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# Genetic variation in a desert aquatic snail (*Nymphophilus minckleyi*) from Cuatro Ciénegas, Coahuila, Mexico

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**Abstract**

*Nymphophilus minckleyi* is a hydrobiid snail endemic to the freshwater spring ecosystem of Cuatro Ciénegas, Mexico. We used seven allozyme loci to examine the genetic substructure of *N. minckleyi* from 14 sites (sub-populations) in the basin and to test the hypothesis that spring pools in Cuatro Ciénegas are separated into seven hydrologically distinct drainages. Hierarchical *F*-statistics suggest significant population structure exists among the fourteen populations but not among the seven proposed drainages. Cluster analysis of Nei's genetic distance did not show populations grouping according to drainages, although it did reveal alternative clusters. We found two distinct morphotypes that were supported as genetically distinct groups by the allozyme data. Genetic studies of vagile species in desert spring ecosystems can be used to reveal hydrologic connections and identify genetically unique sub-populations.

## Introduction

Desert spring ecosystems are havens of endemism because of their stable environmental conditions and long-term geographic isolation from other aquatic systems (Shepard, 1993; O'Brien & Blinn, 1999). Cuatro Ciénegas, Mexico, has an especially rich endemic fauna when compared to other similar-sized desert spring systems (Minckley, 1984b). Ash Meadows, Nevada, U.S.A. (Deacon & Williams, 1991), supported five endemic fishes (one extinct), nine mollusks, and two insects (Williams et al., 1985). Dalhousie Springs, Australia, has four fishes, six mollusks, and a frog (Zeidler & Ponder, 1989; Crowley & Ivantsoff, 1990). In contrast, Cuatro Ciénegas supports twelve endemic fishes (Minckley, 1984a), nine mollusks (Hershler, 1985) and four reptiles (McCoy, 1984).

Cuatro Ciénegas was designated a Natural Protected Area by the Mexican government in 1994. However, managers lack much of the basic scientific data required to make informed conservation decisions (Secretaria de Medio Ambiente, 1999). In addition to species inventories (Minckley, 1984a; Dinger, 2001), managers require information about the dispersal abilities (Barr & Holsinger, 1985; Bohonak, 1999) and population genetic structure of native species (Meyers et al., 2001). Population genetics are especially important in desert springs where the arid environment limits aquatic animal dispersal and leads to isolated populations. Although a number of descriptive genetic projects are underway on hydrobiid snails, pupfish, and amphipods from Cuatro Ciénegas, no published

studies of genetic population structure exist for any species in the basin.

Cuatro Ciénegas is located in the Chihuahuan desert in northern Mexico. The 840 km$^2$ Natural Protected Area is enclosed in an intermontane basin in the Sierra Madre Oriental Mountains (Secretaria de Medio Ambiente, 1994). The surficial geology of the valley is characterized by carbonate rich alluvial material (Meyer, 1973). Due to the extreme faulting and karst geology of the Cuatro Ciénegas area, there are over 200 freshwater pools (pozas), abundant springheads, and streams in the basin as well as sinkholes and underground caves (Secretaria de Medio Ambiente, 1999).

The first biologists to visit Cuatro Ciénegas outlined discrete drainages based on their observations of surface water connections within the valley (Taylor, 1966; Minckley, 1969). A set of aerial photographs was commissioned in 1968, which allowed researchers to refine drainage descriptions (Minckley, 1969). Seven epigean drainages, which did not precisely correlate to Taylor's drainages, were identified from topographic maps and the aerial photos (Fig. 1).

Identifying Evolutionarily Significant Units (geographically discrete populations that differ in molecular and non-molecular traits (e.g. morphology or behavior)) is a high priority for maximizing biodiversity conservation (Moritz, 1994). Conserving genetic variation within a species may provide some resistance to extinction (Avise, 1994). In this study, we used starch gel electrophoresis to define population genetic structure for the endemic spring snail, *Nymphophilus minckleyi.* We then used the genetic data to test the hypothesis that seven hydrologically distinct drainages exist within the Cuatro Ciénegas basin (Minckley, 1969). Hierarchical population genetic and gene flow data from aquatic organisms can be useful in determining biologically important hydrologic connections, especially in areas characterized by complex hydrogeology, such as Cuatro Ciénegas. Traditional hydrologic mapping techniques, such as dye tracing, can be unpredictable in karst regions and many dyes are toxic to some degree (Field et al., 1995). In addition, traditional surface water mapping techniques yield hydrologic maps that may or may not outline biologically important pathways for dispersal. Mapping aquatic connections with genetic divergence data from non-vagile aquatic organisms has the advantage of identifying biologically important hydrologic connections. Genetic studies have been used previously to outline biologically important drainages even when there is no genetic isolation by distance (Ross, 1999). Understanding both the hydrological connections among sites and the genetic structure of endemic taxa will help managers identify which sites need the most protection to maintain genetic diversity.

## Materials and methods

### *Previously hypothesized drainages*

Minckley (1969) outlined seven drainages in an attempt to explain surficial hydrologic connections in Cuatro Ciénegas (Fig. 1). Canals now connect a few of the previously distinct drainages (Secretaria de Medio Ambiente, 1999), and the karstic geology of the basin strongly suggests the importance of underground hydrologic connections that could link drainages that, based on surface topography, appear to be isolated (Minckley, 1969). Unfortunately the groundwater hydrology of the basin has not been explored in detail to date (Tafelski, 1998). Nevertheless, alternatives to the surficial isolation hypothesis have not been proposed. Because hydrologic connections within the basin allow dispersal and thus gene flow among populations, determining biologically important hydrologic connections is important for conservation (Hershler & Ponder, 1998), both in identifying genetically distinct populations or subpopulations, and in describing potential dispersal routes for invasive species.

*N. minckleyi* is an aquatic snail, so gene flow is facilitated by hydrologic connections between spring pools. The influence of passive dispersal during floods or by birds (Darwin, 1878, 1882; Rees, 1965; Jarne & Delay, 1991) is discounted because both flooding and wading birds are rare in Cuatro Ciénegas (Contreras-Balderas, 1984). Therefore, if the proposed surficial drainages constrain gene flow, we would expect the hydrologic drainage pattern to be reflected in population genetic structure, with high levels of gene flow within surficial drainages, but low levels among them. In contrast, if dispersal is not limited by surface connections (i.e. there are underground connections or other modes of dispersal), we would not expect to see correlations in allele frequencies among sub-populations within surface drainages. *N. minckleyi* dispersal through underground connections is unlikely because there are no underground food resources for adults (R. Hershler, pers. comm.).

