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TECHNICAL NOTE

Characterization of 10 polymorphic microsatellite loci in the South American lizard *Liolaemus fitzingerii* with cross-amplification in *L. chehuachekenk*

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Abstract We describe the characterization of ten polymorphic microsatellite loci from the *Liolaemus fitzingerii* species complex with cross-amplification for nine of the 10 loci in *L. chehuachekenk*. The number of alleles within *L. fitzingerii* ranged from 7 to 24 and the observed heterozygosity ranged from 0.311 to 0.956 for 45 individuals. Nine of the ten loci conformed to Hardy–Weinberg equilibrium and we did not detect linkage disequilibrium between loci in *L. fitzingerii.* Cross-species amplification in *L. chehuachekenk* was successful for nine of the ten loci with polymorphisms detected for each successfully amplified locus. These microsatellite loci are the first developed for the *Eulaemus* clade of the genus, and will aid in both ongoing and future studies focusing on gene flow, historical divergence and population structure within the *Liolaemus fitzingerii* species complex.

Keywords *Liolaemus fitzingerii* · *Liolaemus chehuachekenk* · Microsatellites · Heterozygosity · Cross-amplification · Population genetics

Liolaemus is one of the most species-rich genera of lizard throughout southern South America (Cei 1986, 1993; Lobo et al. 2010). In particular, *L. fitzingerii* is distributed throughout central areas of Patagonia, including Santa Cruz and Chubut province, extending as far north as the southern slopes of Somuncura plateau (Avila et al. 2006). Previous work has recognized extreme inter and intra population variation in morphology, coupled with sexual and ontogenetic variation, making species delimitation difficult in some groups (Etheridge 1993; Avila et al. 2006).

Because of the broad range and extensive species diversity within the genus *Liolaemus fitzingeri* complex, DNA was isolated from *L. chehuachekenk* and *L. fitzingeri* using the DNeasy Animal Tissue Kit (QIAGEN). Muscle tissue from the caudal region was used to prevent contamination from potential liver parasites. Microsatellite libraries were enriched using 454 sequencing and primers were designed at the Savannah River Ecology Laboratory. A total of 20 primer pairs were chosen to test for allelic variation, and ten of those primer pairs showed positive amplifications. Rather than use each forward primer with a fluorescent label, we adapted the M13-tailed microsatellite protocol of (Schuelke 2000), where each forward primer is 5′ augmented with an M13 forward sequence (CACGACGTTGTAAAACGAC). This tailed primer was then used in combination with a 6-FAM fluorescently labeled M13 primer. Thus, amplification reactions contained three primers: a forward M13 fluorescent primer, 5′-augmented microsatellite forward primer, and an unmodified microsatellite reverse primer. Reactions consisted of 1 μL of DNA, 0.04 μL of the 10 μM forward M13 tailed primer, 0.45 μL of the 10 μM reverse primer, 0.45 μL of 10 μM fluorescently labeled reverse primer, 1.25 μL of $MgCl_2$, 1.25 μL of PCR Buffer II (Perkin Elmer), 0.21 μL of a 10 μM dNTP mixture, 0.1 μL of taq and 7.75 μL of water for a total volume of 12.5 μL. The thermal profile was 94°C for 2 min followed by 19 cycles of 94°C for 30 s, the primer specific annealing temperature (Table 1) for 30 s and 68°C for 30 s, followed by a 3 min extension at 68°C. A second

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Table 1 Characterization of the ten microsatellite loci isolated from *Liolaemus fitzingerii*, and cross amplified in *L. chehuachekenk*

