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RESEARCH ARTICLE

Chuckwallas of Punta La Cholla

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Introduction

Assessing the impact of habitat fragmentation is a central theme in conservation biology. Anthropogenic landscape change affects habitat connectivity and species' ability to maintain natural patterns of gene flow. As habitat fragments become smaller and smaller so, too, do populations. General principles of population genetics dictate that small populations are affected by increased genetic drift and inbreeding, which decrease genetic variation and heterozygosity and make the population more vulnerable to extinction; called an extinction vortex.

Common Chuckwalla/Cachorón de Roca (*Sauromalus ater*); Punta La Cholla, Sonora, Mexico; March 2017. Photo by T. Edwards.

Many species have populations that are naturally disjunct and these same principles apply, although the time frame of geologic or environmental change may be dramatically different than when humans manipulate the landscape. Consider, however, a species that arrives on an island; here, the few individuals that are now isolated from their mainland population face many challenges to exist. While the vast majority of these events likely lead to death, over evolutionary time some individuals do survive and establish new populations despite the detriments of inbreeding and drift. The Galapagos Islands are the classic example of small population persistence.

The Sonoran Desert's heterogeneous landscape is an excellent location to examine species with small, disjunct populations as well as the modern influence of increasing habitat fragmentation due to anthropogenic landscape change. An example of this can be ob-

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Figure 1. Punta La Cholla, Cholla Bay, Sonora Mexico. Red line indicates approximate areas explored for visual encounter surveys. Markers show locations of samples collected for DNA analysis.

served in Desert tortoise (*Gopherus morafkai*). Tortoises in the Sonoran Desert generally inhabit rocky foothills characteristic of Arizona Upland Sonoran desertscrub. Although foothill populations appear to be isolated by low desert valleys, radiotelemetry and genetic data show that tortoises are capable of making long-distance movements between populations. The most likely scenario for the desert tortoise is that gene flow occurs not at a regular rate, but with varying frequencies over time related to environmental fluctuations (Morafka 1994). For example, there may be increased potential for successful emigration in years of high rainfall.

Another Sonoran Desert reptile that shares a similar distribution and potentially a similar evolutionary history with the desert tortoise is the common chuckwalla, *Sauromalus ater*. Chuckwallas are saxicolous (rock dwelling) lizards that occur in disjunct, rocky environments throughout the Sonoran and Mojave deserts. Chuckwallas are different, however, in that they are less mobile (they cannot travel as great of distances) and they have shorter generation times (so genetic changes in a population may accumulate more quickly). Within the genus *Sauromalus* there are several different species, some even having evolved on islands in the Sea of Cortez and on the Baja peninsula as a result of isolating events over geologic time. We investigate an isolated population of chuckwallas at Punta La Cholla near Cholla Bay, Sonora, Mexico to better understand its origins and its connectivity to other populations in the surrounding landscape. We also assess the impact of increasing human development in the area that is contributing further to the isolation of this small population and the other inhabitants of Punta La Cholla.

Methods and Results

Sample collection

We performed visual encounter surveys for Chuckwallas (*Sauromalus ater*) at Punta La Cholla, Sonora Mexico on 11 and 12 March 2016. We surveyed the entire perimeter of the Punta La Cholla as well as hiked in 2 larger canyons on the east side of the mountain. We observed live animals and sign (tracks in front of burrows and scat) at several different locations. Because of the difficulty in catching animals and the stress caused to the animal by handling and drawing blood, we collected scat samples for DNA analysis. We selected scat samples that were as fresh as possible to reduce the amount of degradation to the DNA caused by heat and UV light. We considered darker samples to be fresher because older scats tend to bleach in the sun. Although scat was observed and collected in the two canyons on the east side of the mountain, no live animals or signs of recent activity were observed at that time. We observed live animals and collected scats for DNA analysis from two locations on the west side of the mountain (Figure 1).

