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# Genetic structuring in the Pyramid Elimia, *Elimia potosiensis* (Gastropoda, Pleuroceridae), with implications for pleurocerid conservation

Russell L. Minton[1], Bethany L. McGregor[2], David M. Hayes[3], Christopher Paight[4], Kentaro Inoue[5]

1 *Department of Biological and Environmental Sciences, University of Houston Clear Lake, 2700 Bay Area Boulevard MC 39, Houston, Texas 77058 USA*

2 *Florida Medical Entomology Laboratory, Institute of Food and Agricultural Sciences, University of Florida, 200 9th Street SE, Vero Beach, Florida 32962 USA*

3 *Department of Biological Sciences, Eastern Kentucky University, 521 Lancaster Avenue, Richmond, Kentucky 40475 USA*

4 *Department of Biological Sciences, University of Rhode Island, 100 Flagg Road, Kingston, Rhode Island 02881 USA*

5 *Texas A&M Natural Resources Institute, 578 John Kimbrough Boulevard, 2260 TAMU, College Station, Texas 77843 USA*

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Corresponding author: *Russell L. Minton* (mintonrl@gmail.com)

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population genetics

## Abstract

The Interior Highlands, in southern North America, possesses a distinct fauna with numerous endemic species. Many freshwater taxa from this area exhibit genetic structuring consistent with biogeography, but this notion has not been explored in freshwater snails. Using mitochondrial 16S DNA sequences and ISSRs, we aimed to examine genetic structuring in the Pyramid Elimia, *Elimia potosiensis*, at various geographic scales. On a broad scale, maximum likelihood and network analyses of 16S data revealed a high diversity of mitotypes lacking biogeographic patterns across the range of *E. potosiensis*. On smaller geographic scales, ISSRs revealed significant population structure, even over the distance of a few hundred meters. Unlike other freshwater mollusks like mussels, *E. potosiensis* showed no evolutionary patterns relating to biogeography. The species does show population-level genetic structure, which may have implications in conservation efforts.

## Introduction

The Interior Highlands, separated by the Arkansas River Valley into the Ozark and Ouachita regions, is a major geographical feature of southern North America and includes the Ozark and Ouachita Mountains (Fenneman 1928, Mayden 1985). As a result of the geological processes that formed it, the Interior Highlands has a distinct fauna with high instances of endemism and drainage patterns that have dictated diversity (Mayden 1985, Mayden 1988, Matthews and Robison 1998, Austin et al. 2004, Bonett and Chippindale 2004). The Interior Highlands also served as a refugium for many species during periods of glaciation and sea level rise. Analyses of Interior Highland fauna demonstrate a specific relationship between southern Ozark fauna (from Arkansas River tributaries) and Ouachita Mountain fauna (Mayden 1988, Crandall and Templeton 1999, Berendzen et al. 2003). For some aquatic species, the Arkansas River may have formed a barrier for those adapted to cold, fast moving waters (Turner et al. 1996, Bonett and Chippindale 2004), thus isolating populations. Genetic structuring at narrow and broad geographic scales have been observed in freshwater bivalves from the Interior Highlands (Inoue et al. 2013, Chong et al. 2016) but has not been explored in freshwater snails in the same region.

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With nearly 170 recognized species (Johnson et al. 2013), the family Pleuroceridae is the second largest group of freshwater snails in North America behind only the Hydrobiidae. As part of the 700 snail species in North America, pleurocerids are increasingly imperiled by river regulation, habitat loss, poor water quality, reduced water quantity, and invasive species (Johnson et al. 2013). Only 22% of all freshwater snail species are stable in a conservation context, and recent extinctions support these rankings (Sada and Vinyard 2002, Hershler et al. 2007, Johnson et al. 2013). Thirty-three pleurocerid species are extinct, and of all federally endangered and threatened species, 55% of the listed snails are freshwater species including eight pleurocerids (Johnson et al. 2013). Only six recognized species occur west of the Mississippi River (NatureServe 2017), with three of them occurring in the Interior Highlands. The Pyramid Elimia, *Elimia potosiensis* (Lea, 1841), is a morphologically variable species found in springs and rivers throughout the Ozark and Ouachita highlands of Arkansas and Missouri, its range stretching westward into eastern Kansas and Oklahoma (Goodrich 1939, Tiemann and Cummings 2007). Unlike many other pleurocerids, populations of *E. potosiensis* are often large and connected to one another within stream flows (Gordon 1980, Gordon 1982), and the species is secure across its range (global heritage rank of G5; NatureServe 2017). Given that freshwater taxa, including mollusks, in the Interior highlands exhibit genetic structure, and that many freshwater snails are of conservation concern, we aimed to answer three research questions. First, on a broad geographic scale, does *E. potosiensis* represent a single clade showing genetic structuring by population and river drainage? Second, on narrower scales, do populations show any degree of genetic structure within single streams of closely connected waterways? Finally, what impact can our findings have on pleurocerid conservation?

We utilized two forms of genetic data for our study, mitochondrial DNA sequences at the species level and inter-simple sequence repeats (ISSRs) at the population level. The bulk of freshwater gastropod genetic studies employ one of two mitochondrial DNA fragments derived from either the 16S or cytochrome oxidase *c* subunit I gene. While these sequences may show utility in population and species-level studies, results are mixed in freshwater cerithioideans including pleurocerids (e.g. Köhler and Deein 2010, Miura et al. 2013, Köhler 2016). Widely divergent mitochondrial haplotypes can be found in single populations and species that can cloud questions of monophyly and relatedness (Dillon and Frankis 2004, Whelan and Strong 2016), and no consistent explanation has been offered as to why these divergent haplotypes exist or persist (Whelan and Strong 2016). Not all studies, however, report this issue (Lydeard et al. 1998, Minton and Lydeard 2003, Minton and Savarese 2005). ISSRs are fragments of DNA separating neighboring microsatellites used as genetic markers amplified using polymerase chain reaction (PCR) (Zietkiewicz et al. 1994). Primers for ISSR amplification are complementary to microsatellite sites, bind to them using a one to three nucleotide anchor sequence on the 5' or 3' end (Bussell et al. 2005), and amplify the ISSR in between (Culley and Wolfe 2001). This approach does not require prior genome sequence information, and leads to banding patterns containing multiple loci and high levels of polymorphism (Tsumura et al. 1996). ISSRs have provided useful population data in a variety of organisms including plants (Lisek and Rozpara 2010), insects (Vijayan et al. 2006), arachnids (Machkour-M'Rabet et al. 2009), and birds (Haig et al. 2003), but have not been utilized extensively in gastropods (Dong et al. 2011, Snegin 2014).

