC	ACC	60	290–305	127	6	0.654	0.691	0.1399
	R: GTTTCTTGAGGCACTTTATTAATTCCTACCTCT								
Cs18*	F: TGTAAAACGACGGCCAGTTGGTGGTTTCTCTGGAAGTTTT	AATT	56	268–276	127	3	0.157	0.159	0.7169
	R: GTTTCTTTTCTGCTTTTAACCTGCACTCA								
Cs19	F: TGTAAAACGACGGCCAGTTTGAGTGGTCTACGGGAACC	AGG	58	194–206	127	5	0.472	0.469	0.3158
	R: GTTTCTTTGACGGTTTCCTAGCGGTAT								
Cs22	F: TGTAAAACGACGGCCAGTCGGCAGAAGATAAGAGGCATT	AAAT	56	321–333	127	4	0.378	0.357	0.4339
	R: GTTTCTTTGGTAGGGTTGCTCATGAAA								
Cs24	F: TGTAAAACGACGGCCAGTTGTTTCCATTCCAAACACCTG	ACC	61	417–432	127	3	0.551	0.575	0.2540
	R: GTTTCTTGCAACACTGCTTCCCTTCAT								
Cs25	F: TGTAAAACGACGGCCAGTTGTGTTGTCACAGGGCACTT	ATC	60	220–229	127	3	0.567	0.560	0.2921
	R: GTTTCTTAAATGGACTGCGGACACTTC								
MteD9	F: TGTAAAACGACGGCCAGTCCAGATGCTAGTCTCACACC	ATCT	60	261–285	127	6	0.740	0.743	0.1058
	R: GTTTCTTGCTTACTGGAATTAACCTCATG								
MteD111*	F: TGTAAAACGACGGCCAGTTCCACAAACTCCCATCTTC	TAGA	60	175–191	120	14	0.558	0.865	0.0324
	R: GTTTCTTCCACACGGAAAAATCTATCTAC								

N number of individuals genotyped, *k* number of alleles, H_o observed heterozygosity, H_e expected heterozygosity, *PI* probability of identity. Primer sequences shown include a 5′ M13 tail (5′-TGT AAA ACG ACG GCC AGT-3′) on forward primers and a 5′ GTTTCTT pigtail on reverse primers. MteD111 and MteD9 are from Hackler et al. (2006), with M13 tail (F) and pigtail (R) added. Loci which are not in Hardy–Weinberg equilibrium ($p < 0.01$) are indicated with a *. The repeat motif for locus MteD9 differs from that reported for *M. temmincki* (Hackler et al. 2006)

forward primer, 0.66 μL each of pigtailed reverse primer (Eurofins MWG Operon) and a 6-carboxyfluorescein dye (6-FAM; Eurofins MWG Operon) and 1.0 μL of DNA eluate (6–9 ng). We also tested cross-amplification of nine microsatellite loci developed for the alligator snapping turtle (*Macrochelys temminckii*; Hackler et al. 2006) using a 12.5 μL PCR reaction containing 0.6 μL of forward primer and 1.0 μL each of reverse primer and 6-FAM. Two of the nine alligator snapping turtle loci amplified successfully in the snapping turtle. PCR cycling parameters followed King and Julian (2004) with annealing temperatures adjusted for each locus as summarized in Table 1. We visualized length of the amplified fragments using a 3730 DNA Analyzer (Applied Biosystems) with GS(500) Liz (Applied Biosystems) as a size standard and scored genotypes using GeneMarker (SoftGenetics, State College, PA). One to two homozygous samples were subsequently sequenced at each locus to confirm identity of the amplified fragments, and five percent of our sampled individuals were genotyped twice at each locus to assess genotyping error.

We successfully amplified ten novel loci and cross-amplified two of the nine alligator snapping turtle loci (MteD9 and MteD111). We genotyped 127 *C. serpentina* from Ontario to characterize these 12 loci. We found no ambiguities in the genotypes of the individuals amplified and genotyped multiple times. Sequencing of homozygotes confirmed the identity and motifs of the amplified fragments.