DNA isolation

To isolate Chuckwalla DNA from the scat, we targeted the epithelial cells on the outside of the scat sample which consists of cells that were shed from the intestinal lining of the animal. The scat itself is composed primarily of plant material (indigestible cellulose / fiber) and contains mostly DNA of the food items. We instead wanted to target the DNA of the study animal. We collected these cells by following the swabbing protocol of Rutledge et al. (2009). We gently swabbed the surface of the scat with a buccal swab (Whatman OmniSwab, WB100035; Whatman Inc., USA) dampened with lysis buffer. We then stored the swab in ~650 µl of the same buffer and returned them to the University of Arizona Genetics Core for processing.

We isolated total DNA from 10 buccal swabs (collected from 10 separate scats) by overnight lysis with proteinase K at 55 °C, followed by robotic extraction using a QIAGEN BioSprint 96 robotic magnetic-par-

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Figure 2. Chuckwalla habitat identified at Punta La Cholla where live animals were observed and samples were collected. We observed chuckwallas to be associated with both sandstones on the west side of the mountains and granitic rock outcrops in the canyons on the east side.

Figure 3. Swabbing chuckwalla scat with a buccal swab soaked in lysis buffer for isolation of DNA.

ticle purification system (Qiagen, Valencia, California USA) and Aline Biosicences Buccal Swab gDNA Kit (Aline Biosciences, Woburn, Massachusetts USA). We quantified recovered DNA using a BioTEK Synergy HT (BioTEK, Vermont, USA). We successfully isolated DNA from all 10 swabs, yielding an average of 500 ng of DNA per sample (87.5–892.5), however, this is total DNA and therefore contains not only chuckwalla DNA, but also that from the plant food and bacteria.

DNA amplification

We selected and designed primers that target just the Chuckwalla DNA in our samples and that could be used to generate DNA sequences for our analysis. Primers are used in Polymerase Chain Reaction (PCR) to target a specific locus (or location) of the DNA and make copies so that the specific DNA regions of interest can be differentiated from other DNA in the sample. Because genetic studies have previously been performed on Chuckwallas, information on DNA sequences and amplifying primers were available in the scientific literature so that we could reproduce the methods of these previous studies. We used the published primers pairs from two of these studies which amplify between 855 and 980 b.p. (basepairs; a measure of DNA sequence length) of DNA (Table 1; Kocher et al. 1989, Corl et al. 2010). Because we suspected that our DNA samples might be degraded from exposure to heat and light, resulting in shorter fragments of DNA available for amplification, we also designed our own primer pairs from sequences available online in the GenBank database (Table 1: GenBank Accession #AF020234; Petren and Case 1997). All PCR amplification targeted the Cytochrome *B* (Cytb) region of the mitochondrial genome. Mitochondrial DNA (mtDNA) is widely used in phylogenetic studies and Cytb is a standard barcoding locus. MtDNA is maternally inherited and males do not pass on their mtDNA to offspring, so this marker only details female genealogies.

Polymerase Chain Reaction is a process of precisely heating and cooling a chemical reaction and it is necessary to optimize each reaction for the specific combination of reagents and temperatures to ensure that the primers are targeting only the region of interest and are not producing additional sequences that may interfere with our ability to 'read' the sequence data. We optimized PCR for each primer pair using all 10 DNA samples by performing a touchdown PCR from 62-52 °C, stepping down 0.5 °C per cycle, and assessing 8 different concentrations of $MgCl_2$ ranging from 1.0-4.5 mM. We then ran an agarose gel on the PCR product to assess if the target amplicon was recovered during the reaction. We were able to identify which reactions were successful by which conditions produced a visible band on an agarose gel (PCR product).

We identified PCR conditions that worked successfully for all primer pairs; including the ones that generate longer DNA sequences. We also noted that not all of our DNA samples had viable DNA and thus failed under all conditions (Table 2). We next selected the 5 samples that were viable and ran the optimized PCR for the 2 longer sequences (855 and 980 b.p). We next

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Table 1. Primers used for PCR amplification. Primers designed from Genbank AF020234 submitted by K. Petren and T.J. Case (Sonoyta, Mexico).