## Materials and methods

### Broad scale phylogenetic analyses

We collected live *E. potosiensis* from six river drainages throughout the Ozark and Ouachita highlands (Figure 1). We followed a standard CTAB/chloroform protocol (Saghai-Maroof et al. 1984) for DNA extraction. We amplified a 500 bp portion of the 16S ribosomal subunit (primers 16sar and 16sbr [Palumbi, 1996]) using Qiagen Taq PCR Core Kit and the following thermal cycling conditions: an initial denaturation cycle of 95°C for three minutes; 40 cycles of 95°C for 35s, 44°C for 45s, 72°C for 45s; and a final five-minute extension period at 72°C. We sequenced gel-purified products on a Beckman CEQ8000 automated sequencer using Sanger dideoxy sequencing. We used Popart (Leigh and Bryant 2015) to calculate nucleotide diversity and Tajima's *D* (Tajima 1989), and to visualize haplotype diversity in *E. potosiensis* by generating a TCS (Clement et al. 2002) network. We then combined our *E. potosiensis* sequences with other pleurocerid 16S data taken from NCBI (Table 1) and aligned them using the Muscle algorithm (Edgar 2004) with default settings. We used Gblocks 0.91b (Castresana 2000) with default settings to remove poorly aligned positions from the data, and used the ModelFinder (Kalyaanamoorthy et al. 2017) function of Iq-tree (Nguyen et al. 2015) to select the appropriate substitution model by Bayesian information criterion. We then analyzed the data under maximum likelihood in Iq-tree and estimated branch support using 10,000 ultra-fast bootstrap replicates (Minh et al. 2013). We also tested the hypothesis that *E. potosiensis* sequences from each separate river drainages represented separate monophyletic groups. We used the approximately unbiased test (Shimodaira 2002) with 1000 RELL bootstrap replicates implemented in Iq-tree to test whether topologies were significantly different ($p<0.05$).

### Narrow scale population genetic analyses

We employed ISSRs as population markers on two different spatial scales. In our first study, herein referred to as the 'small' study, ten individuals from each of 12 *E. potosiensis* populations were collected (Figure 2, white circles). Four populations were in a spring run flowing into Walnut Creek in the Arkansas portion of the Ouachita

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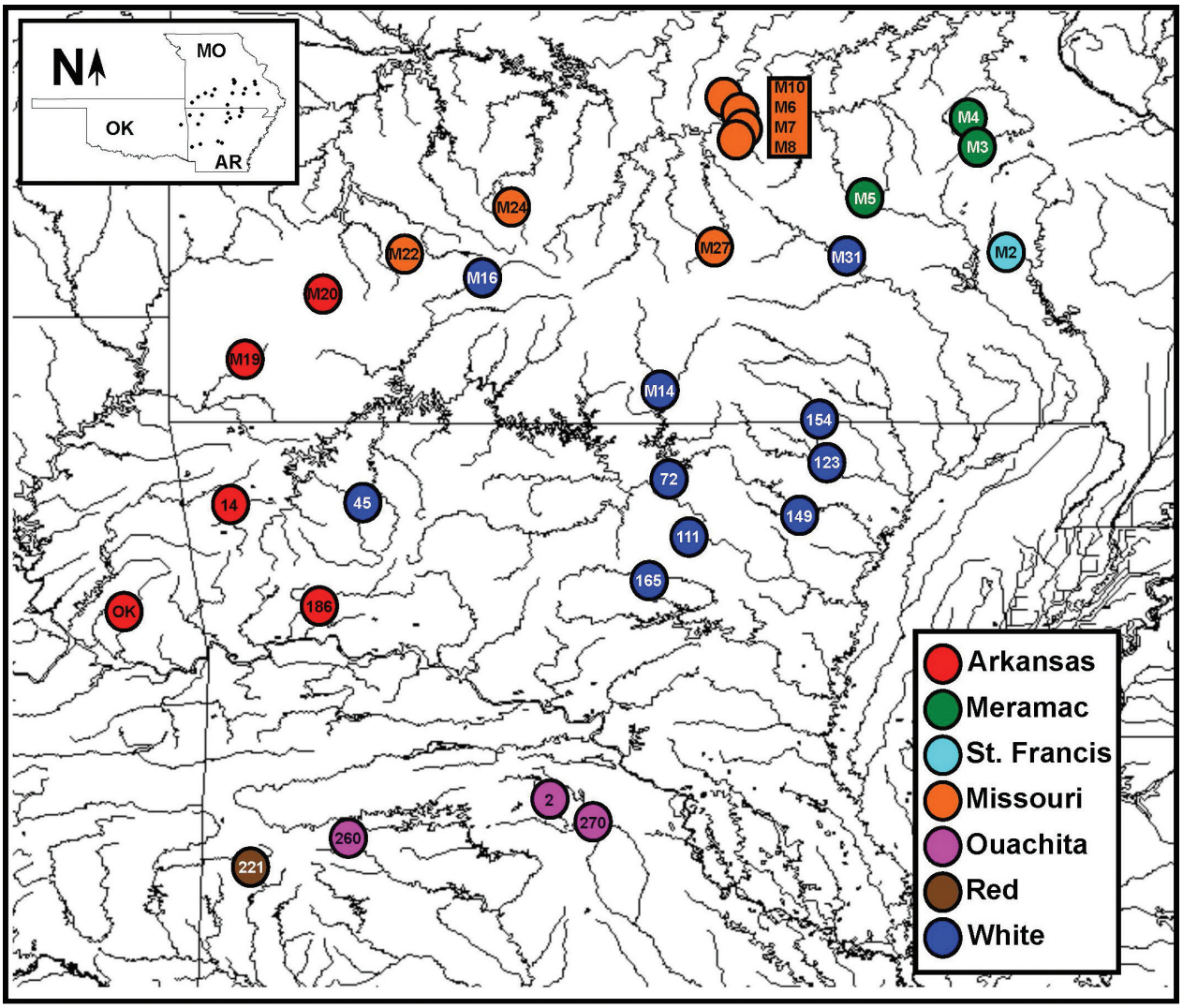


**Figure 1.** Map of *E. potosiensis* collection localities, color coded by river drainage, used in the phylogenetic analysis. Detailed locality information is in Table 1.

National Forest. Populations were sampled at the mouth of the spring run (S4) and in 50 m intervals upstream (S3-S1); at the mouth, the spring run flowed over a 1 m rock ledge into Walnut Creek. Populations from Walnut Creek were sampled as well, using the confluence point with the spring drainage (C3) as a starting point. Populations were sampled in 50 m intervals upstream (C2 and C1) and downstream (B1–B5, below where the creek flows under the road through a culvert).

We followed the 'small' study with a second 'large' study. We collected 50 *E. potosiensis* from each of four localities (Figure 2, gray circles). Three sites were located in the Walnut Creek drainage in the Arkansas portion of the Ouachita National Forest. The first (ARK1) was a small spring feeding the main channel of Walnut Creek. The second and third were in Walnut Creek, 325 m upstream and 350 m downstream of where the ARK1 spring run entered the creek. The final site was Sallisaw Creek, Sequoyah County, Oklahoma near Marble City. For both studies, samples were stored in 95% ethanol or frozen at -20°C.

