

MITOCHONDRIAL DNA DIVERSITY AND PHYLOGEOGRAPHY OF *LUCANIA INTERIORIS* INFORM BIODIVERSITY CONSERVATION IN THE CUATRO CIÉNEGAS BASIN, MÉXICO

Evan W. Carson^{1,4}, Valeria Souza², Hector Espinosa-Pérez³, and Thomas F. Turner¹

ABSTRACT.—The Cuatro Ciénegas killifish *Lucania interioris* is an endemic fundulid in the Chihuahuan Desert valley of Cuatro Ciénegas, Coahuila, México. It occurs in 3 major drainage regions of the basin and is limited primarily to naturally fragmented and physicochemically severe habitats of the valley floor. We assessed mitochondrial DNA variation and population genetic structure in *L. interioris* from 8 populations representative of the entire geographic range of this species, including its distribution within and among major drainage regions (western, central, and southeastern) of the valley. Five populations were invariant, including both populations in the western region (Haplotype A only), whereas 2 haplotypes were observed in each of the remaining populations. Haplotype diversity was low in the central basin ($H_D = 0.10$ and $H_D = 0.19$) and moderate in the southeastern basin ($H_D = 0.52$). Low levels of divergence among haplotypes (0.3%–0.9%), together with high levels of genetic differentiation among regions ($F_{CT} = 0.847$, $P < 0.001$), indicate complete isolation among major drainage regions. These results agree with previous documentation of morphological differentiation between populations from the western and central basins. Populations in the southeastern basin are divergent genetically and, therefore, also may exhibit morphological distinction.

RESUMEN.—El pez *Lucania interioris* del valle de Cuatro Ciénegas en Coahuila, México es endémico al valle. La especie ocurre en 3 cuencas principales dentro del valle donde está limitado a sistemas fragmentados y extremos físico-químicos. Determinamos la diversidad mitocondrial y la estructura genética de 8 poblaciones muestreadas en todo el rango de distribución de la especie. Cinco poblaciones no presentaron diversidad genética, incluyendo ambas poblaciones del Oeste (solo Haplotipo A); 2 haplotipos mas fueron observados en las otras poblaciones. Entre las poblaciones genéticamente mas diversas, la diversidad fue mas baja en la parte central del valle ($H_D = 0.10$ y $H_D = 0.19$) y moderadamente diversa en la zona Sudeste ($H_D = 0.52$). A pesar de que la divergencia entre haplotipos es baja (0.3%–0.9%), la estructura genética es significativa entre regiones ($F_{CT} = 0.847$, $P < 0.001$) indicaron que el aislamiento entre las principales regiones de drenaje se ha completado, pero no antiguo. Estos resultados concuerdan con trabajos morfológicos previos donde se aprecian diferencias visibles entre las poblaciones del Oeste y parte Central del valle sugiriendo que la variación morfológica es mayor que lo que se había reportado.

The Chihuahuan Desert basin of Cuatro Ciénegas, Mexico, is a small, intermontane valley (~1200 km²) renowned for its biodiversity and high incidence of endemism among aquatic organisms (Minckley 1969, Souza et al. 2012). The fish fauna, for example, is unusually diverse for a desert region of North America (Minckley 1984) and is eclectic in taxonomic composition and biogeographic affinities. Nine Nearctic and Neotropical families are represented among 16 species, of which half are endemic (Minckley 1984). Biogeographic relationships include close associations to forms in the Mexican Plateau (pupfish [Cyprinodontidae], Echelle et al. 2005), Mesoamerica (cichlids [Cichlidae], Hulsey et al. 2004; platyfish [*Xiphophorus* spp.], Kang et al. 2013), and the Rio Grande basin (several species,

Miller 1978; Minckley 1978). Similarly, the springsnails (Hydrobiidae) are represented by 9 genera (5 endemic and 3 monotypic) and 12 species. At least 9 springsnails are endemic to the basin (Hershler 1985), and one exhibits substantial morphological variation among populations (Taylor 1966, Hershler 1985). Endemic taxa also are represented among arthropods (Cole 1984), reptiles (McCoy 1984), and vascular plants (Pinkava 1984), while exploration of the spectacular biodiversity of the bacterial communities remains in its infancy (Souza et al. 2006, 2012).

The high concentration of endemic species in Cuatro Ciénegas has drawn comparison to that of the Galápagos Islands (Souza et al. 2012). Although there is wide appreciation of the aquatic biota of Cuatro Ciénegas, few

¹Department of Biology and Museum of Southwestern Biology, MSC03 2020, University of New Mexico, Albuquerque, NM 87131-0001.

²Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, AP 25-275, Coyoacán 04510, México D.F., México.

³Colección Nacional de Peces, Instituto de Biología, Universidad Nacional Autónoma de México, AP. 70-153, Coyoacán 04510, México D.F., México.

⁴E-mail: evan.carson@gmail.com

of these organisms have received research attention since first described. Several phylogeographic studies have, however, provided important insights into hydrogeographic and climatic influences on population structure and evolutionary diversification of endemic and native aquatic species, including representatives from such taxonomically disparate groups as teleost fishes (pupfishes [Cyprinodontidae], Carson and Dowling 2006; cichlids [Cichlidae], Chaves-Campos et al. 2011b; sunfish [Centrarchidae], Coghill et al. 2013), gastropods (springsnails [Rissooidea], Moline et al. 2004, Johnson 2005, Chaves-Campos et al. 2011b), reptiles (box turtles [Emydidae], Howeth et al. 2008), and crustaceans (freshwater shrimp [Palaemonidae], Chaves-Campos et al. 2011a). The spatial distribution of genetic variation in these taxa is varied but includes examples of strong partitioning by drainage region (pupfishes and springsnails); of separation by the east and west divides of the basin (sunfish, springsnails, and box turtles); and of weak or absent signals of geographic structure (cichlids and shrimp). Further, evolutionarily distinct populations are prevalent among isolated populations of pupfishes and springsnails.

Phylogeographic differences among species are unsurprising because organisms differ in ecology, habitats, physicochemical tolerances, and vagility as well as in evolutionary age (i.e., some organisms likely have resided in the basin longer than others). An important question, however, is whether the valley hosts 'hotspots' of biodiversity. If so, then unsustainable exploitation of water resources in Cuatro Ciénegas and the intensification of groundwater extraction in the adjacent basins (Souza et al. 2006, 2012) may threaten biodiversity at a greater scale than recognized. The seriousness of these threats is exemplified by drying of the Río Cañon (former municipal water source for the town of Cuatro Ciénegas [Minckley 1992]) and of most of the Río Churince system (Carson, Souza, and Espinoza-Pérez personal observations). In both cases, degradation occurred rapidly following development of intensive alfalfa-farming operations in adjacent basins (Calaveras and Valle del Huido, respectively), with the decline of Churince also influenced by gradual effects of canalization of Becerra spring in the 1960s (Minckley personal communication). Conservation of the aquatic biodiversity of Cuatro

Ciénegas will in part require a more thorough assessment of evolutionary diversity within and among endemic and native aquatic organisms of the valley.

We used mitochondrial DNA (mtDNA) variation to describe geographic variation in genetic diversity and population genetic structure of the Cuatro Ciénegas killifish *Lucania interioris* (Hubbs and Miller 1965). This fundulid is endemic to and abundant in the Cuatro Ciénegas basin, where the species maintains a broad but fragmented distribution within and among western, central, and southeastern drainage regions of the valley (Fig. 1). *Lucania interioris* primarily inhabits shallow, vegetated areas of environmentally harsh laguna and marsh systems of the valley floor (Hubbs and Miller 1965). These habitats are prone to extreme and rapid fluctuations in temperature, salinity, and size. Consequently, populations of *L. interioris* are subject to large and rapid changes in local abundance ('boom and bust' ecology), to fragmentation and, presumably, to high rates of genetic drift that accompany small populations (Frankham et al. 2002, Allendorf and Luikart 2007). Prolonged isolation in *L. interioris* is evident from morphological differentiation between populations from the Río Garabatal system of the western basin and those from the Río Mezquites system of the central basin (Hubbs and Miller 1965). Morphological variation may be greater than documented because most populations, including those from the remote southeastern basin, remain unstudied. Comprehensive descriptions of spatial patterns of genetic variation in *L. interioris* will improve understanding of the biodiversity of *L. interioris* and will contribute to broader understanding of the biodiversity and population structure of species distributed primarily on the valley floor. These species, of which only the pupfish *Cyprinodon atrorus* has been studied genetically (Carson and Dowling 2006, Carson et al. 2012), typically have high innate potential but rare opportunity for broad dispersal in this arid landscape. Among these species, however, the distribution of genetic diversity may differ depending on their particular life history and biological characteristics. Conservation implications for *L. interioris*, and the relationships of our findings to the broader challenges to conservation of aquatic biodiversity in the Cuatro Ciénegas basin, are discussed.