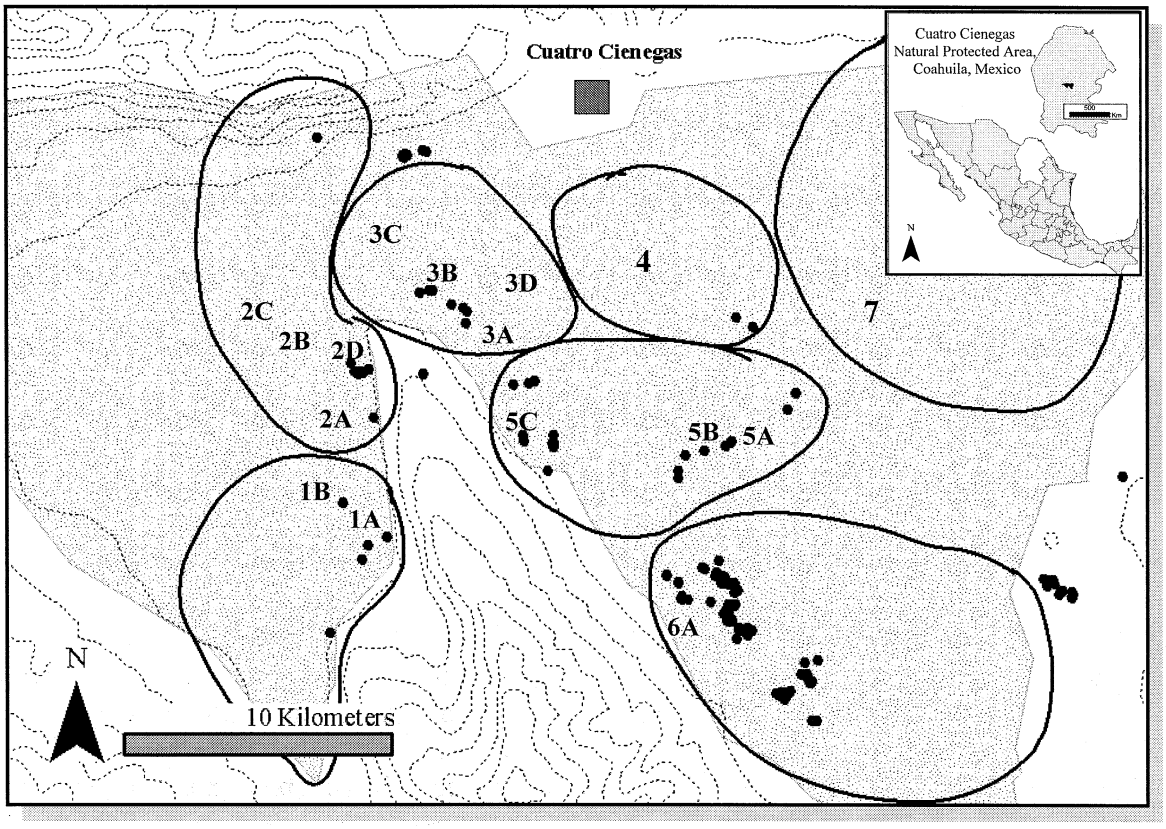


*Figure 1.* Map of collection locations within seven hypothesized drainages (Taylor, 1966; Minckley, 1969) in the Cuatro Ciénegas Basin. A short description of each site as well as geographic coordinates are given in Appendix A. Drainages (.sites) are: **1.A** Churince, **1.B** Laguna Grande, **2.A** La Becerra, **2.B** Garabatal 1, **2.C** Garabatal 2, **2.D** Juan Santos, 3**.A** Los Remojos, **3.B** Mojarral East, **3.C** Rio Mesquites A, **3.D** Rio Mesquites B, **4.0** Charcos Prietos drainage (not sampled), **5.A** La Maroma, **5.B** Los Hundidos, **5.C** Tio Candido, **6.A** Pozas Azules, **7.0** Rio Salado de Nadadores drainage (not sampled).

*Biology of* Nymphophilus minckleyi

There are two described species within the genus *Nymphophilus*, both endemic to Cuatro Ciénegas (Hershler, 1985). *N. minckleyi* is abundant throughout most of the basin in pools with aquatic vegetation; *N. acarinatus* is allopatric to *N. minckleyi* and found only in the far southeastern portion of the basin Santa Tecla area (Hershler, 1985). For this research, we sampled only snails that matched the morphological description of *N. minckleyi.*

*Nymphophilus minckleyi* (Taylor, 1966) is readily discerned from other Cuatro Ciénegas hydrobiids by its large size (9–13 mm) and generally triangular shell morphology. The shells of the species are translucent, with flattened to slightly rounded whorls with a strong spiral keel (Hershler, 1985). Bodies are darkly pigmented.

Two morphotypes of *Nymphophilus minckleyi* were identified based on shell morphology. The primary morph fits the type description of *N. minckleyi* given by Hershler (1985). The secondary (and far less frequent) morphotype has curved (rather than flattened) whorls and lacks a peripheral keel like *N. acarinatus* (Hershler, 1985). Snails of this type were limited to the southeastern lobe of the basin (Los Hundidos, La Maroma and Pozas Azules). Dr R. Hershler examined specimens of both morphotypes and concluded that based on characteristic morphological traits, such as the large shell size, globose shell shape and carinate whorls, that both morphotypes met the description of *N. minckleyi* (Hershler, pers. comm.). The morphotypes were therefore combined for this study.

*Collection methods*

Hershler sampled over 100 sites in the Cuatro Ciénegas Basin (Hershler, 1985) and catalogued *N. minckleyi* from 19 locations, most of which were large springs and a few streams. We revisited eight of these sites (Churince, Laguna Grande inlet, Becerra, Rio Mesquites A, Mojarral East, Los Remojos, Juan Santos, and Tio Cándido) and sampled six novel habitats (Fig. 1). Some of the original sites were not resampled due to difficulty in locating or accessing them. The geographic range of sampling locations for this study spans the known distribution of *N. minckleyi* and includes 5 of the 7 proposed drainages

Most sites were spring-fed pools or streams with abundant aquatic vegetation, generally either *Nymphaea* or *Utricularia*; see Appendix A for habitat descriptions and geographic coordinates. In most cases, snails were collected by hand from submerged aquatic vegetation. All snails were cryogenically preserved in liquid nitrogen in the field, usually within one hour of collection, and were returned to Northern Arizona University in liquid nitrogen and stored at $-80\,^{\circ}$C until they were processed for electrophoresis. For each site voucher specimens were preserved in 90% ethanol and deposited at the University Autonoma of Mexico.

*Electrophoresis*

Individual snails were prepared for electrophoresis by thawing and removing soft body parts with forceps. Each whole snail was placed in a microtiter plate with 100 µl of solution (0.05 M Tris-HCl, pH 7.0) and homogenized by hand with a glass rod. Twenty-five µl of the snail extract was placed in a 10% starch (Sigma) gel. Two different buffer systems were used for electrophoresis: TBE (Tris-borate-EDTA, pH 7.0 (Acquaah, 1992) and CT (citrate-aminopropylmorpholine system at pH 7.2 (Clayton & Tretiak, 1972). In both the gel buffer was a 1:10 dilution of the running buffer (Johnson, 2000).

Twenty-five individuals were run together on a gel, providing a comparison of 2 to 4 populations on any one gel. Gels were run for 3 hours at a constant potential drop of 30 milliamps between electrodes. Gels were sliced horizontally to allow for double staining. Stains were prepared using the tetrazolium method (Acquaah, 1992) and immediately applied to the gels. Gels were incubated at 37 °C until a scorable stain became visible (15–60 min). Most gels were digitized on a desktop scanner to provide a permanent record. Some gels were not scanned due to breakage.

Allozyme phenotypes were scored directly from the gel or digital image. Only presumptive loci that were consistently scorable are reported here. Where more than one locus was present on any one gel, each was designated in order of increasing mobility (e.g. PGM-1, PGM-2). Data were obtained for six enzymes and seven loci. Enzymes, buffers, abbreviations, and numbers assigned by the International Union of Biochemistry are as follows: alkaline phosphatase (CT, ALP, 3.1.3.1), hexokinase (TBE, HEX, 2.7.1.1), malate dehyrogenase (CT, MDH, 1.1.1.37), 6-phosphogluconate dehyrogenase (CT, 6PGDH, 1.1.1.44), and phosphoglucomutase (TBE, PGM, 5.4.2.2). An additional six enzymes were tested (FUM, PGI, AAT, AAM, ADH, GOT) but were not used in this analysis because of poor staining or because of uninterpretable results. Sample sizes are given in Appendix A.

*Data analysis*

Each of the 14 sites was considered a subpopulation; subpopulations were combined into 5 drainages following the surface drainage definitions of Minckley (1969). Descriptive statistics, including heterozygosity and percent polymorphic loci, were calculated with BIOSYS-2 (Swofford et al., 1997). Observed and Hardy-Weinberg expected (HWE) heterozygote frequencies were calculated and tested for conformance to HWE with TFPGA (Miller, 1997).

A Mantel test was performed in TFPGA to test for a correlation between genetic isolation ($F_{ST}$ values) and Euclidian distance among sub-populations (Sokal & Rohlf, 1995). Pairwise $F_{ST}$ values for the Mantel test were generated with FSTAT (Goudet, 2001).

*F*-statistics were calculated to determine population structure among pools with FSTAT according to the methods described in Weir & Cockerham (1984). Standard errors for the *F*-statistics were calculated at each locus by jackknifing over all populations in FSTAT.

To test the hypothesis proposed by Minckley (1969) that seven isolated drainages exist, four-level hierarchical *F*-statistics were calculated with BIOSYS-2 to describe genetic structure among and within drainages (Weir & Cockerham, 1984). The four levels were: total basin ($F_{IT}$), drainage ($F_{DT}$), subpopulation ($F_{ST}$), and individual ($F_{IS}$). Confidence intervals were generated for each statistic from the

jackknifed average and standard error. Standard errors for $F_{SD}$ were calculated by jackknifing over drainages. The variance of $F_{ST}$ was decomposed into the variance due to $F_{SD}$ and $F_{DT}$. These variance contributions were calculated from four-level hierarchical $F$-statistics calculated with the Wright78 step in BIOSYS-2. To estimate gene flow among subpopulations and within and among drainages, the number of migrants per generation ($N_m$) was calculated according to Weir (1996).

