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TECHNICAL NOTE

Fourteen novel microsatellite markers for the gopher frog, *Lithobates capito* (Amphibia: Ranidae)

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Abstract We isolated and characterized a total of 14 microsatellite loci from gopher frogs, *Lithobates capito*. This species is of conservation concern because most populations have gone locally extinct across the geographic distribution. Loci were screened for 21 individuals from a single population in Florida. The number of alleles per locus ranged from 7 to 17, observed heterozygosity ranged from 0.667 to 0.947, and the probability of identity values ranged from 0.011 to 0.077. These new loci provide tools for examining the genetic diversity and population structure of *L. capito* populations and addressing factors associated with their decline.

Keywords *Lithobates* · *Rana* · Ranidae · Gopher frog · Microsatellite · PCR primers · SSR · STR

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Gopher frogs, *Lithobates capito*, are habitat specialists that inhabit periodically burned long-leaf pine (*Pinus palustris*) ecosystems in the non-reproductive season and migrate to depressional wetlands for reproduction (Jensen and Richter 2005). As a result of habitat loss, *L. capito* is now considered endangered, threatened, or of special concern in all states they inhabit (Jensen and Richter 2005), and populations in portions of the geographic distribution may warrant federal protection. Currently, there are eight microsatellite loci developed for *L. sevosus*, dusky gopher frogs, that cross amplify in *L. capito* (Richter and Broughton 2005), but to increase power of analyses we developed additional loci for this species.

Total DNA was extracted from one individual of *L. capito*, using the DNeasy tissue kit protocol (Qiagen, Valencia, CA) for the construction of the microsatellite library. DNA was then serially enriched twice for microsatellites using three probe mixes following Glenn and Schable (2005) with changes described in Lance et al. (2010). There were two primary changes to the Glenn and Schable (2005) protocol. First, a different linker was used (SimpleX-5 Forward 5′-AAAA CGAGCAGCGGAACT and SimpleX-5 Reverse 5′-pAGTTCCGCTGCTCG). Second, the enriched libraries were sequenced on a 454 using titanium chemistry following standard Roche 454 library protocols (454 Life Sciences, a Roche company, Branford CT). All methods for sequencing, microsatellite identification, primer design, and primer screening are as described

Table 1 Details for 14 polymorphic microsatellite loci developed for *Lithobates capito*

Locus	Primer sequence 5′ → 3′	Repeat motif	Size (bp)	*N*	*K*	H_o	H_e	PI	TD
Lica5	F: ACCGTCTATGGTGGTCTAACG	$(AGAT)^{13}$	213–233	17	7	0.765	0.780	0.074	65
	R: TCTAGGGATTCCAAACTGTGC[a]								
Lica7	F: GGGCTGAGAACTAACGTGC	$(AGAT)^{10}$	310–422	18	13	0.722	0.835	0.044	65
	R: GTGTGCATCTACACAAGGGC[a]								
Lica8	F: TCTTCCACTACTCTGGAAAGC[a]	$(AGAT)^{11}$	333–401	19	14	0.789	0.785	0.060	65
	R: TGTGTGATACGCAGTTCCTTC								
Lica11	F: TTTCAGGCCAGCATCAATGG	$(AGAT)^{14}$	140–168	21	9	0.667	0.829	0.051	65
	R: ACTTGCAACGACTAGAGCC[a]								
Lica14	F: AGCAAACTGACACCTCCAG[a]	$(ATCT)^{14}$	232–331	19	17	0.842	0.911	0.014	65
	R: CAGAACTTTGGAAGTAGAAGCCC								
Lica15	F: ATACCGATTGGTCAGAGAGC[a]	$(ATCT)^{9}$	318–354	19	8	0.684	0.791	0.071	65
	R: ACCTGTTCCAGTGACAACAATC								
Lica18	F: TGTACGGATAATGATTGGCG	$(AGAT)^{12}$	179–275	19	13	0.842	0.877	0.026	65
	R: CAGTTTCTGCAGTGTAACC[a]								
Lica25	F: CGAATGAATTTGTCGCTCTACG	$(ATCT)^{13}$	214–338	19	16	0.895	0.904	0.016	65
	R: GGCGACTACACACTGTTCTTATC[a]								
Lica29	F: CTCCTTTCCCACCTAGAGGC	$(ATCT)^{9}(ATCC)^{10}$	200–328	19	16	0.737	0.925	0.011	65
	R: TCCTTTCCACCATGCTCACC[a]								
Lica40	F: CAGTGTGAACCAGGGCTTTG	$(ATCT)^{14}$	244–280	20	10	0.750	0.845	0.042	60
	R: CCTGCCTAGAGAGTCTTCCG[a]								
Lica41	F: GGGTGGATAACACACTAGG	$(AGAT)^{9}$	308–340	19	9	0.947	0.839	0.046	65
	R: CCACCCGGTAATATAAAGCTGTG								
Lica43	F: GCACTGCTCCCTAACACAAG	$(ATCT)^{15}$	164–212	19	8	0.842	0.783	0.077	65
	R: CGTGCCAATGCAATTTCTGC[a]								
Lica44	F: TCTTGTAGCACAAAGCGGTG	$(AGAT)^{11}$	264–356	20	13	0.800	0.869	0.030	65
	R: CCGTGCAATGTATCTCTGG[a]								
Lica47	F: GCCATCAAAGTTCATGTGCG	$(ATCT)^{17}$	153–189	19	10	0.947	0.881	0.026	65
	R: TGTATAGGAGCATGGTTCCAG[a]								