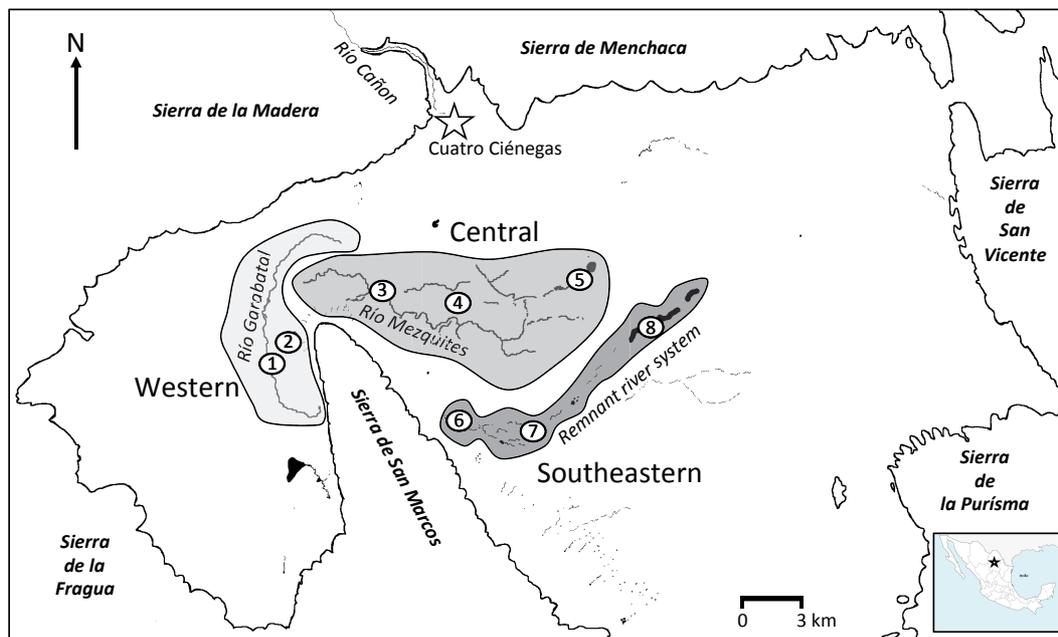


Fig. 1. Map of the Cuatro Ciénegas basin, including 8 collection localities among the 3 major drainage regions in which *Lucania interioris* occurs, with the western region highlighted in light gray, the central basin in medium gray, and the southeastern basin in dark gray. Collection localities are as follows: (1) Río Garabatal; (2) Juan Santos; (3) Las Salinas; (4) El Laberinto; (5) Laguna de los Burros; (6) Tío Cándido; (7) Los Hundidos; and (8) Laguna San Pablo. Map modified from Figure 2 of Minckley (1969).

METHODS

We collected 158 *L. interioris* from 8 sites among 3 major hydrogeographic regions (western, central, and southeastern) of the Cuatro Ciénegas basin (Fig. 1). These localities encompass the entire geographic range of the species, including its distribution among and within major drainage systems. Collections in the western basin were obtained from marsh habitats associated with Juan Santos spring ($n = 20$) and the Río Garabatal ($n = 20$). Collections in the central basin were made in a marsh complex associated with the Río Mezquites and Río Puente Chiquito systems and included Las Salinas ($n = 20$) in the upper system, El Laberinto ($n = 20$) in the middle system, and Laguna de los Burros ($n = 18$) in the lower system. In the southeastern basin, collections were made across a naturally fragmented river system and included marsh habitats associated with Tío Cándido ($n = 20$) in the upper system, Los Hundidos ($n = 20$) in the middle system, and Laguna San Pablo ($n = 20$) in the lower system. Extensive surveys

by E.W. Carson, W.L. Minckley, and D.A. Hendrickson indicated that, for unknown reasons, *L. interioris* is absent from Churince and other systems of the western basin and from the southeastern-basin systems of Pozas Azules and Santa Tecla (see Figs. 2 and 27 in Minckley 1969). Voucher numbers at Universidad Nacional Autónoma de México in Mexico City are as follows: Juan Santos, CNPE-IBUNAM18440; Río Garabatal, CNPE-IBUNAM18441; Las Salinas, CNPE-IBUNAM18442; El Laberinto, CNPE-IBUNAM18443; Laguna de los Burros, CNPE-IBUNAM18444; Tío Cándido, CNPE-IBUNAM18445; Los Hundidos, CNPE-IBUNAM18446; and Laguna San Pablo, CNPE-IBUNAM18447.