Locus	Primer Name	Fwd Primer	Rev Primer	b.p.	Source
cytb	SAAT_Cytb_338f	GGCCTCTACTACGGCTCCTA	GAGTTGAGTCCGGTTGGGTT	338	Designed from AF020234
cytb	SAAT_Cytb_352f	AGCCTTCTCATCAGTTGCCC	GAATCGAGTTAGGGTCGCGT	352	Designed from AF020234
cytb	B1L / B6H	CCATCCAACATCTCAGCATGATGAAA	GTCTTCAGTTTTTGGTTTACAAGAC	980	Kocher et al. (1989)
cytb	SAAT_Cytb_855f	CCACCGTTGTTATTCAACTAC	GGTTTACAAGACCAATGCTTT	855	Corl et al. (2010)

Table 2. Results of PCR optimization assessing 4 different primer pairs and 8 different concentrations of $MgCl_2$ ranging from 1.0-4.5 mM (labeled as A-H). Each optimization reaction was run on each of 10 *Sauromalus* DNA samples isolated from scat.

Samples	Primers			
	SAAT_Cytb_338	SAAT_Cytb_352	SAAT_Cytb_855	B1L / B6H
SAAT_01A	*failed*	*failed*	*failed*	*failed*
SAAT_01B	*failed*	*failed*	*failed*	*failed*
SAAT_01C	*failed*	*failed*	*failed*	*failed*
SAAT_02A	A-H	A-H	A-H	E, F, G, H
SAAT_02B	A-H	A-H	A-H	H
SAAT_02C	A-H	A-H	A-H	E, F, G, H
SAAT_02L1	*failed*	*failed*	*failed*	*failed*
SAAT_02L2	A-H	A-H	A-H	G
SAAT_02L3	*failed*	*failed*	*failed*	*failed*
SAAT_02L4	E, F, G, H	G, H	E, F, G, H	*failed*

purified these PCR products for sequencing using a Rapid PCR Purification System Kit (Marligen Bioscience, Inc.). Before submitting samples for Sanger sequencing, we assessed their quantity and quality using a NanoDrop1000 spectophotometer (ThermoFisher Scientific, Inc.). We selected 2 of these purified samples for the cytb 855 b.p. locus for sequencing.

We submitted PCR products to the University of Arizona Genetics Core for DNA sequencing on a 3730XL DNA Analyzer (Applied Biosystems, Carlsbad, CA). We aligned sequences to a reference sequence using CLC DNA Workbench ver. 5.7.1 (CLC Bio, Aarhus, Denmark). The reference sequence (GenBank accession no. AF020233) was published by Petren and Case in 1997 for study titled A Phylogenetic analysis of body size evolution and biogeography in chuckwallas (*Sauromalus*) and other iguanines. We choose this study and sequence because it had the most available data for comparison to our sample. For each of our 2 samples, we sequenced both strands of the amplified DNA (forward and reverse) and using the CLC software, we were able to match our sequence to the entire 855 b.p. length of the reference sequence and identify positions in the DNA that were similar and different (Figure 4). The 2 samples we sequenced were not different from each other.

Petren and Case (1997) sequenced 28 individuals in their study and we built upon this to add our data to the phylogeny using BEAST v.1.7.5 (Drummond and Rambaut 2007) to reconstruct the phylogenetic relationships including our sample form Punta La Cholla and generate a phylogenetic tree. We used an HKY substitution model with the gamma parameter set to 4 and we chose a relaxed, log-normal clock and Yule pro-