UPGMA dendrograms were created in TFPGA for five measures of genetic distance (Roger's original, Roger's modified (Wright, 1978), Nei's original (1972), Nei's modified (1978), and the coancestry distance (Reynolds et al., 1983)) in order to test whether populations clustered within drainages or if an alternative pattern of genetic relationships exists. For each node of the UPGMA dendrograms, TFPGA calculates confidence levels by bootstrapping over loci (Sokal & Rohlf, 1995).

Tied trees, which occur when two or more topologies exist for the same set of allozyme data, can be a problem in cluster analysis (Backeljau et al., 1996). Tied trees occur relatively frequently for UPGMA topologies that are calculated with small sample sizes (Takezaki, 1998). Generally, bootstrap support at tied nodes is low but occasionally is high (e.g., 70–80%). Therefore confidence levels at each node that are generated by bootstrapping should not be used as p-values but rather as indicators of repeatability (Van Dongen, 1995).

Bootstrapping with small sample sizes (e.g. fewer than 20 samples) can have peculiar properties when calculating confidence levels for topologies, because the number of different resamples is limited (Van Dongen & Backeljau, 1995). Van Dongen (1995) suggests bootstrapping over individuals rather than bootstrapping over loci for allozyme data because of the larger sample sizes and greater independence afforded by individuals. Unfortunately, no computer program exists that bootstraps over individuals for cluster analysis of genetic distance, so we generated confidence levels by bootstrapping over loci in TFPGA.

## Results

In general, *N. minckleyi* subpopulations met Hardy-Weinberg expectations (HWE). When variable loci within subpopulations were tested, only 21% (19/89) of the cases did not meet HWE ($\alpha = 0.05$). Most departures from HWE were due to heterozygote deficiency (16/19), which could be an indicator of inbreeding or selection (Appendix A). Much of the genetic differentiation was found in the PGM loci, which may be subject to selection. The average direct count heterozygosity in subpopulations of *N. minckleyi* was 0.36 ($\pm$ standard error 0.09), higher than most reported values for gastropods. For seven gastropods, the average heterozygosity was 0.06 ($\pm$0.03) (Bandoni et al., 1990; Dillon & Lydeard, 1998; Hodges, 1998; Rankevich et al., 1996; Woodruff et al., 1985). The overall level of polymorphism in *N. minckleyi* was 91%, which is slightly higher than other published values for freshwater snails (e.g. overall polymorphism in *Biomphalaria pfeifferi* is 80% (Bandoni et al., 1990).

### *Population structure and gene flow*

Wright (1978) suggested that $F_{ST}(\theta)$ values be interpreted along the following lines: 0–0.05 indicates little genetic differentiation, 0.05–0.15 indicates moderate genetic differentiation, 0.15–0.25 indicates great genetic variation, and $F_{ST}$ values above 0.25 indicate very great genetic differentiation (Hartl & Clark, 1997). $F$-statistics jackknifed over populations (Table 1) show slight, but significant, levels of population subdivision within the Cuatro Ciénegas basin. Most loci showed little to moderate genetic differentiation among sites ($0 > F_{ST}(\theta) > 0.15$; average $F_{ST} \pm SE = 0.111 \pm 0.033$), while PGM-2 showed very great genetic differentiation ($F_{ST} = 0.259 \pm 0.078$).

When $F$-statistics were jackknifed over populations and over loci, $F_{IT}$ and $F_{ST}$ were significantly different from zero ($f = F_{IS}$, $\theta = F_{ST}$ and $F = F_{IT}$, Table 1). The value of $F(0.192 \pm 0.096)$ was greater than $\theta(0.111 \pm 0.033)$, which was greater than $f(0.096 \pm 0.082)$. This result indicates that there is genetic differentiation among subpopulations (sites) but that each subpopulation is in HW equilibrium. Pairwise $F_{ST}$ values over loci were calculated to determine the degree of genetic structure among the 14 subpopulations (Table 2). Numbers of migrants were calculated from $F_{ST}$ (Weir, 1996). This calculation assumes that the populations are at equilibrium and that selection is not an important evolutionary influence. More than two migrants per generation between populations are considered sufficient to prevent genetic divergence between populations (Wright, 1973).

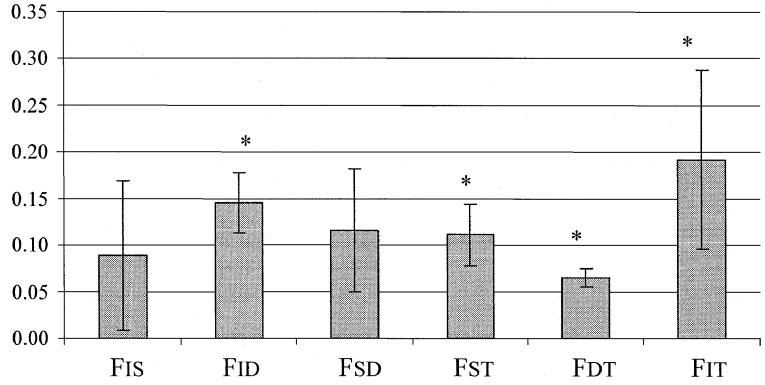


*Figure 2.* Hierarchical $F$-statistics calculated according to (Wright, 1978). Error bars are standard errors. $F_{IS}$ = individuals to subpopulation; $F_{ID}$ = individuals to drainage; $F_{SD}$ = subpopulations to drainage; $F_{ST}$ = subpopulations to total; $F_{DT}$ = drainages to total; $F_{IT}$ = individuals to total. *(asterisks) indicate values that are significantly different from zero ($\alpha = 0.05$).

*Table 1.* $F$-statistics for all alleles jackknifed over populations (Weir & Cockerham 1984). Each value is ± standard error. Weir and Cockerham's equivalent in Wright's notations are as follows: $F = F_{IT}$, $\theta = F_{ST}$, $f = F_{IS}$

| Locus | $f$ (SE) | $\theta$ (SE) | $F$ (SE) |
|---|---|---|---|
| ME | 0.342[a] | 0.106 | 0.405[a] |
| | 0.120 | 0.064 | 0.072 |
| 6PGDH | −0.090 | 0.052 | −0.032 |
| | 0.152 | 0.032 | 0.154 |
| MDH | 0.046 | 0.085[a] | 0.128 |
| | 0.118 | 0.027 | 0.114 |
| ALP | 0.090 | 0.119 | 0.199[a] |
| | 0.062 | 0.065 | 0.084 |
| PGM1 | 0.171[a] | 0.045[a] | 0.208[a] |
| | 0.043 | 0.022 | 0.040 |
| PGM2 | 0.401[a] | 0.259[a] | 0.557[a] |
| | 0.053 | 0.078 | 0.067 |
| HEX | −0.248 | 0.043 | −0.195 |
| | 0.069 | 0.030 | 0.075 |
| All loci | 0.089 | 0.111[a] | 0.192[a] |
| | (0.081) | (0.033) | (0.096) |

[a]Values are significant at the $\alpha = 0.05$ level.

*Drainage structure*

Hierarchical $F$-statistics (Fig. 2) did not show significant subdivision among drainages (average $F_{DT} = 0.030 \pm 0.082$) but subpopulations within drainages were significantly subdivided ($F_{SD} = 0.149 \pm 0.049$). The average number of migrants among drainages for Cuatro Ciénegas is 8.1-migrants/generation, while the average number of migrants among subpopulations within drainages is 1.4/generation. This result does not agree with the prediction made for distinct drainages, which suggested that surficial drainages would confine *N. minckleyi* migration and limit gene flow across drainage boundaries.

The majority of the genetic variation is found within subpopulations. The variance partitioning analysis of $F_{ST}$ found that 84% of the variance can be attributed to subpopulations within drainages, whereas only 17% of the variation is attributable to drainages (Preziosi & Fairbairn, 1992). This reinforces the idea that the subpopulation, rather than the drainage, is the genetically important unit.

*Genetic isolation by distance*

Comparing genetic differentiation and geographic distance provides a picture of dispersal patterns within a given area (Ponder et al., 1995). A simple, straight line relationship between distance and differentiation indicates that genetic exchange among populations is highly correlated to their proximity; a scattered relationship indicates that dispersal is more sporadic (Wright, 1978 *sensu* Ponder et al., 1995).