Fish were captured by seining (4 × 6-ft., 0.25-inch mesh) and dip-netting (0.25-inch mesh) and preserved in 95% ethanol. Whole genomic DNA was extracted using the modified phenol-chloroform method of Tibbets and Dowling (1996). Genetic variation was screened using single-strand conformation polymorphism (SSCP) analysis of a 338-base pair (bp) fragment of the mitochondrial cytochrome *b* gene

(*cyt b*). The SSCP analysis followed the protocol of Carson and Dowling (2006) except that PCR amplifications were conducted with primers LC and HD (Schmidt et al. 1998). From each SSCP gel, 2–5 representatives of each DNA-sequence variant were sequenced for a 601-bp fragment of *cyt b* (SSCP fragment = positions 274–601). This fragment was amplified with primers LA (Schmidt et al. 1998) and HD in 30- μ L reaction volumes of \sim 100 ng whole genomic DNA, 1X GoTaq[®] Flexi Buffer (Promega, Madison, Wisconsin, USA), 1.5 mM MgCl₂, 0.5 μ M of each primer, 250 μ M of each dNTP, and 1.7U GoTaq *Taq* polymerase (Promega). Amplification conditions included initial denaturation at 95 °C for 3 min followed by 30 cycles at 95 °C for 1 min, 48 °C for 1 min, and 72 °C for 2 min, and a final extension for 10 min at 72 °C. Sequencing reactions were performed using the primer LA and a BigDye[®] Terminator Kit (ver. 3.1; Applied Biosystems, Foster City, California, USA). Products were separated on an ABI 3100 capillary sequencer (Applied Biosystems), and visual alignment and editing of sequences were performed in Sequencher 4.0 (Gene Codes Corp., Ann Arbor, Michigan, USA). Protein coding was verified in Mega5 (Tamura et al. 2011).

Number of haplotypes (H_N), haplotype diversity (H_D), and nucleotide diversity (π) were determined using DnaSP 5.10.01 (Rozas et al. 2003); all analyses were based on the SSCP fragment. Homogeneity of haplotype distributions among localities was evaluated through global exact tests as implemented in Arlequin (Excoffier and Lischer 2010; ver. 3.5.1.2, <http://cmpg.unibe.ch/software/arlequin35/>). Pairwise differences (between locations) were assessed via the F_{ST} analogue Φ_{ST} , with estimates based on pairwise genetic distances and with significance determined by exact tests (Raymond and Rousset 1995, Goudet et al. 1996) performed in Arlequin. Sequential Bonferroni correction was used to adjust for multiple simultaneous tests (Rice 1989). Population genetic structure was evaluated using analysis of molecular variance (AMOVA) as implemented in Arlequin. Three population groups were specified based on major drainage regions ii–vi identified by Minckley (1969) as follows: region ii—western basin (Río Garabatal + Juan Santos); regions iii and iv—central basin (Las Salinas + El Laberinto + Laguna

de los Burros); and region v—southeastern basin (Tío Cándido + Los Hundidos + Laguna San Pablo). Based on population genetic structure of the ecologically similar and sympatric pupfish *Cyprinodon atrorus* (Carson and Dowling 2006), for this study regions iii and iv of Minckley (1969) were combined into a single drainage region. To visualize intraspecific relationships of haplotypes within and among populations, Network ver. 4.6.1.1 (fluxus engineering.com) was used to construct minimum spanning networks. Haplotypes were clustered using the full median joining algorithm (Bandelt et al. 1999), and superfluous connections were removed via maximum parsimony (Polzin and Daneshmand 2003).

RESULTS

Five *cyt b* haplotypes (A–E; GenBank accession numbers KF999966–KF999970) were detected among 158 specimens of *L. interioris* (Table 1). All substitutions were synonymous, 3rd-position changes except for a nonsynonymous (A–T), 1st position transversion (from Asn–Tyr) that separated the southeastern basin specimens from those in the central and western basins; an additional substitution (synonymous) unique to southwestern basin samples was detected in the sequenced LA-HD-fragment at site 90 (i.e., outside SSCP fragment). The parsimony network of SSCP haplotype relationships and frequencies is shown in Figure 2. Five populations were invariant including Río Garabatal and Juan Santos (Haplotype A), Laguna de los Burros (Haplotype C), and Tío Cándido and Los Hundidos (Haplotype D). Two haplotypes each were observed in and shared between Las Salinas and El Laberinto (haplotypes B and C). Laguna San Pablo also segregated for 2 haplotypes (D and E). Sequence divergence between haplotypes from the same population or region was 0.3% for all comparisons, whereas divergences between haplotypes from different regions ranged from 0.3% to 0.9%. The largest sequence divergence (0.9%) was between Haplotype E (southeastern region) and haplotypes A (western region) and C (central region). Haplotype diversity (H_D) was higher in Laguna San Pablo (0.52) than in Las Salinas (0.19) and El Laberinto (0.10). Nucleotide diversity (π_D), though difficult to evaluate statistically, was higher in Laguna San Pablo

TABLE 1. The distribution of mtDNA *cyt b* haplotypes within and among populations of *Lucania interioris*. Regions as shown in as in Fig. 1.