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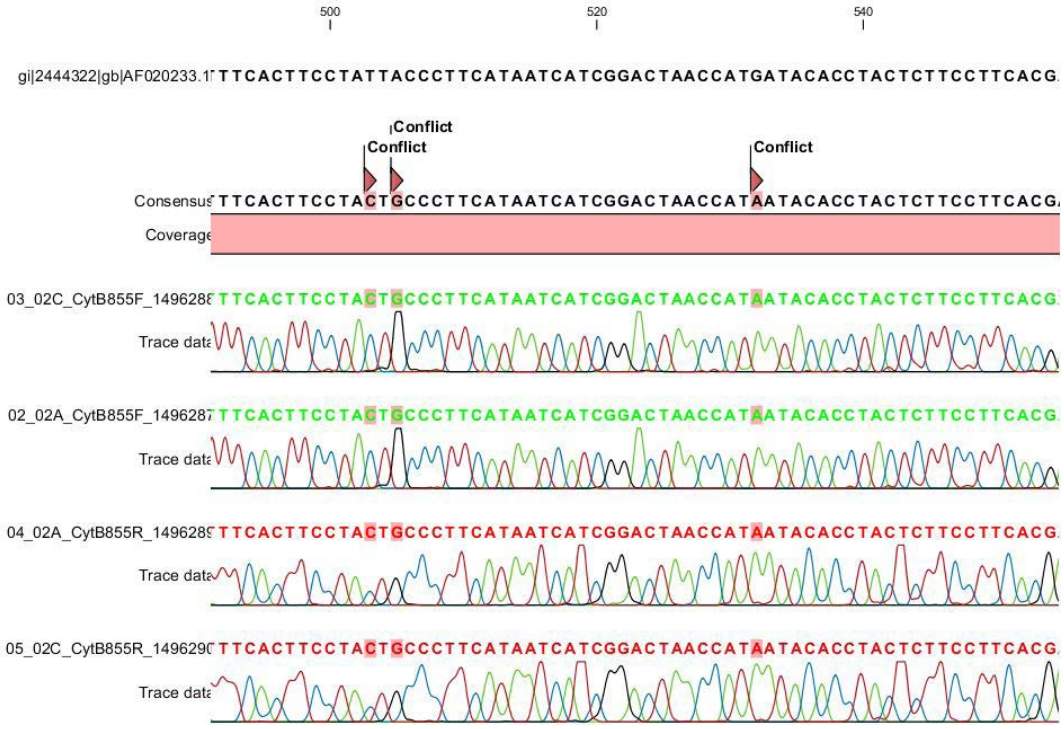

Figure 4. Electropherpgram alignment of two *Sauromalus ater* DNA samples collected at Punta La Cholla (forward and reverse sequences) aligned to a reference sequence AF020233 collected near Sonoyta, Mexico. Conflicts indicate where the sequences are different between the samples and the reference.