We isolated genomic DNA as before, purified it on silica filters (UltraClean PCR Clean-up Kit, MoBio), and diluted it with sterile water to 50 ng/µl concentration. We amplified ISSRs by PCR in 50 µl volumes with the GoTaq PCR Core System I reagents (Promega) at the manufacturer's recommended concentrations. The cycling profile consisted of an initial denaturation cycle of 95°C for three minutes, 30 cycles of 95°C for 30s, annealing for 30s, and 72°C for 60s, followed by a ten-minute extension period at 72°C. We used one primer in each ISSR reaction (Table 2) and optimized each annealing temperature. For the 'small' study we visualized ISSRs by running 10 µl of PCR product on 2% agarose gels in TBE at 100 V for 90 minutes. For the 'large' study, 20 µl of PCR product was run on 10% polyacrylamide-TBE gels at 110V for two hours. In both, a 0.1–1 kb size ladder was loaded, and we stained gels 1% ethidium bromide then de-stained in deionized water. Gels were photographed (Bio-Rad Chemi XRS) and processed (Bio-Rad Quantity One) before analysis. We coded individual bands if they

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**Table 1.** Locality and NCBI accession numbers for sequences used in this study.

| Taxon | Accession | Locality | Identifier | Reference |
|---|---|---|---|---|
| *Elimia alabamensis* | U73761 | | | Lydeard et al. 1997 |
| *E. caelatura* | AF100988 | | | Holzangel and Lydeard 2000 |
| *E. catenaria* | FJ471493 | | | Strong and Kohler 2009 |
| *E. clenchi* | FJ471492 | | | Strong and Kohler 2009 |
| *E. comalensis* | KU052563 | Salado Creek at Interstate 35, Salado, Bell County, TX | | new |
| *E. crenatella* | U73762 | | | Lydeard et al. 1997 |
| *E. cylindracea* | U73765 | | | Lydeard et al. 1997 |
| *E. doolyensis* | DQ311118 | | | Lee et al. 2006 |
| *E. haysiana* | U73763 | | | Lydeard et al. 1997 |
| *E. hydei* | U73764 | | | Lydeard et al. 1997 |
| *E. interrupta* | AY010521 | | | Lydeard et al. 2002 |
| *E. laqueata* | KU052565 | Green River, KY | | new |
| *E. livescens 1* | DQ311116 | | | Lee et al. 2006 |
| *E. livescens 2* | KU052564 | French Creek, PA | | new |
| *E. melanoides* | AF540003 | | | Minton et al. 2003 |
| *E. olivula* | U73766 | | | Lydeard et al. 1997 |
| *E. potosiensis* | KT988910 | Alum Fork Saline River, AR 34.67310N, 92.79920W | 2 | new |
| | KT988911 | | | |
| | KT988965 | Illinois River, AR 36.10320N, 94.34500W | 14 | new |
| | KT988932 | War Eagle Creek, AR 36.12100N, 93.69340W | 45 | new |
| | KT988962 | | | |
| | KT988940 | Otter Creek, AR 36.22380N, 92.25190W | 72 | new |
| | KT988923 | Blanchard Springs, AR 35.95680N, 92.13960W | 111 | new |
| | KT988950 | | | |
| | KT988951 | | | |
| | KT988956 | Spring River, AR 36.31530N, 91.49080W | 123 | new |
| | KT988957 | | | |
| | KT988964 | Mill Creek Spring, AR 36.05720N, 91.60890W | 149 | new |
| | KT988916 | Mammoth Spring, AR 36.49580N, 91.53320W | 154-1 | new |
| | KT988922 | | | |
| | KT988943 | | | |
| | KT988944 | | | |
| | KT988945 | | | |
| | KT988933 | Warm Fork Spring, AR 36.49580N, 91.53320W | 154-2 | new |
| | KT988934 | | | |
| | KT988929 | | | |
| | KT988954 | Mulberry Fork Little Red River, AR 35.74210N, 92.33380W | 165 | new |
| | KT988955 | | | |
| | KT988939 | Mulberry River, AR 35.62275N, 93.91023W | 186 | new |
| | KT988958 | Cossatot River, AR 34.37950N, 94.23680W | 221 | new |
| | KT988967 | | | |
| | KT988963 | Tributary to South Fork Ouachita River, AR 34.51870N, 93.75940W | 260 | new |
| | KT998966 | Saline River, AR 34.58710N, 92.60480W | 270 | new |
| | KT988968 | | | |
| | KT988953 | Big Creek, MO 37.29375N, 90.62775W | M2 | new |
| | KT988927 | Big River, MO 37.81483N, 90.77049W | M3 | new |
| | KT988941 | Brenton Creek, MO 37.93847N, 90.79239W | M4 | new |
| | KT988952 | | | |
| | KT988921 | Meramac River, MO 37.57011N, 91.30293W | M5 | new |
| | KT988938 | | | |
| | KT988942 | | | |
| | KT988918 | Trek Creek, MO 37.95693N, 91.89561W | M6 | new |
| | KT988919 | | | |
| | KT988925 | | | |
| | KT988949 | Little Piney River, MO 37.91063N, 91.90381W | M7 | new |
| | KT988960 | | | |
| | KT988924 | Mill Creek, MO 37.87296N, 91.92921W | M8 | new |
| | KT988928 | | | |

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| Taxon | Accession | Locality | Identifier | Reference |
|---|---|---|---|---|
| *E. potosiensis* | KT988947 | Clifty Creek, MO 38.04173N, 91.96089W | M10 | new |
| | KT988948 | | | |
| | KT988914 | North ForkWhite River, MO 36.66723N, 92.28129W | M14 | new |
| | KT988915 | | | |
| | KT988926 | | | |
| | KT988931 | James River, MO 37.19040N, 93.12660W | M16 | new |
| | KT988937 | Indian Creek, MO 36.79320N, 94.24380W | M19 | new |
| | KT988930 | Spring River, MO 37.11560N, 93.89420W | M20 | new |
| | KT988961 | Clear Creek, MO 37.30831N, 93.50060W | M22 | new |
| | KT988970 | | | |
| | KT988959 | Niangua River, MO 37.51970N, 92.98420W | M24 | new |
| | KT988969 | | | |
| | KT988917 | Big Piney River, MO 37.32720N, 92.00210W | M27 | new |
| | KT988946 | | | |
| | KT988935 | Current River, MO 37.27990N, 91.40600W | M31 | new |
| | KT988936 | | | |
| | KT988912 | Sallisaw Creek, OK 35.57660N, 94.83047W | OK | new |
| | KT988913 | | | |
| | KT988920 | | | |
| *E. showalteri* | U73767 | | | Lydeard et al. 1997 |
| *E. virginica 1* | DQ311117 | | | Lee et al. 2006 |
| *E. virginica 2* | AF100989 | | | Holzangel and Lydeard 2000 |
| *Io fluvialis* | AF100999 | | | Holzangel and Lydeard 2000 |
| *Juga plicifera* | AF101004 | | | Holzangel and Lydeard 2000 |
| *Leptoxis ampla 1* | U73768 | | | Lydeard et al. 1997 |
| *Leptoxis ampla 2* | KF680604 | | | unpublished |
| *Le. crassa anthonyi* | AF101001 | | | Holzangel and Lydeard 2000 |
| *Le. dilatata* | DQ311122 | | | Lee et al. 2006 |
| *Le. foremani* | KF680592 | | | unpublished |
| *Le. picta 1* | KF680596 | | | unpublished |
| *Le. picta 2* | U73769 | | | Lydeard et al. 1997 |
| *Le. plicata* | U73770 | | | Lydeard et al. 1997 |
| *Le. praerosa* | AF101002 | | | Holzangel and Lydeard 2000 |
| *Le. taeniata 1* | U73771 | | | Lydeard et al. 1997 |
| *Le. taeniata 2* | KF680600 | | | unpublished |
| *Le. virgata* | AF101000 | | | Holzangel and Lydeard 2000 |
| *Lithasia armigera* | AF100998 | | | Holzangel and Lydeard 2000 |
| *Li. duttoniana* | AF100997 | | | Holzangel and Lydeard 2000 |
| *Li. geniculata fuliginosa* | AF100996 | | | Holzangel and Lydeard 2000 |
| *Li. geniculata geniculata* | AF100995 | | | Holzangel and Lydeard 2000 |
| *Pleurocera acuta 1* | AF100994 | | | Holzangel and Lydeard 2000 |
| *P. acuta 2* | MF357697 | Mulberry Fork Little Red River, AR 35.74210N, 92.33380W | | new |
| *P. acuta 3* | MF357698 | Warm Fork Spring, AR 36.49580N, 91.53320W | | new |
| *P. annulifera* | U73772 | | | Lydeard et al. 1997 |
| *P. prasinata 1* | U73774 | | | Lydeard et al. 1997 |
| *P. prasinata 2* | U73773 | | | Lydeard et al. 1997 |
| *P. pyrenella 1* | AF100990 | | | Holzangel and Lydeard 2000 |
| *P. pyrenella 2* | DQ311123 | | | Lee et al. 2006 |
| *P. pyrenella 3* | KT164352 | | | Whelan and Strong 2016 |
| *P. uncialis hastata* | AF100993 | | | Holzangel and Lydeard 2000 |
| *P. vestita* | U73775 | | | Holzangel and Lydeard 2000 |
| *P. walkeri* | AF100992 | | | Holzangel and Lydeard 2000 |