Locus	Motif	Primer sequence	Annealing temp (°C)	Allele size range (bp)	N_A	H_O	H_E
DI-7938	$(AC)_{13}$	F-TCACAGCTTTCTTTATTTCAAC R-AAGCAAGACCTTAGCACAAC	49	346–358 (348–360)	7 (6)	0.533 (0.626)	0.838 (0.866)
DI-159	$(AC)_{11}$	F-TGCTCAATAAGGGACAGTTC R-AATTAATGACTCAGTGCATAGG	49	260–274 (260–280)	6 (5)	0.311 (0.875)	0.644 (0.786)
TET-3500	$(AAAG)_{12}$	F-GGTTACCCTGCATATTACACC R-ATTAACGAGGACCAAGTTTG	49	308–364 (332–364)	17 (9)	0.733 (0.875)	0.916 (0.902)
DI-1570	$(AC)_{10}$	F-TTACTACACCACACCGTCTG R-TTTCAGAGCCACAACATACC	49	354–366 (354–372)	6 (6)	0.512 (0.625)	0.740 (0.777)
TET-2216	$(AGAT)_{12}$	F-GACTCTTGCCATTTGTGAAG R-AATTCATGCGTGTTCTGAG	49	227–308 (228–313)	18 (11)	0.614 (0.750)	0.891 (0.964)
DI-3138	$(AC)_{11}$	F-CCTTCACTCCTTTCCTATTTC R-ATGTGAAGCTGGAAGTGTTC	55	263–290 (264–284)	21 (10)	0.667 (0500)	0.944 (0.964)
TET-3105*	$(AAAG)_{12}$	F-ATCCATTTACCTGCCATATG R-CCTGCTTCTGTTCAAACTTC	55	260–322 (260–304)	23 (11)	0.956 (0.625)	0.941 (0.982)
TET-1177	$(AAAG)_{12}$	F-ATGACTTGCCCAATATTACC R-TTTGTAGCCCTCAATATGC	49	280–310 (NA)	16 (NA)	0.689 (NA)	0.861 (NA)
TET-1501	$(AAAG)_{12}$	F- AGAGCTTTCTTGGTGCTTAG R-ATCTGGTAGGTGTGCACATC	49	316–380 (322–373)	24 (7)	0.778 (0.375)	0.943 (0.848)
TET-1102	$(AAAG)_{14}$	F-AGCCAGAGGATCTTTGAGAG R-TCATTCTCCCTTTGTCAGTG	48	228–242 (240–246)	8 (3)	0.575 (0.00)	0.839 (0.464)

Numbers in parenthesis are values from *L. chehuachekenk* (n = 9). All loci for *L. chehuachekenk* showed significant deviation from HWE

N_A number of alleles, H_O observed heterozygosity, H_E expected heterozygosity

* Locus showing significant departure from HWE for *L. fitzingerii*

round of 10 cycles was run with an annealing temperature of 53°C to ensure proper annealing of the fluorescently labeled primer. Loci were first sequenced to ensure the amplification of the correct microsatellite locus.

All ten loci produced appropriate sized bands when visualized via electrophoresis using a 2% agarose gel. Depending on the overall strength of the amplification reaction, PCR products were diluted 1:5 or 1:7 with water into a single plate for genotyping. One microliter of the diluted PCR product was added to a plate containing 0.09 μL of the GeneScan 500 LIZ genotyping standard and 9.91 μL of HiDi Formamide (Applied Biosystems). Reactions were genotyped on an ABI 3730XL automated sequencer and preliminarily scored using PEAKSCANNER (Applied Biosystems). Final scoring of microsatellite alleles was verified by eye for each sample.

We used GENODIVE (Meirmans and Van Tienderen 2004) to calculate the number of alleles, allele size range, expected and observed heterozygosity (Table 1). All ten loci scored showed varying levels of allelic diversity. Using sequential Bonferroni corrections, we calculatded deviations from hardy–Weinberg equilibrium and linkage disequilibria using GENEPOP 4.0.10 (Raymond and Rousset 1995). Incidence of null alleles and scoring errors due to stuttering or large allele drop-out were assessed on MICRO-CHECKER 2.2.2 (Oosterhout et al. 2004).

Microsatellite loci isolated from *L. fitzingerii* showed medium to high levels of allelic diversity. The number of alleles per locus varied from 7 to 24 (mean = 16.2), ranging in size from 227 to 380 bp long. The expected heterozygosity ranged from 0.644 to 0.944 and the observed heterozygosity ranged from 0.311 to 0.956. We did not detect deviation from Hardy–Weinberg equilibrium or linkage disequilibrium between pairs of loci after sequential Bonferroni correction, except in locus TET 3105 ($P > 0.01$; Table 1). The expected distribution of homozygote size classes calculated with MIICRO-CHECKER suggested no incidence of null alleles or scoring errors due to large allelic drop-out or stuttering. We used the same procedure as above to successfully amplify nine out the ten loci in *L. chehuachekenk* (n = 9). We were not able to amplify locus TET_1177 in any sample of *L. chehuachekenk* despite multiple attempts with different annealing temperatures and *Taq* polymerase enzymes. Of the remaining nine loci that successfully amplified, the number of alleles ranged from 3 to 11 (mean 7.5). We found significant deviation from Hardy–Weinberg equilibrium for each of the nine loci, however this is likely due to high

allelic richness compared to the sample size. Our ongoing microsatellite-based studies should contribute to better characterization of species boundaries, hybrid zone interactions, and the formulation of adequate conservation measures.

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