The Mantel test (999 randomizations) for pooled *N. minckleyi* populations showed a significant ($p < 0.01$), but scattered (correlation coefficient, $r = 0.46$), relationship between $F_{ST}$ and geographic distance. Individual Mantel tests were performed for the drainages that had sufficient sample sizes (drainage 2 and drainage 3). Neither of these tests was significant ($p > 0.4$) and there was considerable scatter in the plots ($r = 0.29$ and $r = -0.36$, respectively). This indicates that gene flow and geographic distance are not tightly coupled.

*Table 2.* Number of migrants ($N_m$, below the diagonal) and $F_{ST}$ values (above the diagonal) for all pairs of populations. $F_{ST}$ values between 0–0.15 indicate little to moderate genetic differentiation

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | La Becerra | | 0.0091 | 0.0481 | 0.0605 | 0.1359 | 0.0289 | 0.1586 | 0.1834 | 0.1250 | 0.0922 | 0.0657 | −0.0144 | 0.1465 | 0.1179 |
| 2 | Churince | 27.22 | | 0.0986 | 0.0680 | 0.1910 | 0.0838 | 0.1154 | 0.2763 | 0.1837 | 0.0971 | 0.0743 | 0.0785 | 0.1111 | 0.2346 |
| 3 | Garabatal 1 | 4.95 | 2.29 | | 0.0824 | 0.1171 | 0.0195 | 0.1533 | 0.1840 | 0.0751 | 0.1794 | 0.0843 | 0.0412 | 0.1441 | 0.0325 |
| 4 | Garabatal 2 | 3.88 | 3.43 | 2.78 | | 0.1518 | 0.0916 | 0.0223 | 0.1893 | 0.0882 | 0.1215 | −0.0351 | −0.0174 | 0.0184 | 0.1059 |
| 5 | Los Hundidos | 1.59 | 1.06 | 1.88 | 1.40 | | 0.3486 | 0.1283 | 0.2051 | 0.0416 | 0.0195 | 0.0929 | 0.1660 | 0.3282 | 0.1166 |
| 6 | Juan Santos | 8.40 | 2.73 | 12.57 | 2.48 | 0.47 | | 0.1698 | 0.3788 | 0.1976 | 0.2336 | 0.1281 | 0.0574 | 0.1499 | 0.1831 |
| 7 | Laguna Grande | 1.33 | 1.92 | 1.38 | 10.96 | 1.70 | 1.22 | | 0.1459 | 0.1133 | 0.0457 | 0.0136 | 0.0457 | 0.0561 | 0.1815 |
| 8 | La Maroma | 1.11 | 0.65 | 1.11 | 1.07 | 0.97 | 0.41 | 1.46 | | 0.1977 | 0.1164 | 0.1741 | 0.1982 | 0.2826 | 0.2665 |
| 9 | Mojarral East | 1.75 | 1.11 | 3.08 | 2.58 | 5.76 | 1.02 | 1.96 | 1.01 | | 0.1270 | 0.0557 | 0.0954 | 0.2144 | 0.0303 |
| 10 | Pozas Azules | 2.46 | 2.32 | 1.14 | 1.81 | 12.57 | 0.82 | 5.22 | 1.90 | 1.72 | | 0.0860 | 0.1385 | 0.2039 | 0.2122 |
| 11 | Los Remojos | 3.56 | 2.93 | 2.72 | 0 | 2.44 | 1.70 | 18.13 | 1.19 | 4.24 | 2.66 | | −0.0154 | 0.0408 | 0.0898 |
| 12 | Rio Mesquites A | 0 | 2.93 | 5.82 | 0 | 1.26 | 4.11 | 5.22 | 1.01 | 2.37 | 1.56 | 0 | | 0.0418 | 0.0810 |
| 13 | Rio Mesquites B | 1.46 | 2.00 | 1.48 | 13.34 | 0.51 | 1.42 | 4.21 | 0.63 | 0.92 | 0.98 | 5.88 | 5.73 | | 0.2582 |
| 14 | Tio Candido | 1.87 | 0.82 | 7.44 | 2.11 | 1.89 | 1.13 | 1.13 | 0.69 | 8.00 | 0.93 | 2.53 | 2.84 | 0.72 | |

*Genetic distance cluster analyses*

Cluster analyses performed on a various genetic distance metrics (Roger's modified genetic distance, Nei's genetic distance, $F_{ST}$) showed that populations did not cluster according to their drainages nor did they cluster strictly by geographic proximity. We calculated confidence levels for each node of the dendrograms by bootstrapping over loci (100 replicates). Although the five dendrograms were similar with identical nodes appearing in most topologies, bootstrap support was generally low (<60%), which suggests that alternate topologies are viable. Two nodes consistently received high bootstrap support (65–85%), which suggests that an alternative drainage structure to the one being tested for this paper (Fig. 1) exists in Cuatro Ciénegas. The dendrogram that resulted from Nei's genetic distance is shown in Figure 3. This topology illustrates the two nodes that consistently grouped together: the first node includes three sites, Garabatal 2, Los Remojos, and Rio Mesquites A (67–85% bootstrap support), and the second node includes Mojarral East and Tio Cándido (67–83% support). Neither of these two nodes group populations strictly by geographic proximity nor by drainage; for example, Mojarral East lies between the Rio Mesquites A and Los Remojos (Fig. 1).

The dendrogram of Nei's genetic distance (Fig. 3) shows a phylogeographic break that separates the two previously mentioned *N. minckleyi* morphotypes. The cluster of atypical *Nymphophilus* includes the Pozas Azules, Los Hundidos and La Maroma populations in the southeastern lobe of the basin. The divergence point occurred at $D = 0.131$ and was supported by 27% of bootstrap randomizations. For context, interspecific values of Nei's genetic distance are typically greater than 0.5 (range 0.20–0.60), whereas intraspecific comparisons are usually less than 0.10 (Ponder et al., 1995). Although this study shows only moderate support for this phylogeographic break characterized by a difference in allelic frequencies (as opposed to fixed differences) and the two morphotypes probably represent natural phenotypic plasticity within the species, it warrants further investigation with a more sensitive technique.

## Discussion

Inbreeding coefficients ($F_{IS}$ and $F_{IT}$) for *N. minckleyi* are comparable to values observed for other freshwater gastropods. The overall inbreeding coefficient for *N. minckleyi* ($F \pm$ SE) was $0.192 \pm 0.096$ compared to the average value of $0.413 \pm 0.10$ for freshwater snails (Table 1). The mean within-population inbreeding coefficient ($f$) was $0.089 \pm 0.081$ for *N. minckleyi* compared to $0.165 \pm 0.043$ for other aquatic gastropods. Significant inbreeding coefficients have been observed in other freshwater snail species, such as *Biomphalaria*, which has a high number of homozygotes relative to heterozygotes due to the slower growth rate and longer life span of homozygotes (Falniowski & Szarowska, 1999).

Levels of overall population differentiation as indicated by $F_{ST}$ are similar for *N. minckleyi* and other freshwater snails (Table 3). There is a general expectation, particularly for non-vagile species, that population differentiation increases with increasing geographic distance between populations because dispersal, and thus gene flow, is distance limited. Population differentiation as calculated by $F_{ST}$does not take into consideration the geographic distance separating sampled populations, which can make comparisons among studies difficult. To make cross study comparisons easier, we suggest a normalized genetic differentiation index where the mean $F_{ST}$ is divided by the distance between the two most distant sampling sites in the study ($F_{ST}$/scale). When this index was applied to values for various freshwater snails (Table 3), we find that population substructure in *N. minckleyi* is comparable to *Fluvidona* sp., which is a hydrobiid with 'very restricted gene flow' that is endemic to Wilsons Promontory in southern Australia (Colgan & Ponder, 1994).

*Refuting the distinct drainage hypothesis*

Our results did not support the drainage hypothesis, indicating that drainages do not restrict gene flow. Other studies have examined levels of genetic divergence among freshwater snail populations within and among drainages (Colgan & Ponder, 1994). *Goniobasis*, for example, showed a high degree of genetic diversity between populations in the same drainage system, which indicated low levels of gene flow; inter-drainage gene flow levels were even lower (Chambers, 1980). The Australian hydrobiids of the genus *Fluvidona* show lower levels of gene flow within drainages than among drainages (Ponder, 1994; Colgan & Ponder, 1994).

