

Appendix 1: Genetic Data Collection Methods

***Chionactis occipitalis* Microsatellite Data**

We amplified 9 microsatellite loci for 63 individuals from 11 populations throughout the U.S. species range (including 26 individuals from 7 populations within the Mojave Desert study region). Tissues were obtained and DNA was extracted following Wood et al. [1]. Loci were developed from a repeat-enriched genomic library sequenced on a 454 automated DNA sequencer (Roche) at the Sequencing and Genotyping Facility at Cornell University Core Laboratory Center. Of the 11,514 sequences obtained, 3,847 contained microsatellite repeats. We tested 32 loci for variability across 6 individuals, and selected 10 variable loci that amplified consistently for further analysis. These 10 loci were amplified in 3 multiplex reactions using a Qiagen multiplex PCR kit. Each 10 µL reaction contained 5 µL of Qiagen multiplex PCR Master Mix, 1 µL primer mix (containing 2 µM of each primer) and 1 µL Q-solution. Fragment analysis was performed on an ABI 3100 in the CSUPERB Microchemical Core Facility at San Diego State University. We used GENE-MARKER v1.90 (SoftGenetics) to edit the raw allelic data and score allele sizes. We used MICROCHECKER [2] to test for the presence of null alleles and scoring errors. Primer sequences, individual genotypes and associated locality data were deposited in Dryad (doi:10.5061/dryad.t0j7s).

***Dipsosaurus dorsalis dorsalis* mtDNA Sequence Data**

We gathered 12 tissue samples of *Dipsosaurus dorsalis dorsalis* from museum specimens and wild caught individuals, from 8 localities within the study area and 4 localities near the border of the study area. Museums that loaned tissue for the study are the Museum of Zoology at the University of California, Berkeley, Museum of Vertebrate Zoology (MVZ137449, MVZ150059, MVZ230560), California Academy of Sciences (CAS208708), and the Yale Peabody Museum of Natural History (YPM13364, YPM13527). Specific locality data were included with the Genbank haplotype submissions (see below).

DNA extraction and sequencing of the mitochondrial ND4 locus was accomplished using the methods outline in Stephen et al. 2012 [3], resulting in 8 haplotypes of length 815 bp. Haplotypes were deposited in Genbank, along with the corresponding geographical information (Genbank Accession Numbers: KC433320-KC433331).

***Uma scoparia* Nuclear Sequence Data**

DNA samples from 93 *Uma scoparia* were collected during 2008 from throughout the species range in California and Arizona. Lizards were live-captured with a noose. Tail tips were removed at an inter-vertebral break point and preserved in 95% ethanol on ice. We used a GPS unit to record location and elevation before photographing and releasing each lizard.

We developed a small-insert genomic library from an individual *U. scoparia* (BDH061, San Diego Natural History Museum, California), which allowed us to design PCR primers for five new anonymous loci, three of which were used in this study (Uma06, Uma07, Uma08) [4]; Gottscho 2010). DNA extraction and amplification methods are also detailed in Gottscho 2010 [4]. PCR products were sequenced using an ABI3730 sequencer (Applied Biosystems, Foster City, CA, USA) at the University of Washington High-Throughput Genomics Center. We used CodonCode Aligner v3.5.2 (CodonCode

Inc., Dedham, MA) to resolve heterozygous indels, call heterozygous SNPs, and create sequence alignments for each locus. Resulting sequences and corresponding geographical information were deposited in Genbank (KC526641 - KC526868)

References

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2. Van Oosterhout, C.; Hutchinson, W.F.; Wills, D.P.M.; Shipley, P. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* **2004**, *4*, 535–538.
3. Stephen, C.L.; Reynoso, V.H.; Collett, W.; Hasbun, C.R.; Breinholt, J.W., Geographical structure and cryptic lineages within common green iguanas, *Iguana iguana*. *J.Biogeogr* **2012**, in press.
4. Gottscho, A.D. Coalescent analysis of fifteen nuclear loci reveals Pleistocene speciation and low genetic diversity in the Mojave fringe-toed lizard, *Uma scoparia*. M.A. Thesis, Humboldt State University, Humboldt, CA. USA, 2010. Available online: <http://humboldt-dspace.calstate.edu/xmlui/handle/2148/581/> (accessed on 14 December 2012).