were clear and reliably scored in each individual. Bands were identified and sized using GelAnalyzer2010 (http://www.gelanalyzer.com/), then binned into markers with 5 bp tolerance, For both studies bands were scored as present or absent in each individual to produce separate data matrices.

We analyzed our data matrices using GenAlEx 6.052 (Peakall and Smouse 2006) and the 'poppr' 2.2.1 package (Kamvar et al. 2014, Kamvar et al. 2015) in R 3.3.1 (R Core Team 2016). For each, we generated a genotype accumulation curve (Kamvar et al. 2015) to determine the minimum number of loci necessary to discriminate

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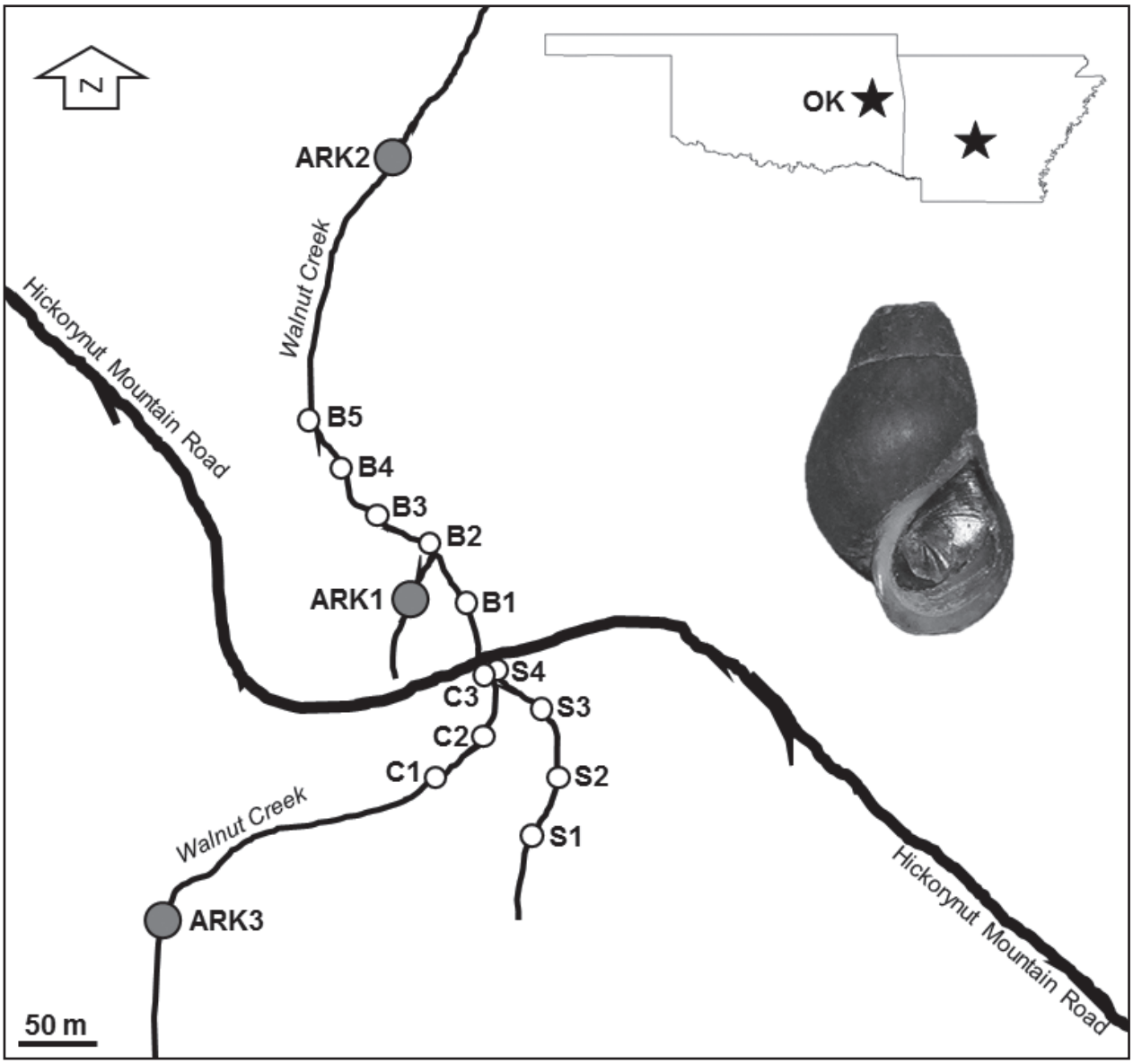


**Figure 2.** Map showing collecting localities for both ISSR studies. Inset shows position of Oklahoma (OK) relative to Arkansas (ARK1-3) populations. Shell of *E. potosiensis* from population OK is shown.

**Table 2.** Sequences, annealing temperatures (ºC), and number of bands produced for ISSR primers used in each study.

| 'small' study | | | 'large study' | | |
|---|---|---|---|---|---|
| **Primer sequence** | **Annealing temperature** | **number of bands** | **Primer sequence** | **Annealing temperature** | **number of bands** |
| 5'-$(AG)_8$T | 50° | 6 | 5'-$(AC)_8$C | 53° | 47 |
| 5'-$AC_8$ | 49° | 2 | 5'-$(CCA)_5$ | 54° | 27 |
| 5'-BHB$(GA)_7$ | 49° | 1 | 5'-$(CA)_7$RG | 53° | 26 |
| 5'-RY$(CA)_7$ | 49° | 2 | | | |
| 5'-$CA_7$ | 54° | 7 | | | |
| 5'-WB$(GACA)_4$ | 50° | 4 | | | |

between individuals in a population. We calculated Simpson diversity and evenness (Grünwald et al. 2003) at each locus, and Nei's gene diversity (Nei 1973) for each population. An analysis of molecular variance (Excoffier et al. 1992) was performed on the data by populations in regions (spring [S] versus creek upstream [C] versus creek downstream [B], or Arkansas versus Oklahoma) to partition the overall genetic variation. The Φ statistic was used to describe ratios of between and among population and region variance to total variance; Φ values are comparable to F statistics calculated for dominant-only genotype markers (Excoffier et al. 1992). Significance of

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Φ was determined through standard permutation (10,000 replicates) of the entire dataset. We also used principal coordinates analysis (PCoA) based on Nei's genetic distance to visualize population structure.