In Cuatro Ciénegas, gene flow for *Nymphophilus minckleyi* is not constrained by the seven hypothesized drainages (Minckley, 1969; Taylor, 1966; Hershler &

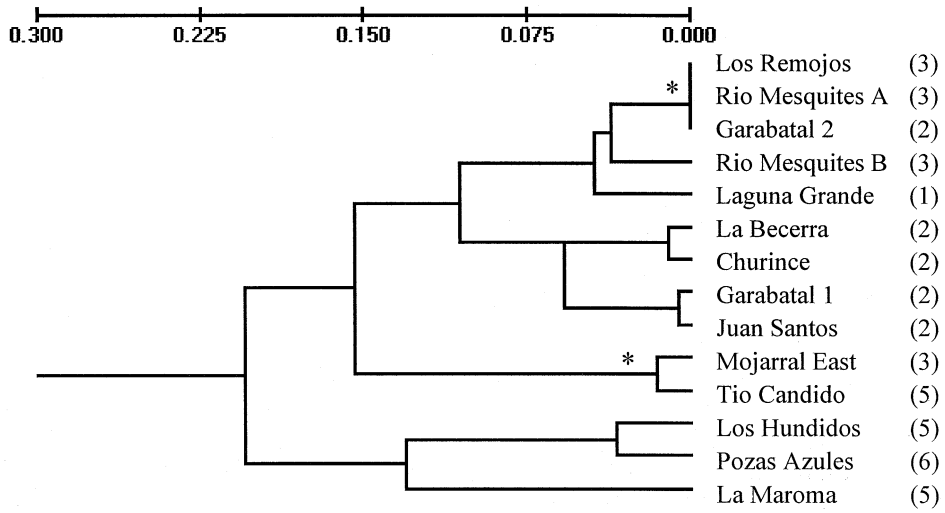


*Figure 3.* UPGMA dendrogram of Nei's genetic distance (Nei, 1978) between populations. Drainage numbers for each site are in parenthesis. *(asterisks) designate the two nodes with consistently high bootstrap values (65–85%) that are discussed in the text.

*Table 3.* Average $F_{ST}$ and $N_m$ (number of migrants) for various freshwater mollusks. $F_{ST}$/scale is the normalized genetic differentiation to aid in comparing levels of population substructure across studies with different geographical distances between sites

| Species | No. loci | No. samples | Scale (km) | $F_{ST}$ (mean) | $N_m$ (mean) | Reference | $F_{ST}$/scale |
|---|---|---|---|---|---|---|---|
| *Goniobasis (2 sp.)* | 14 | 12 | 1000 | 0.408 | 0.36 | Chambers, 1980 | 0.0004 |
| *Lymnaea peregra* | 6 | 4 | 50 | 0.018 | 13.64 | Jarne & Delay, 1990 | 0.0004 |
| *Biomphalaria glabrata* | 13 | 6 | 1000 | 0.805 | 0.06 | Mulvey et al., 1998 | 0.0008 |
| *Biomphalaria pfeifferi* | 7 | 12 | 500 | 0.589 | 0.17 | Bandoni et al., 1990 | 0.0012 |
| *Fluvidonia sp. Straight-s* | 22 | 8 | 7.8 | 0.130 | 1.67 | Coglan & Ponder 1994 | 0.0167 |
| *Fluvidonia sp. 3* | 22 | 12 | 7.8 | 0.086 | 2.66 | Coglan & Ponder 1994 | 0.0110 |
| *Fluvidonia sp. 4* | 22 | 26 | 9.4 | 0.075 | 3.08 | Coglan & Ponder 1994 | 0.0080 |
| *Fluvidonia sp. 5* | 22 | 14 | 17.8 | 0.161 | 1.30 | Coglan & Ponder 1994 | 0.0090 |
| *Fonscochela accepta* | 24 | 12 | 250 | 0.116 | 1.91 | Coglan & Ponder 1994 | 0.0005 |
| *Fonscochela aquatica* | 24 | 16 | 150 | 0.110 | 2.02 | Coglan & Ponder 1994 | 0.0007 |
| *Fonscochela zeidleri* | 23 | 16 | 205 | 0.360 | 0.44 | Coglan & Ponder 1994 | 0.0018 |
| *Fonscochela variabilis* | 27 | 19 | 55 | 0.196 | 1.03 | Coglan & Ponder 1994 | 0.0036 |
| *Trochidrobia punicea* | 24 | 17 | 112.5 | 0.143 | 1.50 | Coglan & Ponder 1994 | 0.0013 |
| *Trochidrobia smithi* | 18 | 7 | 150 | 0.426 | 0.34 | Coglan & Ponder 1994 | 0.0028 |
| *Trochidrobia minuta* | 18 | 3 | 25 | 0.086 | 2.66 | Coglan & Ponder 1994 | 0.0034 |
| *Bythinella* | 7 | 20 | 300 | 0.490 | 0.26 | Falniowski & Szarowska, 1999 | 0.0016 |
| *Nymphophilus minckleyi* | **7** | **14** | **17** | **0.111** | **2.00** | present study | **0.0065** |
| Average | 17.65 | 12.82 | 226.90 | 0.25 | 2.06 | | 0.0041 |
| St. Deviation | 7.18 | 6.04 | 319.56 | 0.22 | 3.13 | | 0.0046 |

Hayek, 1988). First, if the drainages were isolated, one would expect to see very few snails migrating across the drainage boundaries; however the migration rate among drainages is relatively high. Second, the majority of the genetic variance is found within subpopulations rather than within drainages, which indicates that genetic significance of the drainages may be an artifact of pooling subpopulations. Third, the dendrograms of genetic distances between *N. minckleyi* populations do not cluster by drainage (Fig. 3).

The *N. minckleyi* gene flow data corroborates evidence from *Mexipyrgus churinceanus* shell morphology and *Astyanax mexicanus* dispersal. Hershler & Hayek (1988) tested the hypothesis that geographic patterns in *Mexipyrgus* shell morphology derived from the seven evolutionarily distinct drainages; however they failed to find a correlation between the two. The population density of the Mexican tetra (*A. mexicanus*) fluctuates from one pool to another on a very rapid basis, suggesting that individuals move readily among drainages (Minckley, 1984a). The groundwater hydrology of the basin has not been studied so we are unable to determine whether dispersal occurs through underground connections, although the possibility exists (Tafelski, 1998).

*Alternative drainages*

*N. minckleyi* migration rates and genetic distance dendrograms suggest that alternative drainages may be structuring gene flow. Although the *Nymphophilus* data do not support the drainage hypothesis proposed by Minckley (1969), the pairwise $F_{ST}$ values show heterogeneous gene flow among populations indicating that corridors for and barriers against snail migration exist in Cuatro Ciénegas. The cluster analysis for each of the five genetic distance measures included two nodes with high bootstrap support: (1) the Mojarral East-Tio Candido cluster, (2) the Garabatal 2-Rio Mesquites A-Los Remojos cluster. Genetic data from additional aquatic organisms, or direct hydrologic data, are needed to confirm or refute the existence of alternative drainages at Cuatro Ciénegas. In addition, the genetic data support the presence of an evolutionarily significant unit in the southeast portion of the basin where *N. minckleyi* populations are geographically isolated and have a distinct morphology.

*Managing naturally fragmented populations*

Desert springs are habitats for endemic species in a variety of groups which have presumably speciated in relatively stable and isolated conditions. This study shows how identifying genetically distinct subpopulations on an endemic snail provides insight into the underlying hydrological structure of a complex spring ecosystem. This is an important first step in helping scientists and managers understand dispersal patterns of a suite of endemic taxa which can be used to design management plans to protect sites which harbor genetically unique taxa.

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## References

Acquaah, G., 1992. Practical Protein Electrophoresis for Genetic Research. Dioscorides Press, Portland, OR.

Avise, J. C., 1994. Molecular Markers, Natural History, and Evolution. Chapman & Hall, New York, NY.

Backeljau, T., L. De Bruyn, H. De Wolf, K. Jordaens, S. Van Dongen & B. Winnepenninckx, 1996. Multiple UPGMA and Neighbor-joining trees and the performance of some computer packages. Molecular Biology and Evolution 13: 309–313.

Bandoni, S. M., M. Mulvey, D. K. Koech & E. S. Loker, 1990. Genetic structure of Kenyan populations of *Biomphalaria pfeifferi* (Gastropoda: Planorbidae). Journal of Molluscan Studies 56: 383–391.

Barr, T. C., Jr. & J. R. Holsinger, 1985. Speciation in cave faunas. Annual Review of Ecology and Systematics 16: 313–337.