Haplotype	Western			Central				Southeastern				Total
	Río Carabatal	Juan Santos	Laguna Salada	Central marshes	Charcas Prietas	Tío Cándido	Los Hundidos	Los Gatos				
A	20	20	—	—	—	—	—	—	—	—	—	40
B	—	—	2	1	—	—	—	—	—	—	—	3
C	—	—	18	19	18	—	—	—	—	—	—	55
D	—	—	—	—	—	20	20	11	—	—	—	51
E	—	—	—	—	—	—	—	9	—	—	—	9
TOTAL	20	20	20	20	18	20	20	20	20	20	20	158

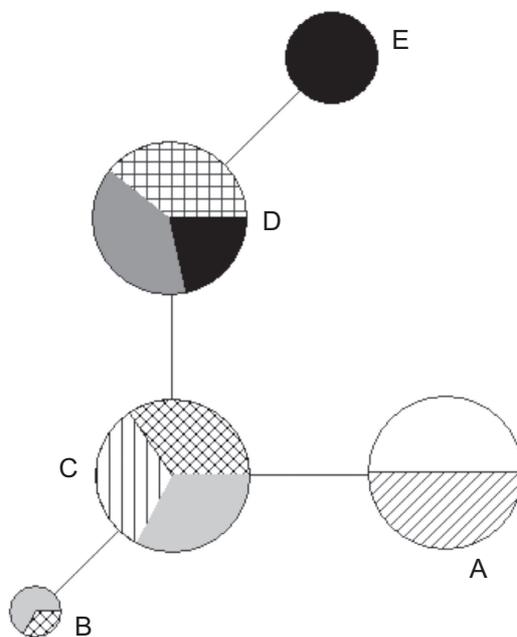


Fig. 2. Median-joining network of *cyt b* haplotypes of *Lucania interioris* sampled from the 8 sites that represent the entire geographic distribution of the species within and among drainage regions of the Cuatro Ciénegas basin. Circles represent SSCP haplotypes (A–E) with diameter scaled to haplotype frequency. Haplotype occurrence by major drainage region: western basin, Haplotype A; central basin, haplotypes B and C; and southeastern basin, haplotypes D and E. Haplotype occurrence by site: Garabatal, white; Juan Santos, forward diagonal; Las Salinas, light gray; El Laberinto, diamond crosshatched; Laguna de los Burros, vertical bars; Tío Cándido, square crosshatched; Los Hundidos, dark gray; Laguna San Pablo, black.

(0.0015) than in Las Salinas (0.0006) and El Laberinto (0.0003).

Global exact tests revealed significant heterogeneity of haplotype distributions among sites ($P < 0.001$), and pairwise estimates of Φ_{ST} (range 0.0–1.0; Table 2) were significant in 23 of 28 comparisons ($P < 0.001$ for all significant tests; Table 2), including all 21 between-region comparisons (Φ_{ST} range 0.7–1.0). Within regions, significant pairwise differences were found between Laguna San Pablo and Los Hundidos ($\Phi_{ST} = 0.42$) and Tío Cándido ($\Phi_{ST} = 0.42$). These differences resulted from fixation of Haplotype D in Los Hundidos and Tío Cándido versus similar frequencies of haplotypes D (55%) and E (45%) in Laguna San Pablo. Analysis of molecular variance revealed that genetic variation was associated with differences among populations ($F_{ST} = 0.953$,

other drainage regions and, potentially, from each other. A thorough assessment of the relationships between genetic and morphological variation in *L. interioris* is warranted.

Low levels of sequence diversity within populations of *L. interioris* are consistent with the high rates of genetic drift in small and isolated populations (Soulé 1980, Lynch et al. 1995, Frankham 1996). Further, given the aridification of southwestern North America during the Holocene (Smith 1981), *L. interioris* may have maintained low genetic diversity and, presumably, low effective population size (N_e) for a substantial period. Although genetic risks of extinction, such as inbreeding depression (Frankham 1995) and mutational meltdown (Lynch et al. 1995), appear to be low for *L. interioris*, this assessment could change with further loss of habitat. In fact, agricultural intensification in adjacent basins has been linked to decreases in surface-water flows and extensive loss of habitat in the Cuatro Ciénegas basin (Souza et al. 2006), while continued efforts toward sustainable development of water resources in Cuatro Ciénegas and adjacent basins have met with low success. Notably, habitat loss was implicated in increased demographic and genetic risk of extinction in the Cuatro Ciénegas platyfish *Xiphophorus gordonii* (Kallman 1964, Carson et al. 2013), a microendemic (IUCN 2014) that exhibits critically low genetic diversity at histocompatibility genes (Kallman 1964) and low to absent mtDNA sequence variance in remaining populations (Carson et al. 2013).