# Results

### Broad scale phylogenetic analyses

Within *E. potosiensis* we observed nucleotide diversity $\pi = 0.066$ and Tajima's D = 0.894 (p [D > 0.894] = 0.38) using aligned sequences prior to processing with Gblocks. Our parsimony network (Figure 3) connected the 30 resulting 16S haplotypes with a maximum of 83 mutations. Our final data matrix processed through Gblocks consisted of 110 sequences and 317 nucleotide sites. Under maximum likelihood we generated a single tree (log likelihood = -2454.1915, se = 186.2554) under the HKY+R3 model (Figure 4). The *E. potosiensis* sequences did not form a single clade but rather resolved in four well-supported clades. Constraining the monophyly of *E. potosiensis*, however, did not produce a significantly different topology (p = 0.63). Individuals from single localities were not necessarily recovered as sister taxa, and constraining them to be sister produced a significantly less likely topology (p < 0.001). Also, no grouping of sequences by river drainage was observed in the overall tree nor in the four *E. potosiensis* clades, and constraining the monophyly of each river drainage generated significantly less likely topologies (p < 0.001).

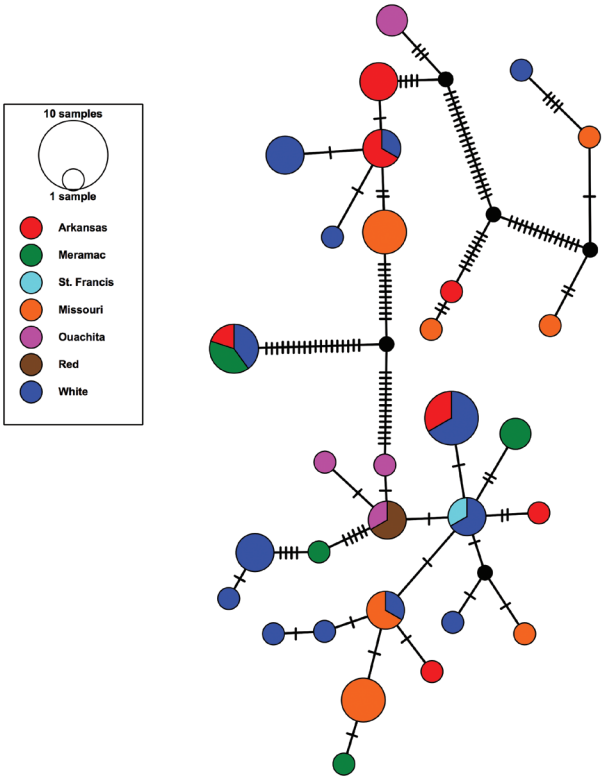


**Figure 3.** TCS parsimony network of *E. potosiensis* 16S haplotypes color coded by river drainage. Hash marks represent mutational steps between haplotypes.

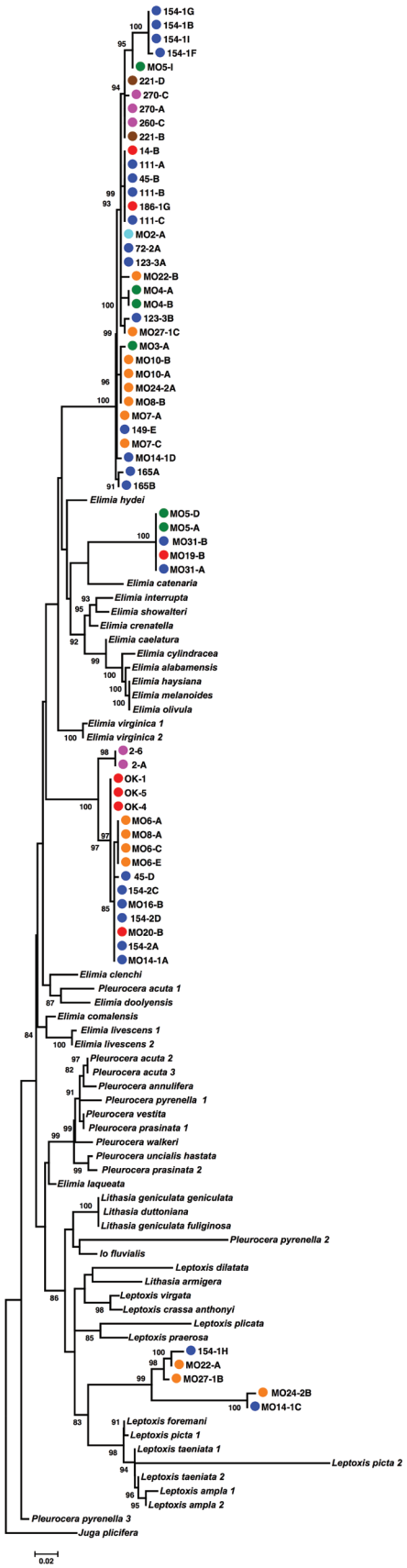


**Figure 4.** Maximum-likelihood phylogeny of *E. potosiensis* sequences. Individual snails are color-coded by river drainage and labelled according to Figure 1 and Table 1. Branches with bootstrap support ≥ 80% are labeled.

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**Population genetics**

In the 'small' study, we were able to bin and identify 22 markers. There were 110 unique genotypes identified among the 120 total individual snails. A genotype accumulation curve suggested that the minimum number of markers needed to discriminate individuals was 19, so our analyses were close to the detection limit for those markers. Simpson diversity for individual loci ranged from 0.033 to 0.500, while evenness ranged from 0.383 to 1. Nei's gene diversity by population ranged from 0.248 (B3) to 0.394 (C3). AMOVA (Table 3) showed that most of the variation in the data was within populations (95.86%), followed by between the regions (spring and creek sites) (3.91%) and within each region (0.23%). Permutation tests suggested significant ($p < 0.01$) genetic structuring between populations and between regions (spring or creek), but not between populations within the same region ($p = 0.28$). Pairwise comparisons of populations suggested low genetic differentiation between populations ($\Phi \leq 0.133$), and PCoA showed no clustering by proximity or region (Figure 5).

In the 'large' study, we were able to bin and identify a total of 100 markers. Each of the 200 snails possessed its own unique genotype. A genotype accumulation curve suggested that a minimum of 31 polymorphic markers was needed to discriminate individuals, so our dataset of 98 was sufficient for further analysis. Simpson diversity for individual loci ranged from 0.303 to 0.500, while evenness ranged from 0.376 to 1. Nei's gene diversity for the populations ranged from 0.254 for ARK1 to 0.328 for ARK3. An AMOVA (Table 4) showed that most of the variation in the dataset was seen between individuals of a population (66.8% of total). The least variation (12.1%) was seen between populations in a region (Arkansas or Oklahoma), and the remainder (21.1%) existed between regions. Permutation tests indicated significant genetic structuring within populations, between populations, and between regions ($\Phi = 0.133$ to 0.367); pairwise comparisons at all levels were significant at $p<0.01$. PCoA suggested complete separation of OK from the ARK populations, and little overlap between the three ARK populations (Figure 6).