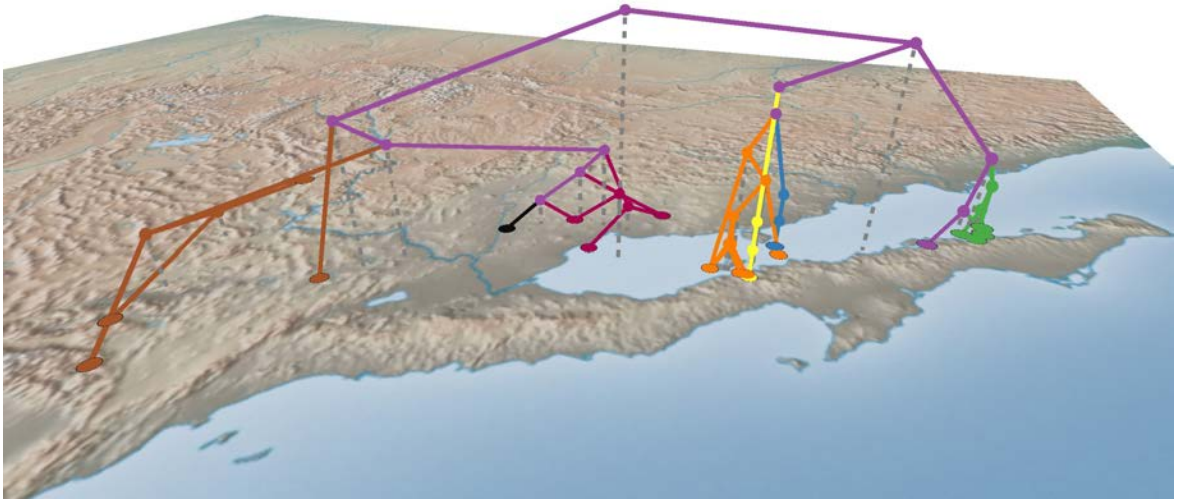

Figure 5. MtDNA sequence distributions and geographical associations of haplotypes of *Sauromalus* spp. Unrooted network generated using BEAST. The distribution of haplotypes exhibited a strong association with geography (MC permutation test, $P \leq 0.005$). Generated using Gengis 2.1.1 (Parks et al. 2009). Maroon-colored sites which cluster together represent lineages from Punta La Cholla, Sonoyta and Caborca.

cess model. We ran the MCMC for 500,000,000 generations, sampling every 5,000 with a burnin of 10%. We viewed results in TRACER: MCMC Trace Analysis Tool Version v1.6.0 (Rambaut et al. 2003-2013) and used TreeAnnotator v1.7.5 (Rambaut and Drummond 2002–2013) to select the Maximum Clade Credibility tree which has the highest product of the posterior probability of all its nodes from the BEAST analysis. We used FigTree Version 1.4.0 (Rambaut 2006-2012) to visualize the tree.

We then mapped this phylogenetic 'network' which represents the evolutionary relationships among these matrilines of *Sauromalus* to their geographic locations. We assigned geographic location information to the 28 samples from the Petren and Case (1997) study using Google Earth and used GenGIS v.2.1.1 (Parks et al. 2009) to visualize mtDNA sequence distributions and to test for geographic association of haplotypes (Figure 5). We used GenGIS to perform a Monte Carlo permutation test to determine if the fit of ordered leaf nodes at geographic locations from the tree topology was significantly greater than expected by chance alone (Parks and Beiko, 2009). As expected these samples, including the sample from La Cholla fit an expected geographic distribution with distribution of haplotypes exhibiting a strong association with geography (MC permutation test, $P \leq 0.005$).

We used DnaSP (v.5.10.01; Librado and Rozas, 2009) to estimate nucleotide diversity, polymorphism and other descriptive statistics among maternal lineages. Nucleotide divergence (Jukes Cantor) between N. Mexico and Punta La Cholla was 0.92% with 3 fixed differences unique to Punta La Cholla. In contrast, between California and northwestern Mexico (including Punta La Cholla), nucleotide divergence was 4.277%. Comparing differences just between the closest localities with sequence data to Punta La Cholla (Figure 6), we observed differentiation consistent with historic geographic isolation among sites (Table 3).

Table 3. Differentiation among *S. ater* mitochondrial Cytochrome *B* sequences from three sample locations in Northwestern Sonora, Mexico; Sonoyta ($n = 2$), Caborca ($n = 2$) and Punta La Cholla ($n = 2$). Sequence divergence (Dxy) estimates above the diagonal (Jukes and Cantor 1969) and number of fixed nucleotide differences between 902 b.p. total sequence below diagonal.

Pop	Sonoyta	Caborca	LaCholla
Sonoyta	—	0.011	0.009
Caborca	1	—	0.009
LaCholla	3	3	—

Although it would be desirable to have a precise estimate the amount of time that chuckwallas at Punta La Cholla and the surrounding area have been genetically isolated from regional populations (such as Sonoyta and Caborca), it is not accurate to do so using genetic data without calibrating it to geological or palaeontological references, such as by using fossil evidence (Benton and Ayala 2003, Reisz and Müller 2004). Unfortunately, the fossil record for iguanid lizards in western Mexico is very limited (Davy et al. 2011). As a comparison, however, Davy et al. (2011) studied spiny-tailed iguanas (*Ctenosaura* spp.) in this region and within *C. macrolopha* (located on the mainland in southern Sonora), they generated similar estimates of divergence (average pairwise genetic divergence - percentage uncorrected p-distances; 0.1 ± 0.0) to what we observed among northwestern, Mexican *Sauromalus* populations (Table 3). They estimate the time to most recent common ancestor for this node of their tree to be 1.66 Ma (95% HPD; 3.41–0.36). Assuming similar generation time, population size and dispersal ability, we might project that populations of *Sauromalus* in northwestern Mexico have undergone divergence over a similar timeframe and that individuals at Punta La Cholla and surrounding areas have been isolated at least since the Last Glacial Maximum, ~25,000 BCE. During this time, the Central Gulf