With increased threats from exploitation of water resources, differences in local or regional environmental characteristics within the Cuatro Ciénegas basin also may become relevant to conservation of *L. interioris* and other aquatic organisms of this valley. For example, subtle among-population differences in levels of genetic variation may, at least in part, reflect differences in local environmental conditions across the basin. Of the 3 populations of *L. interioris* that maintained genetic variability (Las Salinas, El Laberinto, and Laguna San Pablo), all were associated with expansive and relatively stable marsh systems (see Minckley 1969). In contrast, 4 of the 5 populations that exhibited haplotype fixation occurred either in small, remnant habitats of a naturally fragmented river system (Tío Cándido and Los Hundidos) or in habitats of the

western basin (Río Garabatal and Juan Santos), which lies in the rain shadow of the basin-dividing Sierra San Marcos. Nearly identical results were reported from throughout the basin for the endemic pupfishes, especially in *C. atrorus*, collected from the same localities or systems (Carson and Dowling 2006). Further, Johnson (2005) reported that the extremely low levels of mtDNA diversity in western basin populations of the Cuatro Ciénegas endemic springsnail *Mexipyrgus churinceanus* were consistent with a severe population bottleneck ~50,000 years before present. Thus, conservation of *L. interioris* and other aquatic organisms of Cuatro Ciénegas may, in part, depend on the attention given to relationships between habitat loss and prevailing local environmental characteristics within the basin.

Prolonged isolation of major drainage regions of Cuatro Ciénegas is evident in the geographic distribution of morphological and genetic variation in *L. interioris*. Similar findings have been reported for pupfishes (Carson and Dowling 2006, Tobler and Carson 2010, Carson et al. 2012), cichlids (Chaves-Campos et al. 2011b), and hydrobiid snails (Taylor 1966, Hershler 1985, Johnson 2005, Chaves-Campos et al. 2011b). As agricultural intensification continues in the region, this biota is becoming more imperiled. While species extinctions, as well as loss of local populations, are of concern, habitat loss also could threaten local hotspots of biodiversity. Biological conservation in Cuatro Ciénegas will depend in part on a more thorough understanding of the distribution of biodiversity within and among the indigenous aquatic organisms of the valley.

Priorities for future research include comprehensive evaluations of morphological and multilocus-DNA variation within and among populations throughout the geographic range of *L. interioris*. Most urgent, however, are conservation actions to arrest unsustainable development of water resources in Cuatro Ciénegas and surrounding basins. Habitat improvements, including restoration of flow to the Río Garabatal, are conservation imperatives as well. The hypothesis that biodiversity hotspots occur in the Cuatro Ciénegas should be tested rigorously, and consideration should be given to how the presence of such areas, should they exist, might shape water management policy and conservation strategies in the valley.

ACKNOWLEDGMENTS

We thank Hailey Conover for assistance in the laboratory and Alexandra Snyder for help with collections. The Carlos Slim Foundation and World Wildlife Fund México provided funding. Specimens were collected under Pesca de Fomento permit no. DGOPA00889.280211-0349 granted to H.E.-P. and followed IACUC protocol number 10-100492-MCC.