Bohonak, A. J., 1999. Dispersal, gene flow and population structure. The Quartarterly Review of Biology 74: 21–45.

Chambers, S. M., 1980. Genetic divergence between populations of *Goniobasis* (Pleuroceridae) occupying different drainage systems. Malacologia 20: 63–81.

Clayton, J. W. & D. N. Tretiak, 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. Journal of the Fisheries Research Board of Canada 29: 1169–1172.

Colgan, D. J. & W. F. Ponder, 1994. The evolutionary consequences of restrictions on gene flow: Examples from hydrobiid snails. The Nautilus, Supplement 2: 25–43.

Contreras-Balderas, A. J., 1984. Birds from Cuatro Ciénegas, Coahuila, México. Journal of the Arizona-Nevada Academy of Science 19: 77–79.

Crowley, L. E. L. M. & W. Ivantsoff, 1990. A second hardyhead, *Craterocephalus gloveri* (Pisces: Atherinidae), from Dalhousie Springs, central Australia. Ichthyological Exploration of Freshwaters 1: 113–122.

Darwin, C., 1878. Transplantation of shells. Nature 18: 120–121.

Darwin, C., 1882. On the dispersal of fresh-water bivalves. Nature 25: 529–530.

Deacon, J. E. and C.D. Williams, 1991. Ash Meadows and the legacy of the Devils Hole pupfish. In Minckley, W. L. & J. E. Deacon (eds), Battle Against Extinction: Native Fish Management in the American West. University of Arizona Press, Tucson, AZ.: 69–87.

Dillon, R. T. J. & C. Lydeard, 1998. Divergence among mobile populations of the pleurocerid snail genus, *Leptoxis*, estimated by allozyme electrophoresis. Malacologia 39: 113–121.

Dinger, E. C., 2001. Aquatic Invertebrates of Cuatro Ciénegas, Coahuila, Mexico and Effects of Fish on Stromatolite Invertebrate Assemblages. Department of Biology. Northern Arizona University, Flagstaff, AZ.

Falniowski, A. M. & K. M. Szarowska, 1999. Homozygote excess and gene flow in the spring snail *Bythinella* (Gastropoda: Prosobranchia). Journal of Zoological Systematics and Evolutionary Research 37: 165–175.

Field, M. S., R. G. Wilhelm, J. F. Quinlan & T. J. Aley, 1995. An assessment of the potential adverse properties of fluorescent tracer dyes used for groundwater tracing. Environmental Monitoring and Assessment 38: 75–96.

Goudet, J, 2001. FSTAT, a program to estimate and test gene diversities and fixation indices. Updated from Goudet (1995). Available from http://www.unil.ch/izea/softwares/fstat.html.

Hartl, D. L. & A. G. Clark, 1997. Principles of Population Genetics. Sinauer Associates, Inc., Sunderland, MA.

Hershler, R., 1985. Systematic revision of the Hydrobiidae (Gastropoda: Rissoacea) of the Cuatro Ciénegas basin, Coahuila, Mexico. Malacologia 26: 31–123.

Hershler, R. and L. C. Hayek, 1988. Shell variation of springsnail populations in the Cuatro Ciénegas Basin, Mexico: Preliminary analysis of limnocrene fauna. The Nautilus 102: 56–64.

Hershler, R. & W. F. Ponder, 1998. A review of morphological characters of hydrobioid snails. Smithsonian Contributions to Zoology 600: 55.

Hodges, M. A., F. W. Allendorf, 1998. Population Genetics and Pattern of Larval Dispersal of the Endemic Hawaiian Freshwater Amphidromous Gastropod *Neritina granosa* (Prosobranchia: Neritidae). Pacific Science 52: 237–249.

Jarne, P. & B. Delay, 1990. Population genetics of *Lymnaea peregra* (Mueller) (Gastropoda: Pulmonata) in Lake Geneva. Journal of Molluscan Studies 56: 317–321.

Jarne, P. & B. Delay, 1991. Population genetics of freshwater snails. Trends in Ecology and Evolution 6: 383–386.

Johnson, K. M., 2000. An Analysis of the Genetic Population Structure of *Paracerceis sculpata* in the Gulf of California. Department of Biology, Northern Arizona University, Flagstaff, AZ.

Kimura, M. and J. F. Crow, 1964. The number of alleles that can be maintained in a finite population. Genetics 49: 725–738.

McCoy, C. J., 1984. Ecological and zoogeographic relationships of amphibians and reptiles of the Cuatro Ciénegas Basin. Journal of the Arizona-Nevada Academy of Science 19: 49–60.

Meyer, E. R., 1973. Late-Quaternary paleoecology of the Cuatro Ciénegas basin, Coahuila, México. Ecology 54: 983–995.

Meyers, M. J., F. A. H. Sperling & V. H. Resh, 2001. Dispersal of two species of Trichoptera from desert springs: Conservation implications for isolated vs. connected populations. Journal of Insect Conservation 5: 207–215.

Miller, M. P., 1997. Tools for population genetic analyses (TFPGA) 1.3: A Windows program for the analysis of allozyme and molecular population data. Computer software distributed by the author.

Minckley, W. L., 1969. Environments of the Bolsón of Cuatro Ciénegas, Coahuila, Mexico, with special reference to the aquatic biota. Texas Western Press., El Paso, Texas. Science Series 2: 1–65.

Minckley, W. L., 1984a. 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., 1984b. 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.

Moritz, C., 1994. Defining 'Evolutionarily Significant Units' for conservation. Trends in Ecology and Evolution 9: 373–375.

Mulvey, M., M. C. Newman & D. S. Woodruff, 1998. Genetic differentiation among West Indian populations of the shistosome-transmitting snail *Biomphalaria glabrata*. Malacologia 29: 309–317.

Nei, M., 1972. Genetic distance between populations. American Naturalist 106: 283–292.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–390.

O'Brien, C. & D. W. Blinn, 1999. The endemic spring snail *Pyrgulopsis montezumensis* in a high CO sub$_2$ environment: importance of extreme chemical habitats as refugia. Freshwater Biology 42: 225–234.

Ponder, W. F., 1994. Australian freshwater Mollusca: conservation priorities and indicator species. Memoirs of the Queensland Museum 36: 191–196.

Ponder, W. F., P. Eggler & D. J. Colgan, 1995. Genetic differentiation of aquatic snails (Gastropoda: Hydrobiidae) from artesian springs in arid Australia. Biological Journal of the Linnaean Society 56: 553–596.

Preziosi, R. F. & D. J. Fairbairn, 1992. Genetic population structure and levels of gene flow in the stream dwelling waterstrider, *Aquarius* (= *Gerris remigis* (Hemiptera: Gerridae). Evolution 46: 430–444.

Rankevich, D. L., B. E. Nevo, A. Beiles & Z. Arad, 1996. Genetic and physiological adaptations of the prosobranch landsnail *Pomatias olivieri* to microclimatic stresses on Mount Carmel, Israel. Israel Journal of Zoology 42: 425–441.

Rees, W. J., 1965. The aerial dispersal of mollusca. Proceedings of the Malacological Society of London 36: 269–282.

Reynolds, J., B. S. Weir & C. C. Cockerham, 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. Genetics 105: 767–779.

Ross, T. K., 1999. Phylogeography and conservation genetics of the Iowa Pleistocene snail. Molecular Ecology 8: 1363–1373.

Secretaria de Medio Ambiente, R. N. y. P. S., 1994. Decree of the Cuatro Ciénegas Natural Protected Area.

Secretaria de Medio Ambiente, R. N. y. P. S., 1999. Programa de Manejo del Area de Protección de Flora y Fauna Cuatro Ciénegas. Instituto Nacional de Ecología, Tlacopac, México.

Shepard, W. D., 1993. Desert springs – both rare and endangered. Aquatic Conservation: Marine and Freshwater Ecosystems 3: 351–359.

Sokal, R. R. & F. J. Rohlf, 1995. Biometry: the Principles and Practice of Statistics in Biological Research. W. H. Freeman and Co., New York.

Swofford, D. L., R. B. Selander & W. C. Black IV, 1997. BIOSYS-2: A Computer Program for the Analysis of Allelic Variation in Genetics, Ft. Collins, CO.

Tafelski, R. D., 1998. Unpublished report to the Nature Conservancy, 'Groundwater reconnissance of the Cuatro Ciénegas area.' HRS Consultants, Inc. Lakewood, CO.