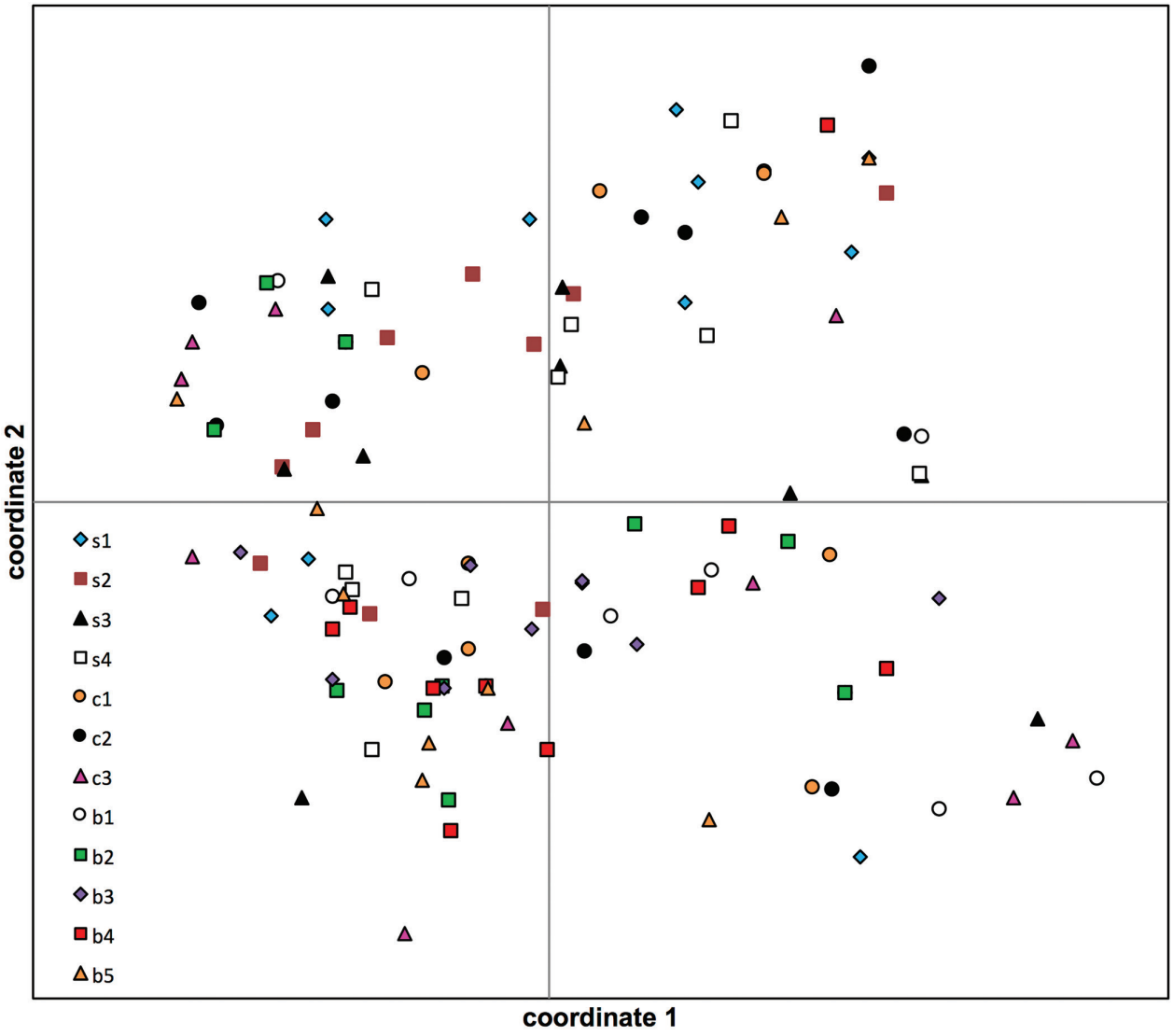


**Figure 5.** Principal coordinates analysis of ISSR genetic distances from the 'small' study showing overlap of populations. Populations labeled as in Figure 2.

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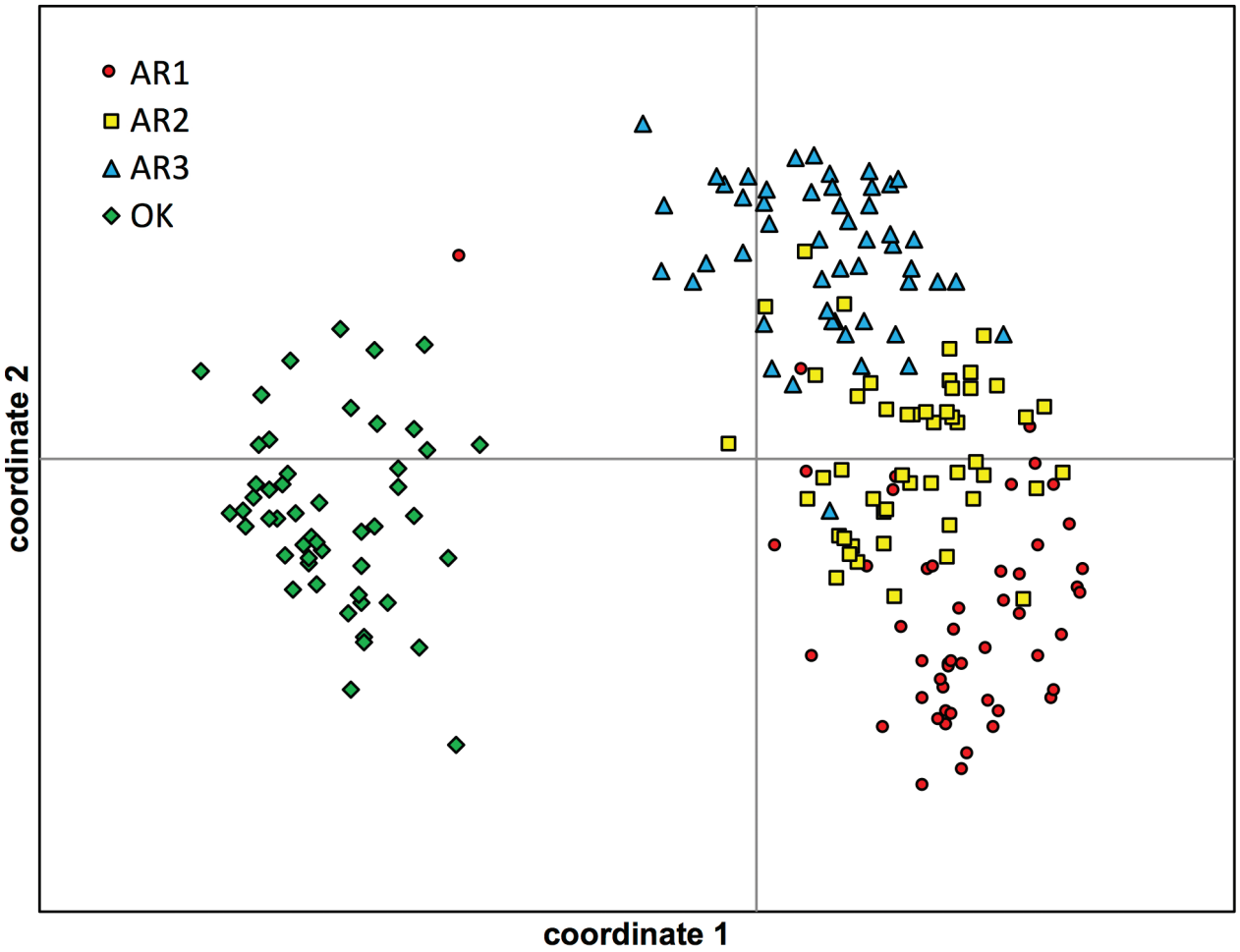


**Figure 6.** Principal coordinates analysis of ISSR genetic distances from the 'large' study showing separation of populations. Populations labeled as in Figure 2.