LITERATURE CITED

- ALLENDORE, F.W., AND G. LUIKART. 2007. Conservation and the genetics of populations. Blackwell Publishing, Malden, MA.
- BANDELT, H.-J., P. FORSTER, AND A. RÖHL. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16:37–48.
- CARSON, E.W., AND T.E. DOWLING. 2006. Influence of hydrogeographic history and hybridization on the distribution of genetic variation in the pupfishes *Cyprinodon atrorus* and *C. bifasciatus*. *Molecular Ecology* 15:667–679.
- CARSON, E.W., H. ESPINOSA-PÉREZ, AND V. SOUZA. 2013. Low mitochondrial DNA sequence variation in the microendemic Cuatro Ciénegas platyfish *Xiphophorus gordonii*. *Western North American Naturalist* 73: 224–229.
- CARSON, E.W., M. TOBLER, W.L. MINCKLEY, R.J. AINSWORTH, AND T.E. DOWLING. 2012. Relationships between spatio-temporal environmental and genetic variation reveal an important influence of exogenous selection in a pupfish hybrid zone. *Molecular Ecology* 21: 1209–1222.
- CHAVES-CAMPOS, J., S.G. JOHNSON, F.J. GARCÍA DE LEÓN, AND C.D. HULSEY. 2011a. Phylogeography, genetic structure, and gene flow in endemic freshwater shrimp *Palaemonetes suttkusi* from Cuatro Ciénegas, Mexico. *Conservation Genetics* 12:557–567.
- CHAVES-CAMPOS, J., S.G. JOHNSON, AND C.D. HULSEY. 2011b. Spatial geographic mosaic in an aquatic predator-prey network. *PLOS ONE* 6:e22472.
- COGHILL, L.M., C.D. HULSEY, J. CHAVES-CAMPOS, F.J. GARCÍA DE LEÓN, AND S.G. JOHNSON. 2013. Phylogeography and conservation genetics of a distinct lineage of sunfish in the Cuatro Ciénegas Valley of Mexico. *PLOS ONE* 8:e77013.
- COLE, G.A. 1984. Crustacea from the bolsón of Cuatro Ciénegas, Coahuila, Mexico. *Journal of the Arizona-Nevada Academy of Science* 19:3–12.
- ECELLE, A.A., E.W. CARSON, A.F. ECELLE, R.A. VAN DEN BUSSCHE, T.E. DOWLING, AND A. MEYER. 2005. Historical biogeography of the New World pupfish genus *Cyprinodon* (Teleostei: Cyprinodontidae). *Copeia* 2005:320–339.
- EXCOFFIER, L., AND H.E.L. LISCHER. 2010. Arlequin suite ver. 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10:564–567.
- FRANKHAM, R. 1995. Inbreeding and extinction: a threshold effect. *Conservation Biology* 9:792–799.
- FRANKHAM, R. 1996. Relationship of genetic variation to population size in wildlife. *Conservation Biology* 10:1500–1508.
- FRANKHAM, R., J.D. BALLOU, AND D.A. BRISCOE. 2002. Introduction to conservation genetics. Cambridge University Press, New York, NY.
- GOUDET, J., M. RAYMOND, T. DE MEEÛS, AND F. ROUSSET. 1996. Testing differentiation in diploid populations. *Genetics* 144:1933–1940.
- HERSHLER, R. 1985. Systematic revision of the Hydrobiidae (Gastropoda: Rissoacea) of the Cuatro Ciénegas Basin, Coahuila, México. *Malacologia* 26:31–123.
- HOWETH, J.G., S.E. MCGAUGH, AND D.A. HENDRICKSON. 2008. Contrasting demographic and genetic estimates of dispersal in the endangered Coahuilan box turtle: a contemporary approach to conservation. *Molecular Ecology* 17:4209–4221.
- HUBBS, C.L., AND R.R. MILLER. 1965. Studies of cyprinodont fishes, XXII: variation in *Lucania parva*, its establishment in western United States, and description of a new species from an interior basin in Coahuila, México. *Miscellaneous Publications, Museum of Zoology, University of Michigan* 127:1–111.
- HULSEY, C.D., J.F. GARCÍA DE LEÓN, Y. SÁNCHEZ JOHNSON, D.A. HENDRICKSON, AND T.J. NEAR. 2004. Temporal diversification of Mesoamerican cichlid fishes across a major biogeographic boundary. *Molecular Phylogenetics and Evolution* 31:754–764.
- [IUCN] INTERNATIONAL UNION FOR CONSERVATION OF NATURE. 2014. IUCN red list of threatened species (Version 2014.2); [accessed 18 December 2014]. <http://www.iucnredlist.org/details/23157/0>
- JOHNSON, S.G. 2005. Age, phylogeography and population structure of the microendemic banded spring snail, *Mexipyrghus churinceanus*. *Molecular Ecology* 14: 2299–2311.
- KALLMAN, K.D. 1964. Genetics of tissue transplantation in isolated platyfish populations. *Copeia* 1964:513–522.
- KANG, J.H., M. SCHARTL, R.B. WALTER, AND A. MEYER. 2013. Comprehensive phylogenetic analysis of all species of swordtails and platies (Pisces: genus *Xiphophorus*) uncovers a hybrid origin of a swordtail fish, *Xiphophorus monticolus*, and demonstrates that the sexually selected sword originated in the ancestral lineage of the genus, but was lost again secondarily. *BMC Evolutionary Biology* 2013, 13:25.
- LYNCH, M., J. CONERY, AND R. BÜRGER. 1995. Mutation meltdown and the extinction of small populations. *American Naturalist* 146:489–518.
- MCCOY, C.J. 1984. Ecological and zoogeographic relationships of amphibians and reptiles of the Cuatro Cienegas basin. *Journal of the Arizona-Nevada Academy of Science* 19:49–59.
- MILLER, R.R. 1978. Composition and derivation of the native fish fauna of the Chihuahuan Desert region. Pages 365–381 in R.H. Wauer and D.H. Riskind, editors, Transactions of the symposium on the biological resources of the Chihuahuan Desert Region, United States and Mexico. Volume 3. National Park Service Transactions and Proceedings Series.
- MINCKLEY, W.L. 1969. Environments of the bolsón of Cuatro Ciénegas, Coahuila, México, with special reference to the aquatic biota. Texas Western Press, University of Texas El Paso Science Series 2:1–65.
- MINCKLEY, W.L. 1978. Endemic fishes of the Cuatro Ciénegas basin, northern Coahuila, México. Pages 383–404 in Transactions of the symposium on the biological resources of the Chihuahuan Desert Region, United States and Mexico. Volume 3. National Parks Service Transactions and Proceedings Series.