Takezaki, N., 1998. Tied trees generated by distance methods of phylogenetic reconstruction. Molecular Biology and Evolution 15: 727–737.

Taylor, D. W., 1966. A remarkable snail fauna from Coahuila, Mexico. The Veliger 9:152–223.

Van Dongen, S., 1995. How should we bootstrap allozyme data? Heredity 74: 445–447.

Van Dongen, S. & T. Backeljau, 1995. One- and two-sample tests on single locus inbreeding coefficients using the bootstrap. Heredity 74: 127–133.

Weir, B. S., 1996. Genetic Data Analysis II: Methods for Discrete Population Genetic Data. Sinauer Associates, Inc. Publishers, Sunderland, MA.

Weir B. S. and C. C. Cockerham, 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38: 1358–1370.

Williams, J. E., D. B. Bowman, J. E. Brooks, A. A. Echelle, R. J. Edwards, D. A. Hendrickson & J. J. Landye, 1985. Endangered aquatic ecosystems in North American deserts with a list of vanishing fishes of the region. Journal of the Arizona-Nevada Academy of Science 20: 1–61.

Woodruff, D., M. Mulvey & M. Yipp, 1985. Population genetics of *Biomphalaria straminea* in Hong Kong. Journal of Heredity 76: 355–360.

Wright, S., 1973. The Origin of the *F*-statistics for Describing the Genetic Aspects of Population Structure. University of Hawaii Press, Honolulu.

Wright, S., 1978. Evolution and the Genetics of Populations. University of Chicago Press, Chicago.

Zeidler, W. and W. F. Ponder (eds), 1989. Natural History of Dalhousie Springs. South Australian Museum, Adelaide.

*Appendix A.* Genetic characteristics for each locus in each population. N is population size, na is the actual number of alleles, ne is the effective number of alleles (Kimura & Crow, 1964), H_obs is the direct count heterozygosity, H_exp is the Hardy–Weinberg expected heterozygostiy (Nei, 1972), *p*-values are the Chi-squared results. HWE is yes if the population $p > 0.025$ and $p < 0.975$, meaning that the locus meets Hardy-Weinberg expectations.

| | | *N* | na | ne | % Het | H_obs | H_exp | *p*-value | HWE |
|---|---|---|---|---|---|---|---|---|---|
| Poza la Becerra | *26.8800, 102.1384* | | *Drainage # 2* | | | | | | |
| | *ME* | 0 | – | - | – | – | – | – | |
| | *6PGDH* | 30 | 3 | 2.01 | 0.53 | 16.00 | 20.20 | 0.183 | Yes |
| | *MDH* | 34 | 3 | 2.17 | 0.62 | 21.00 | 16.99 | 0.168 | Yes |
| | *ALP* | 47 | 3 | 2.02 | 0.57 | 27.00 | 21.97 | 0.116 | Yes |
| | *PGM-1* | 30 | 3 | 2.07 | 0.40 | 12.00 | 15.00 | 0.273 | Yes |
| | *PGM-2* | 20 | 2 | 1.88 | 0.45 | 9.00 | 14.47 | 0.035 | Yes |
| | *HEX* | 3 | 2 | 2.00 | 0.33 | 1.00 | 1.50 | 0.564 | Yes |
| Poza Churince | *26.8397, 102.1341* | | *Drainage # 1* | | | | | | |
| | *ME* | 5 | 2 | 1.47 | 0.40 | 2.00 | 1.60 | 0.576 | Yes |
| | *6PGDH* | 17 | 3 | 2.10 | 0.12 | 2.00 | 8.47 | 0.002 | No |
| | *MDH* | 19 | 2 | 1.95 | 0.63 | 12.00 | 9.26 | 0.198 | Yes |
| | *ALP* | 19 | 2 | 2.00 | 0.63 | 12.00 | 10.00 | 0.371 | Yes |
| | *PGM-1* | 17 | 2 | 1.84 | 0.47 | 8.00 | 7.77 | 0.901 | Yes |
| | *PGM-2* | 13 | 3 | 1.59 | 0.31 | 4.00 | 4.62 | 0.631 | Yes |
| | *HEX* | 10 | 2 | 1.83 | 0.50 | 5.00 | 4.55 | 0.754 | Yes |
| El Garabatal 1 | *26.8952, 102.1419* | | *Drainage # 2* | | | | | | |
| | *ME* | 20 | 2 | 1.47 | 0.30 | 6.00 | 6.40 | 0.780 | Yes |
| | *6PGDH* | 22 | 3 | 1.39 | 0.32 | 7.00 | 5.89 | 0.375 | Yes |
| | *MDH* | 21 | 2 | 1.69 | 0.29 | 6.00 | 8.57 | 0.169 | Yes |
| | *ALP* | 13 | 2 | 1.74 | 0.31 | 4.00 | 5.54 | 0.317 | Yes |
| | *PGM-1* | 10 | 2 | 1.98 | 0.50 | 5.00 | 4.95 | 0.975 | Yes |
| | *PGM-2* | 0 | – | - | – | – | – | – | |
| | *HEX* | 0 | – | - | – | – | – | – | |
| El Garabatal 2 | *26.8896, 102.1580* | | *Drainage # 2* | | | | | | |
| | *ME* | 6 | 2 | 1.95 | 0.17 | 1.00 | 2.92 | 0.107 | Yes |
| | *6PGDH* | 32 | 4 | 2.28 | 0.63 | 20.00 | 15.75 | 0.127 | Yes |
| | *MDH* | 36 | 3 | 2.02 | 0.56 | 20.00 | 17.78 | 0.453 | Yes |
| | *ALP* | 28 | 2 | 1.69 | 0.36 | 10.00 | 11.43 | 0.508 | Yes |
| | *PGM-1* | 37 | 4 | 2.02 | 0.30 | 11.00 | 17.64 | 0.022 | No |