**Table 3.** Results of the 'small' study AMOVA. Regions were spring (S populations), and creek above (C) and below (B) confluence with the spring.

| Source | d.f. | Φ | % total variation | p value |
|---|---|---|---|---|
| Within populations | 108 | 0.054 | 95.86 | 0.014 |
| Between populations within regions | 10 | 0.007 | 0.23 | 0.274 |
| Between regions | 1 | 0.047 | 3.91 | 0.005 |

**Table 4**. Results of the 'large' study AMOVA. Regions were either Arkansas or Oklahoma.

| Source | d.f. | Φ | % total variation | p value |
|---|---|---|---|---|
| Within populations | 196 | 0.332 | 66.80 | <0.01 |
| Between populations within regions | 2 | 0.153 | 12.10 | <0.01 |
| Between regions | 1 | 0.211 | 21.10 | <0.01 |

## Discussion

Freshwater taxa from the same region tend to share evolutionary history, resulting in replicated patterns of biogeography and speciation (Walker and Avise 1998, Carini and Hughes 2006). This is true in the interior highlands, where the Ozark and Ouachita drainages represent separate areas of endemism (reviewed in Hoagstrom et al. 2014). Phylogenies of freshwater mussels reflect these patterns (Serb and Barnhart 2008, Inoue et al. 2013, Chong et al. 2016), but they remained unexplored in freshwater snails. Our nuclear ISSR data suggest that *Elimia potosiensis* exhibits genetic population differentiation, even at small geographic scales, while our mitochondrial data show no evidence of phylogeography across the species range.

Our analyses of mitochondrial 16S sequences suggested no genetic structuring. Mitochondrial haplotypes did not group together in our TCS network, and we recovered *E. potosiensis* in four well-supported clades instead of a single clade. However, we could not reject the monophyly of *E. potosiensis* based on our data. No statistical support existed for genetic structure within populations, within river drainages, or within populations or drainages in each of the four clades. We observed no evidence of heteroplasmy to suggest nuclear analogs of mitochon-

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drial sequences ("numts") in our data, nor did we find any biogeographic groupings of sequences that would suggest cryptic taxa. What we did observe, however, was a pattern where *E. potosiensis* sequences comprised multiple well-supported divergent clades. As mentioned previously, this is frequently observed in freshwater snails including pleurocerids using mitochondrial markers. Whelan and Strong (2016) showed that the pattern exists across multiple valid species, is not limited to specific taxa, and becomes more apparent as additional sequences from each population are included in an analysis. The maximum number of individuals we sequenced for a single population was five, and two of these individuals exhibited the highest pairwise sequence divergence. We predict that additional *E. potosiensis* samples would only increase the observed sequence diversity while not increasing phylogenetic resolution. Whelan and Strong (2016) proposed that balancing selection might keep divergent haplotypes in pleurocerid populations. Our Tajima's *D* of 0.894 suggests balancing selection of 16S haplotypes in *E. potosiensis*, though population reduction and migration can generate similar results (Maruyama and Fuerst 1985, Simonsen et al. 1995).

Four nominal morphospecies comprise the modern notion of *E. potosiensis* (Burch and Tottenham 1980): *E. potosiensis* (Lea, 1841), a narrow-range species from Missouri; *E. crandalli* (Pilsbry, 1890), endemic to Mammoth Springs, Arkansas; *E. ozarkensis* (Call, 1886), found in springs in north Arkansas; and *E. plebejus* (Gould, 1850), the most widespread and the shell form currently associated with *E. potosiensis*. While *E. potosiensis* does possess shell variation that may have an environmental component (Minton et al. 2011), no additional data supports recognition of any separate morphological entities. Jones and Branson performed detailed examinations of internal anatomy and radula structure of all four nominal *E. potosiensis* taxa, noting that, without their shells, internal structures of the morphotypes "...can not be separated" (Jones and Branson 1964: 60), and that the shell variations seen among the forms could be found throughout the species' range. They thus treated *E. potosiensis* as single species exhibiting shell plasticity, where morphological "...variation is the rule rather than the exception" (Jones and Branson 1964: 60). This anatomical data, combined with our inability to reject the monophyly of *E. potosiensis*, lead us to support the current notion (Burch and Tottenham 1980, Johnson et al. 2013) that *E. potosiensis* is a single widespread species.

While we saw no broad scale genetic patterns in *E. potosiensis*, our two ISSR studies suggested population-level genetic structuring. Our 'small' study of ten individuals from each of 12 populations represented a pilot study as well as our first effort with ISSRs. In it, we were able to characterize 22 bands visualized on agarose gels using six primers. The AMOVA suggested that most of the genetic variation was within each population, but that a small (Hartl and Clark 1997) yet significant amount of difference existed between the spring and two creek regions. We predict that the spring is isolated from the creek by the one meter of elevation, as the spring run flows over a rocky ledge and into the creek below. This normally serves as an isolating mechanism between populations in the two channels. Snails within each channel can migrate upstream and downstream freely, and during heavy rains and flooding conditions, the creek can crest above the spring. This could potentially carry individuals from the spring into the creek and vice-versa, and may explain the low pairwise Φ values observed between populations and lack of PCoA clustering (Wilmer et al. 2011). Our 'large' study showed complete differentiation of the four populations examined with high levels of genetic differentiation at all population and region levels (Hartl and Clark 1997). We used large numbers of individuals and markers, both of which increase the resolution and accuracy of population genetic studies (Ruzzante 1998, Kalinowski 2005), and utilized polyacrylamide gels to better visualize bands. Our AMOVA and PCoA suggested that each population is genetically distinct from the others.

Two issues we identified in our 'small' study were the low number of bands resolved by our primers and the small sample sizes from each population. Our band total for six primers was comparable to the number generated by a single primer in other studies, and is likely indicative of two factors. First, choice of our primer sequences was probably not ideal, employing dinucleotide repeats and/or degenerate 5'-bases ahead of the repeated portion. Results from the literature suggest that trinucleotide repeats and 3'-degenerate anchors after the repeats increase resolution and repeatability (Godwin et al. 1997). Second, we visualized our bands on agarose instead of polyacrylamide. Agarose has far less resolving power, and multiple bands in a small range of sizes might not have been identifiable on our gels (Stift et al. 2003). In regards to sample size, many studies recommend thirty individuals per population (Pruett and Winker 2008, Hale et al. 2012). Our AMOVA suggested treating the regional groupings as populations, where we were able to resolve significant structuring on a small spatial scale given the now larger sample sizes. The differences in our 'small' and 'large' studies highlighted the importance of having the appropriate number of markers and individuals.