The size indicates the range of observed alleles in base pairs and includes the length of the CAG tag; number of individuals genotyped is *N*; *k* is number of alleles observed; H_o and H_e are observed and expected heterozygosity, respectively; PI is the probability of identity for each locus, and TD refers to the touchdown protocol used for PCR (see text)

[a] Indicates CAG tag (5′-CAGTCGGGCGTCATCA-3′) label

in Lance et al. (2010) with the exception that the sequence GTTT was added to primers without the universal CAG tag addition.

Forty-eight primer pairs were tested for amplification and polymorphism using DNA obtained from eight individuals. PCR amplifications were performed in a 12.5 µl volume (10 mM Tris pH 8.4, 50 mM KCl, 25.0 µg/ml BSA, 0.4 µM unlabeled primer, 0.04 µM tag labeled primer, 0.36 µM universal dye-labeled primer, 3.0 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 units AmpliTaq Gold® Polymerase (Applied Biosystems), and 20 ng DNA template) using an Applied Biosystems GeneAmp 9700. Touchdown thermal cycling programs (Don et al. 1991) encompassing a 10°C span of annealing temperatures ranging between 65–55°C (TD65) was used for all loci. Touchdown cycling parameters consisted of an initial denaturation step of 5 min at 95°C followed by 20 cycles of 95°C for 30 s, highest annealing temperature (decreased 0.5°C per cycle) for 30 s, and 72°C for 30 s; and 20 cycles of 95°C for 30 s, lowest annealing temperature for 30 s, and 72°C for 30 s. PCR products were run on an ABI 3130*xl* Genetic Analyzer (Applied Biosystems, Inc.) and sized with Naurox size standard prepared as described in DeWoody et al. (2004), except that unlabeled primers started with GTTT. Results were analyzed using GeneMapper version 3.7 (Applied Biosystems, Inc.). Fourteen of the tested primer pairs amplified high quality PCR product that exhibited polymorphism.

We assessed the variability of the 14 polymorphic loci in 21 road-killed specimens collected from Ross Prairie (Marion County, Florida). Conditions and characteristics of the loci are provided in Table 1. We estimated the number

of alleles per locus (k), observed and expected heterozygosity (H_o and H_e), and probability of identity (PI) using GenAlEx v6.4 (Peakall and Smouse 2006). Tests for deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium were conducted using GENEPOP v4.0 (Rousset 2008). After Bonferroni correction for multiple comparisons all loci were in HWE, and no linkage disequilibrium was detected between loci. All 14 loci contained suitable variability for population genetic studies in terms of number of alleles (range = 7–16), H_o (range = 0.667–0.947), H_e (range = 0.780–0.925), and probability of identity (range = 0.011–0.077; Table 1).

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