*Continued on p. 191*

*Appendix A*. Continued

| | | *N* | na | ne | % Het | H_obs | H_exp | *p*-value | HWE |
|---|---|---|---|---|---|---|---|---|---|
| | | *N* | na | ne | % Het | H_obs | H_exp | *p*-value | HWE |
| | *PGM-2* | 33 | 3 | 2.05 | 0.36 | 12.00 | 16.12 | 0.142 | Yes |
| | *HEX* | 32 | 2 | 1.98 | 0.53 | 17.00 | 15.86 | 0.684 | Yes |
| | | *N* | na | ne | % Het | H_obs | H_exp | *p*-value | HWE |
| Los Hundidos | *26.8665, 102.0321* | | *Drainage # 5* | | | | | | |
| | *ME* | 0 | – | - | – | – | – | – | |
| | *6PGDH* | 29 | 2 | 1.92 | 0.48 | 14.00 | 14.40 | 0.879 | Yes |
| | *MDH* | 19 | 2 | 1.99 | 0.63 | 12.00 | 9.47 | 0.245 | Yes |
| | *ALP* | 30 | 3 | 2.27 | 0.37 | 11.00 | 14.98 | 0.145 | Yes |
| | *PGM-1* | 6 | 2 | 1.80 | 0.00 | 0.00 | 2.67 | 0.014 | No |
| | *PGM-2* | 0 | – | - | – | – | – | – | |
| | *HEX* | 0 | – | - | – | – | – | – | |
| Juan Santos | *26.8979, 102.1462* | | *Drainage # 2* | | | | | | |
| | *ME* | 26 | 2 | 1.30 | 0.27 | 7.00 | 6.06 | 0.428 | Yes |
| | *6PGDH* | 30 | 3 | 2.02 | 0.80 | 24.00 | 14.73 | 0.001 | No |
| | *MDH* | 34 | 2 | 1.23 | 0.09 | 3.00 | 6.28 | 0.002 | No |
| | *ALP* | 12 | 2 | 1.95 | 0.50 | 6.00 | 5.83 | 0.921 | Yes |
| | *PGM-1* | 2 | 2 | 2.00 | 1.00 | 2.00 | 1.00 | 0.157 | Yes |
| | *PGM-2* | 0 | – | - | – | – | – | – | |
| | *HEX* | 13 | 2 | 1.74 | 0.62 | 8.00 | 5.54 | 0.109 | Yes |
| La Maroma | *26.8704, 102.0205* | | *Drainage # 5* | | | | | | |
| | *ME* | 2 | 2 | 1.60 | 0.00 | 0.00 | 1.00 | 0.157 | Yes |
| | *6PGDH* | 30 | 3 | 2.18 | 0.10 | 3.00 | 6.18 | 0.005 | No |
| | *MDH* | 28 | 3 | 2.08 | 0.54 | 15.00 | 13.84 | 0.657 | Yes |
| | *ALP* | 26 | 3 | 2.36 | 0.27 | 7.00 | 7.44 | 0.762 | Yes |
| | *PGM-1* | 31 | 2 | 1.95 | 0.39 | 12.00 | 11.87 | 0.952 | Yes |
| | *PGM-2* | 14 | 3 | 2.91 | 0.00 | 0.00 | 1.86 | 0.000 | No |
| | *HEX* | 4 | 2 | 2.00 | 0.75 | 3.00 | 1.88 | 0.230 | Yes |
| Laguna Grande | *26.8513, 102.1487* | | *Drainage # 1* | | | | | | |
| | *ME* | 4 | 1 | 1.00 | 0.50 | 2.00 | 1.50 | 0.505 | Yes |
| | *6PGDH* | 20 | 3 | 1.27 | 0.25 | 5.00 | 9.78 | 0.029 | Yes |
| | *MDH* | 25 | 3 | 2.21 | 0.60 | 15.00 | 12.50 | 0.317 | Yes |
| | *ALP* | 21 | 2 | 1.40 | 0.33 | 7.00 | 10.50 | 0.127 | Yes |
| | *PGM-1* | 30 | 3 | 1.66 | 0.43 | 13.00 | 14.58 | 0.552 | Yes |
| | *PGM-2* | 28 | 2 | 1.15 | 0.04 | 1.00 | 13.55 | 0.000 | No |
| | *HEX* | 29 | 2 | 1.88 | 0.62 | 18.00 | 14.48 | 0.191 | Yes |
| Mojarral East | *26.9228, 102.1189* | | *Drainage # 3* | | | | | | |
| | *ME* | 57 | 2 | 1.98 | 0.28 | 16.00 | 28.28 | 0.001 | No |
| | *6PGDH* | 101 | 2 | 1.98 | 0.73 | 74.00 | 49.90 | 0.000 | No |
| | *MDH* | 102 | 4 | 1.69 | 0.23 | 23.00 | 40.17 | 0.000 | No |
| | *ALP* | 6 | 3 | 2.57 | 0.33 | 2.00 | 3.00 | 0.414 | Yes |
| | *PGM-1* | 21 | 3 | 2.71 | 0.43 | 9.00 | 10.41 | 0.536 | Yes |
| | *PGM-2* | 20 | 2 | 1.28 | 0.05 | 1.00 | 4.38 | 0.001 | No |
| | *HEX* | 64 | 2 | 1.84 | 0.64 | 41.00 | 29.18 | 0.001 | No |
| Pozas Azules | *26.8140, 102.0197* | | *Drainage # 6* | | | | | | |
| | *ME* | 0 | – | – | – | – | – | – | |
| | *6PGDH* | 18 | 3 | 2.11 | 0.33 | 6.00 | 9.00 | 0.157 | Yes |
| | *MDH* | 22 | 2 | 1.94 | 0.36 | 8.00 | 10.64 | 0.245 | Yes |
| | *ALP* | 10 | 2 | 1.60 | 0.50 | 5.00 | 3.75 | 0.292 | Yes |

*Continued on p. 192*

*Appendix A*. Continued

| | | *N* | na | ne | % Het | H_obs | H_exp | *p*-value | HWE |
|---|---|---|---|---|---|---|---|---|---|
| | *PGM-1* | 22 | 2 | 1.90 | 0.41 | 9.00 | 10.43 | 0.520 | Yes |
| | *PGM-2* | 21 | 3 | 1.98 | 0.29 | 6.00 | 9.33 | 0.102 | Yes |
| | *HEX* | 21 | 3 | 2.36 | 0.81 | 17.00 | 10.50 | 0.005 | No |
| Los Remojos | *26.9126, 102.1072* | | *Drainage # 3* | | | | | | |
| | *ME* | 5 | 3 | 1.92 | 0.40 | 2.00 | 2.40 | 0.709 | Yes |
| | *6PGDH* | 20 | 2 | 2.06 | 0.60 | 12.00 | 9.60 | 0.264 | Yes |
| | *MDH* | 18 | 2 | 1.99 | 0.50 | 9.00 | 8.97 | 0.990 | Yes |
| | *ALP* | 7 | 2 | 1.69 | 0.57 | 4.00 | 2.86 | 0.290 | Yes |
| | *PGM-1* | 20 | 3 | 2.18 | 0.55 | 11.00 | 9.98 | 0.646 | Yes |
| | *PGM-2* | 21 | 3 | 2.19 | 0.33 | 7.00 | 10.50 | 0.127 | Yes |
| | *HEX* | 16 | 2 | 1.93 | 0.44 | 7.00 | 7.72 | 0.710 | Yes |
| Rio Mesquites A | *26.9300, 102.1274* | | *Drainage # 3* | | | | | | |
| | *ME* | 0 | – | – | – | – | – | – | |
| | *6PGDH* | 22 | 3 | 2.07 | 0.59 | 13.00 | 10.43 | 0.248 | Yes |
| | *MDH* | 23 | 2 | 1.94 | 0.74 | 17.00 | 11.15 | 0.012 | No |
| | *ALP* | 23 | 3 | 1.68 | 0.43 | 10.00 | 8.87 | 0.541 | Yes |
| | *PGM-1* | 18 | 3 | 1.98 | 0.50 | 9.00 | 8.31 | 0.723 | Yes |
| | *PGM-2* | 18 | 3 | 2.07 | 0.33 | 6.00 | 8.89 | 0.168 | Yes |
| | *HEX* | 15 | 3 | 2.07 | 0.60 | 9.00 | 7.37 | 0.390 | Yes |
| Rio Mesquites B | *26.9333, 102.1276* | | *Drainage # 3* | | | | | | |
| | *ME* | 7 | 2 | 1.69 | 0.29 | 2.00 | 2.86 | 0.427 | Yes |
| | *6PGDH* | 32 | 3 | 2.13 | 0.25 | 8.00 | 15.00 | 0.008 | No |
| | *MDH* | 29 | 2 | 2.00 | 0.62 | 18.00 | 14.48 | 0.191 | Yes |
| | *ALP* | 22 | 3 | 2.29 | 0.45 | 10.00 | 10.91 | 0.696 | Yes |
| | *PGM-1* | 28 | 2 | 1.62 | 0.36 | 10.00 | 11.43 | 0.508 | Yes |
| | *PGM-2* | 19 | 3 | 1.17 | 0.16 | 3.00 | 2.76 | 0.709 | Yes |
| | *HEX* | 20 | 2 | 2.00 | 0.70 | 14.00 | 10.00 | 0.074 | Yes |
| Tio Candido | *26.8701, 102.0784* | | *Drainage # 5* | | | | | | |
| | *ME* | 32 | 2 | 1.64 | 0.28 | 9.00 | 12.48 | 0.114 | Yes |
| | *6PGDH* | 43 | 3 | 1.88 | 0.72 | 31.00 | 19.83 | 0.000 | No |
| | *MDH* | 44 | 3 | 1.43 | 0.36 | 16.00 | 13.09 | 0.140 | Yes |
| | *ALP* | 20 | 3 | 2.12 | 0.20 | 4.00 | 9.60 | 0.009 | No |
| | *PGM-1* | 17 | 2 | 1.94 | 0.35 | 6.00 | 8.24 | 0.263 | Yes |
| | *PGM-2* | 16 | 2 | 1.44 | 0.13 | 2.00 | 4.88 | 0.018 | No |
| | *HEX* | 22 | 2 | 1.37 | 0.32 | 7.00 | 5.89 | 0.375 | Yes |

| | | N ± SE | SE | na | % Het | ne |
|---|---|---|---|---|---|---|
| Over all Populations | | | | | | |
| | *ME* | 12 | 4.44 | 3 | 0.29 | 2.18 |
| | *6PGDH* | 32 | 5.65 | 3 | 0.46 | 2.02 |
| | *MDH* | 32 | 5.73 | 3 | 0.48 | 1.96 |
| | *ALP* | 20 | 2.89 | 2 | 0.42 | 1.69 |
| | *PGM-1* | 21 | 2.69 | 4 | 0.43 | 2.03 |
| | *PGM-2* | 16 | 2.68 | 4 | 0.22 | 2.10 |
| | *HEX* | 18 | 4.47 | 4 | 0.57 | 2.13 |