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Development of 24 polymorphic microsatellite markers for the island night lizard (*Xantusia riversiana reticulata*) of San Clemente Island, California.

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## Abstract

The island night lizard (*Xantusia riversiana*) was delisted from the Endangered Species Act in May 2014 and is required to be monitored throughout its range over the next 10 years. *Xantusia riversiana* is endemic to three of the California Channel Islands and consists of two subspecies: the San Nicolas island night lizard (*X. r. riversiana*) and the San Clemente Island night lizard (*X. r. reticulata*). We isolated and characterized 24 microsatellite loci from the San Clemente Island night lizard. Loci were screened in 32 individuals collected from a single site on San Clemente Island, CA. The number of alleles per locus ranged from 7 to 16, observed heterozygosity ranged from 0.467 to 0.967, and the probability of identity ranged from 0.017 to 0.11. These new loci will be used to estimate effective population sizes, population subdivision, and population connectivity to help inform population monitoring and management strategies.

The island night lizard, *Xantusia riversiana*, is an endemic to three California Channel Islands: Santa Barbara, San Clemente, and San Nicolas. *Xantusia riversiana* was delisted from its threatened status under the Endangered Species Act in May 2014 by the United States Fish and Wildlife Service (2014); contingent on monitoring efforts over the next decade. The island night lizard consists of a single species with three evolutionary units distinct to each island (USFWS 2014). Two subspecies are recognized on the basis of morphological differences (Smith 1946): the San Nicolas island night lizard (*X. r. riversiana*), which recently had 12 polymorphic loci developed by O'Donnell et al (2014), and the San Clemente island night lizard (*X. r. reticulata*).

The San Clemente Island night lizard is endemic to San Clemente and Santa Barbara Islands. These islands experienced severe habitat degradation by introduced grazing animals in the 19<sup>th</sup> Century; however, these grazing animals have been eliminated on both islands and prime habitat is being rehabilitated. San Clemente Island is an active military installation and is estimated to contain 21 million lizards, or ~99.85% of the total number of individuals present in the species (USFWS 2014). Night lizards on all three islands are characterized by long life spans, low reproductive rates, low dispersal distances, and high site fidelity. These factors limit the scope of information available from traditional capture-mark-recapture studies; thus, genetic resources are needed to estimate population-level parameters involving population size and connectivity for effective monitoring to assess the security of the species.

Total DNA was extracted from one individual of *X. r. reticulata* from San Clemente Island for use in isolation of microsatellite loci. Forty-eight primer pairs were tested for amplification and polymorphism using methods described in Carson et al (2013). Results were analyzed using GeneMapper version 3.7 (Applied Biosystems). Twenty four of the tested primer pairs amplified high quality PCR product that exhibited polymorphism.

We assessed the variability of the 24 polymorphic loci in 32 individuals from a single collection site on San Clemente Island, CA captured in February 2013. Loci were characterized with Arlequin version 3.5 (Excoffier and Lischer, 2010). After correcting for multiple tests, two loci (Xari6, Xari30) showed significant deviations from expectations under HWE and LD was detected for one of the 276 paired loci comparisons (Xari22-Xari46).

These loci will allow for the estimation of effective population size, population connectivity, population subdivision, and other population-genetic parameters for San Clemente Island. This information will be used to guide management and monitoring of this species on an island actively utilized by the military and additional populations of *X. r. reticulata* on Santa Barbara Island.

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## References

- Carson EW, Beasley RR, Jones KL, Lance SL, Lozano-Vilano ML, Vela-Valladares L, Banda-Villanueva I, Turner TF, Maza-Benignos M (2013) Development of polymorphic microsatellite markers for the microendemic pupfishes *Cyprinodon julimes* and *C. pachycephalus*. Conservation Genetics Resources. doi 10.1007/s12686-013-9925-5
- Excoffier, L, Lischer HEL (2010) Arlequin suite vers 3. 5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564-567.
- O'Donnell RP, Drost CA, Mock KE (2014) Development and characterization of 12 microsatellite markers for the island night lizard (*Xantusia riversiana*), a threatened species endemic to the Channel Islands, California, USA. Conservation Genetics Resources. doi 10.1007/s12686-014-0189-5.
- Smith A (1946) A subspecies of the lizard *Xantusia riversiana*. Journal of the Washington Academy of Sciences 36:392-393.
- United States Fish and Wildlife Service (2014) Island night lizard (*Xantusia riversiana*) final Post-Delisting Monitoring Plan. U.S. Fish and Wildlife Service, Carlsbad Fish and Wildlife Office, Carlsbad, California.

**Table 1.** Details for 24 polymorphic microsatellite loci developed for *Xantusia riversiana reticulata*. 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.