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Figure 6. Topographic relationship among *Saourmalus* populations occurring at Punta La Cholla, Sonoyta and Caborca. *Saourmalus* are saxicolous and occur in areas with rocky outcrops (darker areas). Lighter, tan areas representing low, sandy desert pose inhospitable environments for chuckwallas.

Coast within the Sonoran Desert likely maintained more xeric vegetation while surrounding areas were converted to woodlands during cooling trends (Anderson and Van Devender, 1995), allowing for chuckwalla populations to expand and facilitate gene flow among disjunct populations. In contrast, one core area of desert throughout most of the Quaternary may have occurred in the Gran Desierto and other surrounding, very arid parts of the Lower Colorado River Valley of the Sonoran Desert (Van Devender et al. 1987) and chuckwallas may instead have used these areas as refugia during these glacial periods and then expanded their populations during the interglacials.

Discussion

Chuckwallas around the coastal area of Rocky Point and Cholla Bay are isolated and have been for a long time. Our data suggests that the Punta La Cholla chuckwallas are closely related to other regional populations (Caborca and Sonoyta) but we cannot accurately estimate a precise date of divergence. It is likely on the order of several thousands of years. It is certainly possible they were moved there by native people (there is documentation that the Seri moved *Sauromalus* and *Ctenosaura* around) and there were definitely native peoples in the Penasco area. Otherwise the lizards had to cross a lot of sand and dunes to get to these coastal areas (unless they got there when there was no sand; >5 million years ago). It would be highly unlikely for chuckwallas to maintain gene flow across these landscapes under current conditions.

These coastal areas, however, likely have maintained gene flow among small populations and this helps explain their persistence. There are quite a few museum records and specimens from Puerto Penasco, Cholla Bay, Las Chollas Point, Granite Mountain near Cholla Bay, Cerro La Cholla 6 mi WNW of Puerto Pensaco, Cerro Prieto 5 mi NE of Puerto Penasco, and near Cholla Bay. Jim Rorabaugh (pers. comm.) states that; "*in the 80s we found Chuckwallas at what we call El Capitan, which is small, mostly slate mountain ~25 miles ESE of San Luis Rio Colorado. Those animals are also very isolated by a sea of sand. There is a big slate mine in that range so hopefully the chucks are still extant. We have looked for saxicolous species on the small range of hills about 6 miles NW of El Capitan just over the border in Arizona and have not seen chucks there. As far as I know, no one has found them at the Sierra del Rosario (and I have not seen them there on my several trips to that range)*". Chuckwallas also occur in the Sierra Pinacate and adjacent ranges north and west to the Sierra Tinajas Altas. Because population sizes of chuckwallas is likely small at each of these sites, dispersal events probably play an important role in the long-term maintenance of these populations.

The current urbanization and development around Cholla Bay and Puerto Penasco has by all indications isolated the small population of chuckwallas at Punta La Cholla from its neighboring, coastal popu-

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Figure 7. Art installation, *Isle of Sauromalus*, exhibited at the University of Arizona Museum of Art (UAMA); December 22, 2018 – March 31, 2019 as part of the as part of the Next Generation Sonoran Desert Researchers (N-Gen) 6&6 Arts and Science Initiative. See: *https://www.heathergreen-art.com/6-6-six-artists-six-scientists* UAMA Photo Documentation by Wilson Graham.

lations (and contributed to the extirpation of animals from Puerto Penasco). This population is essentially an island completely surrounded by development and impenetrable barriers such as ORV recreation areas. It is inevitable that this small population will someday experience a decline, if it is not already. This may be through natural causes such as drought or from human-related activities including habitat degradation from invasive species. Since there is no dependable way for new individuals to naturally immigrate into the population due to the roads and development that fragment the landscape, the population has a high probability of becoming extirpated.