We used Genalex v6.0 (Peakall and Smouse 2006) to quantify the number of alleles per locus (k), calculate observed and expected heterozygosity (H_o and H_e) and probability of identity (PI) for each locus. We used Genepop 4.0.10 (Raymond and Rousset 1995; Rousset 2008) to test for linkage disequilibrium and deviations from Hardy–Weinberg equilibrium (HWE).

The number of alleles per locus ranges from 1 to 14. Heterozygosity ranges from 0.157 to 0.850. Table 1 summarizes the primer sequences, amplification conditions and characteristics of the 12 characterized loci. None of the 66 pairwise comparisons between loci show evidence of linkage disequilibrium after Bonferroni correction for multiple comparisons. Two loci (Cs18 and MteD111) show significant deviations from HWE ($p < 0.01$).

Three alleles at the tri-nucleotide locus Cs16 differ by only one base pair. Sequencing of homozygous individuals and successful replication of these genotypes in independent amplifications both demonstrate these are unique alleles and are not the result of stutter. One locus (Cs14) is monomorphic in our samples. Because they are all from the northern limits of this species' range low genetic diversity is expected, but it may be variable in southern populations.

The family Chelydridae contains two other species (Phillips et al. 1996): the Central American Snapping Turtle (*Chelydra rossignoni*), listed by the IUCN as Vulnerable, and the South American snapping turtle (*C. acutirostris*), which remains to be assessed. Cross-amplification of these markers could facilitate conservation genetic analyses of these two closely related and poorly understood species.

Acknowledgments This project was supported by a Canada Collection grant from Wildlife Preservation Canada (WPC) to C.D. and a National Science and Engineering Research Council (NSERC) Discovery Grant (A3148) to R.W.M. Genotyping costs were offset by the generous assistance of the Schad Foundation. C.D. and I.D. are funded by Canada Graduate Scholarships from NSERC; A.L. is funded by WPC through a Science Horizons grant from Environment Canada. We thank Dr. C. Kyle, E. Kerr and M. Harnden at the NRDPFC, Trent University, for assistance with 454 sequencing. Sample collection was conducted with the permission of the Government of Ontario and Ontario Parks and followed Animal Use Protocol 2010-14 (Royal Ontario Museum).

References

Brownstein MJ, Carpten JD, Smith JR (1996) Modulation of nontemplated nucleotide addition by tag DNA polymerase: primer modifications that facilitate genotyping. BioTech 20: 1004–1010

Ernst C, Lovich J (2009) Turtles of the United States and Canada, 2nd edn. Johns Hopkins University Press, Baltimore

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

Hackler JC, van den Bussche RAV, Leslie DM (2006) Characterization of microsatellite DNA markers for the alligator snapping turtle, *Macrochelys temminckii*. Mol Ecol Notes 7:474–476

King TL, Julian SE (2004) Conservation of microsatellite DNA flanking sequences across 13 Emydid genera assayed with novel bog turtle (*Glyptemys muhlenbergii*) loci. Conserv Genet 5:719–725

Peakall R, Smouse PE (2006) Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Phillips CA, Dimmick WW, Carr JL (1996) Conservation genetics of the common snapping turtle (*Chelydra serpentina*). Conserv Biol 10:397–405

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

Rousset F (2008) Genepop'007: a complete reimplementation 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. Available at http://fokker.wi.mit.edu/primer3/

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning—a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York

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

Smith LM, Burgoyne LA (2004) Collecting, archiving and processing DNA from wildlife samples using FTA® databasing paper. BMC Ecol 4:4. http://www.biomedcentral.com/1472-6785/4/4

van Dijk PP (2011) *Chelydra serpentina*. In: IUCN 2011. IUCN red list of threatened species. Version 2011.2. www.iucnredlist.org. Downloaded on 12 December 2011