Locus	Primer Sequence 5' --> 3'	Repeat motif	Size (bp)	N	K	$H_o$	$H_e$	PI
Xari1	F: *ATATCAGGTGCGGATTTGGG R: AAAGGCTTCACTTGCCAGC	AAAG	195-239	32	11	0.813	0.866	0.032
Xari5	F: *CACACTCAAGCTCTTACATATGGG R: AAGGATGTCATTACTTCATGCCC	ATCT	164-204	32	10	0.844	0.803	0.062
Xari6 <sup>a</sup>	F: *GGAGCAGGCAACTATGTCACC R: ACTTTCTGTGGAAGTATCTTGAAGG	AAAG	172-212	30	9	0.467	0.805	0.063
Xari7	F: *AACGCCAAGGATTATGGAGG R: AGAATTTGGAAGGGCGGC	AACT	182-226	32	10	0.844	0.851	0.039
Xari9	F: *AATTGTCCTGTCCCAAAGGG R: AGCTTCCCATCTCCACAGC	AAAG	264-312	32	11	0.781	0.860	0.035
Xari10	F: *TTTGTGTGTGTGAAATTGATTGG R: AGGGCTGTGGAGTTTCATGC	ATCT	172-220	32	13	0.844	0.855	0.037
Xari11	F: *TTACTAGACCTGTGTTTCTCGGG R: CACTTtagcCAAAGGAAGAGTGC	AAAG	336-392	30	12	0.900	0.871	0.030
Xari13	F: *TTATGGAGAGCCATTACAG R: CATCTTCAAGTGGTACAATATCTTCC	AAAG	223-283	30	14	0.967	0.903	0.017
Xari18	F: *AGAGCTGGCTCACAAGGAGG R: CTCCAATCAAAGCAAAGCCC	AAAG	168-208	28	9	0.893	0.818	0.057
Xari20	F: *GTGAAGGCTGCAGTTCCGG R: CTGTGTGGGTCCAGAAGTGG	ATCT	190-218	30	7	0.667	0.794	0.071
Xari21	F: *GTTTAGTTTCAGAACAAGCCAGGG R: CTGTAAACATTTACCATTCCAGC	AAAG	180-240	32	13	0.938	0.867	0.031
Xari22 <sup>b</sup>	F: *TCTTTCCTTCACACCCACCC R: GAAGACAAGCGCCCTTCC	AAAG	246-322	32	16	0.844	0.887	0.022
Xari30 <sup>a</sup>	F: *TTGGTGAACGTGCCTGG R: GCTCTTTCTCACCTGCCTGG	TTCC	176-228	32	10	0.719	0.843	0.043
Xari31	F: *CAGGAATGTACTGATACAGAATATGG R: GCTTCTATCTTTACATCCTGTGATCC	ATCT	167-211	32	8	0.906	0.805	0.064
Xari33	F: *TCAGAACAATAATTAACATGTGGC R: GGCTAGTACCATCTCCCTCCC	ATCT	148-176	31	8	0.742	0.793	0.069
Xari35	F: *CAAATTGTGAGTGTTATGCAAATAGC R: TCCAGAATAAGGATTCGCC	AAAG	186-246	31	14	0.903	0.883	0.025
Xari38	F: *TGCATGTTATGTGAAGCAGCC R: TGCATGCATGGAATCAAGC	AAAG	153-201	32	8	0.781	0.811	0.061
Xari41	F: *TCTGTATGTAGTGCTTTGACTATGCC R: TGGTTACCTATCCCAAAGGAGC	AAAG	131-155	32	7	0.563	0.718	0.11

Xari42	F: *TAAGCCTGTGGGAAGAGTGG R: TTATAAATTGAGGAAAGTCTCTAAACTAGG	ATCT	350-374	32	7	0.781	0.783	0.078
Xari44	F: *TGCCTCCACTTATGTTCTACAAGG R: TTGCACACTCTCCACATCCC	ATCT	158-198	31	9	0.677	0.838	0.046
Xari45	F: *TTGCAGTGTTAAGGTGTCATAGG R: TTTCCCTTCTGGCTTGTTGG	AAAG	158-206	29	12	0.931	0.880	0.026
Xari46 <sup>b</sup>	F: *GACCCTCCTCTTTCTACAGTGC R: TTTCTGAACTACACGGAAATGC	AAAG	132-164	30	9	0.900	0.857	0.036
Xari47	F: *TTAAGCAGAAATGCACCCTCC R: TTTGCAGAAAGTAGCAAACCTGC	AAAG	199-259	32	13	0.844	0.875	0.028
Xari48	F: *GCAATAATATCAAACCAACAAGCC R: TTTGGGCACTTGCTGACG	ATCT	215-247	32	9	0.844	0.857	0.037

\* indicates CAG tag (5'- CAGTCGGGCGTCATCA-3') label; significant deviations after sequential Bonferroni correction are indicated by <sup>a</sup> for HWE and <sup>b</sup> for linkage disequilibrium.