We used these data to help tell the story of this small, isolated population of chuckwallas and their home in at Punta La Cholla as part of the Next Generation Sonoran Desert Researchers (N-Gen) 6and6 Arts and Science Initiative. In a series of artists' books, we shared a narrative about our experience searching for these elusive creatures, and the increasing impact of human development that is compounding their isolation. Along the walls of the installation, a display of the unique sequence of La Cholla chuckwalla DNA was engraved upon sheets of mica; expressing the close tie between the population's evolutionary history and the geologic constraints of their habitat. However, the exhibit expanded beyond just chuckwallas to tell a more complete story of the flora, fauna, and past and present visitors of Punta La Cholla. We strived to create a sense of place and describe its magnificent and idiosyncratic natural and cultural history so that Punta La Cholla and all its inhabitants might not go unnoticed as the world changes around them.

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RESEARCH ARTICLE

Night lizards (*Xantusia*) and their discoverers on the Baja California Peninsula

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For three decades after Spencer Fullerton Baird (1859; Fig. 1) described *Xantusia vigilis* (Desert Night Lizard), the species was considered rare. This changed dramatically when John Van Denburgh (Fig. 2) and Charles Gilbert (Fig. 3) discovered that the lizards live in fallen branches of Joshua Trees (*Yucca brevifolia*; Van Denburgh 1895). Gilbert's role in the discovery of this unique reptile-plant association surely was among the reasons that Van Denburgh (1897) chose to name a *Xantusia* species for his Stanford mentor.

The type specimen of *Xantusia gilberti* (Gilbert's Night Lizard; Fig. 4) was collected in 1892 at "San Francisquito" by Gustav Eisen (1897; Fig. 5), the intrepid Swedish-American naturalist who climbed, named, and mapped the peaks of the rugged Sierra Laguna on the Cape of the Baja California Peninsula. Type localities are intriguing, particularly when they represent the site of the original discovery of a new species. In 1965, with the help of a guide from Rancho La Burrera, Wade Sherbrooke and Robert Bezy (Fig. 6) made a pilgrimage to this obscure locality in the Sierra Laguna to collect specimens (topotypes) of *X. gilberti* (UAZ 17521-22). From Eisen's (1897) map it seems likely that the spring visited was indeed his San Fancisquito.

After 125 years, *Xantusia gilberti* remains known only from localities atop the Sierra Laguna (Galina-Tessaro et al. 1995, 2003; Segura-Trujillo et al. 2012). These are in oak (Fig. 7) and pine-oak woodlands that share many species with the Sierra Madre Occidental of the mainland. The lizards live under rocks and beneath the logs and bark of piñons, oaks, and nolinas, and they give birth in August (Luja and Bruno Granados 2011).

From the Sierra Laguna, the Magdalena Plain stretches 500 km north along the Pacific to Laguna San Ignacio (Shreve 1937, Fig. 8; Brown et al. 2007, Fig. 9). Over much of the length of this strip of Sonoran Desert, night lizards are unknown (Fig. 10). Cape Yuccas (*Yucca*

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Fig. 1. (left) Spencer Fullerton Baird (1823-1887), naturalist, first curator of the National Museum, Smithsonian Institution. Photo, ca 1850, nine years before he described *Xantusia vigilis* (Desert Night Lizard). Smithsonian Institution Archives.
Fig. 2. (center) John Van Denburgh (1872-1924), herpetologist, California Academy of Sciences. Photo ca 1894, about the time he and Gilbert discovered the Joshua Tree habitat of *Xantusia vigilis* (Desert Night Lizard). Stanford Historical Photograph Collection.
Fig. 3. (right) Charles H. Gilbert (1859-1928), ichthyologist, Stanford University, who discovered the habitat of *Xantusia vigilis* with John Van Denburgh and persuaded him to concentrate on herpetology. Photo 1880, Gilbert Ichthyological Society.