- MINCKLEY, W.L. 1984. Cuatro Ciénegas fishes: research review and a local test of diversity versus habitat size. *Journal of the Arizona–Nevada Academy of Science* 19:13–21.
- MINCKLEY, W.L. 1992. Three decades near Cuatro Ciénegas, Mexico: photographic documentation and a plea for area conservation. *Journal of the Arizona–Nevada Academy of Science* 26:89–118.
- MOLINE, A.B., S.M. SHUSTER, D.A. HENDRICKSON, AND J.C. MARKS. 2004. Genetic variation in a desert aquatic snail (*Nymphophilus minckleyi*) from Cuatro Ciénegas, Coahuila, Mexico. *Hydrobiologia* 522: 179–192.
- PINKAVA, D.J. 1984. Vegetation and flora of the bolsón of Cuatro Ciénegas region, Coahuila, Mexico: IV. Summary, endemism, and corrected catalogue. *Journal of the Arizona–Nevada Academy of Science* 19:23–47.
- POLZIN, T., AND S.V. DANESCHMAND. 2003. On Steiner trees and minimum spanning trees in hypergraphs. *Operations Research Letters* 31:12–20.
- RAYMOND, M., AND F. ROUSSET. 1995. An exact test for population differentiation. *Evolution* 49:1280–1283.
- RICE, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225.
- ROZAS, J., J.C. SÁNCHEZ-DEL BARRIO, X. MESSEGUER, AND R. ROZAS. 2003. DnaSP: DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19:2496–2497.
- SCHMIDT, T.R., J.P. BIELAWSKI, AND J.R. GOLD. 1998. Molecular phylogenetics and evolution of the cytochrome *b* gene in the cyprinid genus *Lythrurus* (Actinopterygii: Cypriniformes). *Copeia* 1998:14–22.
- SMITH, G.R. 1981. Effects of habitat size on species richness and adult body sizes of desert fishes. Pages 125–272 in R.J. Naiman, and D.L. Soltz, editors, *Fishes in North American deserts*. Wiley, New York, NY.
- SOULÉ, M.E. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. Pages 151–169 in M.E. Soulé and B.A. Wilcox, editors, *Conservation biology: an evolutionary-ecological perspective*. Sinauer Associates, Sunderland, MA.
- SOUZA, V., L. ESPINOSA-ASUAR, A.E. ESCALANTE, L.E. EGUIARTE, J. FARMER, L. FORNEY, L. LLORET, J.M. RODRÍGUEZ-MARTÍNEZ, X. SOBERÓN, R. DIRZON, ET AL. 2006. An endangered oasis of aquatic microbial biodiversity in the Chihuahuan Desert. *Proceedings of the National Academy of Sciences, USA* 103: 6565–6570.
- SOUZA, V., J.L. SIEFERT, A.E. ESCALANTE, J.J. ELSER, AND L.E. EGUIARTE. 2012. The Cuatro Ciénegas basin in Coahuila, Mexico: an astrobiological Precambrian park. *Astrobiology* 12:641–647.
- TAMURA, K., D. PETERSON, N. PETERSON, G. STECHER, M. NEI, AND S. KUMAR. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739.
- TAYLOR, D.W. 1966. A remarkable snail fauna from Coahuila, México. *Veliger* 9:152–228.
- TIBBETS, C.A., AND T.E. DOWLING. 1996. Effects of intrinsic and extrinsic factors on population fragmentation in three species of North American minnows (Teleostei: Cyprinidae). *Evolution* 50:1280–1292.
- TOBLER, M., AND E.W. CARSON. 2010. Environmental variation, hybridization, and phenotypic diversification in Cuatro Ciénegas pupfishes. *Journal of Evolutionary Biology* 23:1475–1489.

Received 8 April 2014
Accepted 12 January 2015