Our study also highlighted the need for novel and useful genetic markers for pleurocerid conservation. Nearly 80% of all pleurocerids are imperiled (Johnson et al. 2013), and most species designations still rely on shell morphology. The genetic species and population structure of most pleurocerid taxa remains unknown. Our study provides the latest example where gene sequences are not useful in delineating neither population nor species-level boundaries. Results from analyses of mitochondrial gene sequences are mixed, generating far less resolution (e.g. Minton and Savarese 2005, Minton 2013) than uncertainty (reviewed in Whelan and Strong 2016). Single mitochondrial gene sequences, even from the same population, can differ by over 20%, which can lead to hypotheses of species non-monophyly and aberrant biogeography

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(Dillon and Robinson 2009). Nuclear gene sequences, on the other hand, are too highly conserved in pleurocerids to be useful (Whelan and Strong 2016). Other forms of nuclear data, however, such as allozymes and now ISSRs, have demonstrated utility in population genetic studies. Allozymes have been used to show genetic connectivity in various pleurocerid genera (Chambers 1980, Dillon 1984, Stein and Stansbery 1984, Dillon 2014), and herein our data suggests ISSRs show promise across large and small geographic levels. No published applications of more modern population genetic techniques (microsatellites, SNPs, etc.) are available, but we hope that they too will be useful in understanding pleurocerids. The continued development of consistently useful tools is needed to help properly understand genetic population and species structure in pleurocerids, especially in a conservation contextS before more taxa are lost.

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## References

Austin JS, Lougheed SC, Boag PT (2004) Discordant temporal and geographic patterns in maternal lineages of eastern North American frogs, *Rana catesbeiana* (Ranidae) and *Pseudacris crucifer* (Hylidae). Molecular Phylogenetics and Evolution 32(3): 799–816. https://doi.org/10.1016/j.ympev.2004.03.006

Berendzen PB, Simons AM, Wood RW (2003) Phylogeography of the northern hogsucker, *Hypentelium nigricans* (Teleostei: Cypriniformes): genetic evidence for the existence of the ancient Teays River. Journal of Biogeography 30(8): 1139–1152. https://doi.org/10.1046/j.1365-2699.2003.00888.x

Bonett RM, Chippindale PT (2004) Speciation, phylogeography and evolution of life history and morphology in plethodontid salamanders of the *Eurycea multiplicata* complex. Molecular Ecology 13(5): 1189–1203. https://doi.org/10.1111/j.1365-294X.2004.02130.x

Burch JB, Tottenham JL (1980) North American freshwater snails. Species list, ranges and illustrations. Walkerana 1: 81-215. http://molluskconservation.org/PUBLICATIONS/WALKERANA/Vol1/walkerana%20vol1%20no3%2081-216.PDF

Bussell JD, Waycott M, Chappill JA (2005) Arbitrarily amplified DNA markers as characters for phylogenetic inference. Perspectives in Plant Ecology, Evolution and Systematics 7(1): 3–26. https://doi.org/10.1016/j.ppees.2004.07.001

Carini G, Hughes JM (2006) Subdivided population structure and phylogeography of an endangered freshwater snail, *Notopala sublineata* (Conrad, 1850) (Gastropoda: Viviparidae), in Western Queensland, Australia. Biological Journal of the Linnean Society 88(1): 1–16. https://doi.org/10.1111/j.1095-8312.2006.00594.x

Castresana J (2000) Selection of conserved block from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17(4): 540–552.https://doi.org/10.1093/oxfordjournals.molbev.a026334

Chambers SM (1980) Genetic divergence between populations of *Goniobasis* (Pleuroceridae) occupying different drainage systems. Malacologia 20(1): 63–82. http://biodiversitylibrary.org/page/13148738

Chong JP, Harris JL, Roe KJ (2016) Incongruence between mtDNA and nuclear data in the freshwater mussel genus *Cyprogenia* (Bivalvia: Unionidae) and its impact on species delineation. Ecology and Evolution 6(8): 2439–2452. https://doi.org/10.1002/ece3.2071

Clement M, Snell Q, Walke P, Posada D, Crandall K (2002) TCS: estimating gene genealogies. Proceeding 16th International Parallel Distributed Processing Symposium, 184 pp.

Crandall KA, Templeton AR (1999) The zoogeography and centers of origin of the crayfish subgenus *Procericambarus* (Decapoda: Cambaridae). Evolution 53(1): 123–134. https://doi.org/10.1111/j.1558-5646.1999.tb05338.x

Culley TM, Wolfe AD (2001) Population genetic structure of the cleistogamous plant species *Viola pubescens* Aiton (Violaceae), as indicated by allozyme and ISSR molecular markers. Heredity 86: 545–556. https://doi.org/10.1046/j.1365-2540.2001.00875.x

Dillon RT (1984) Geographic distance, environmental different, and divergence between isolated populations. Systematic Zoology 33(1): 69–82. https://doi.org/10.1093/sysbio/33.1.69

Dillon RT (2015) Cryptic phenotypic plasticity in populations of the North American freshwater gastropod, *Pleurocera semicarinata*. Zoological Studies 53: 31. https://doi.org/10.1186/s40555-014-0031-5

Dillon RT, Frankis R (2004) High levels of mitochondrial DNA sequence divergence in isolated populations of freshwater snails of the genus *Goniobasis* Lea, 1862. American Malacological Bulletin 19(1–2): 69–77. http://www.fwgna.org/dillonr/dillon&-frankis.pdf

Dillon RT, Robinson JD (2009) The snails the dinosaurs saw: are the pleurocerid populations of the Older Appalachians a relict of the Paleozoic Era? Journal of the North American Benthological Society 28(1): 1–11. https://doi.org/10.1899/08-034.1

Dong S, Shentu X, Pan Y, Bai Y, Yu X, Wang H (2011) Evaluation of genetic diversity in the golden apple snail, *Pomacea canaliculata* (Lamarck), from different geographical populations in China by inter simple sequence repeat (ISSR). African Journal of Biotechnology 10(10): 1777–1783. http://www.ajol.info/index.php/ajb/article/download/93084/82495

Edgar RC (2004) Muscle: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5):1792–1797. https://doi.org/10.1093/nar/gkh340

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131(2): 479–491. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1205020/

Fenneman NM (1928) Physiographic regions of the United States. Annals of the Association of American Geographers 18(4): 261–353. https://doi.org/10.1080/00045602809357034

Creative Commons Attribution 4.0 licence (CC-BY); original download https://pensoft.net/journals



Godwin ID, Aitken EA, Smith LW (1997) Application of inter simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis 18(9): 1524–1528. https://doi.org/10.1002/elps.1150180906

Goodrich C (1939) Pleuroceridae of the Mississippi River basin exclusive of the Ohio River system. University of Michigan, Occasional Papers of the Museum of Zoology 406: 1–4. https://deepblue.lib.umich.edu/bitstream/handle/2027.42/56845/OP406.pdf

Gordon ME (1980) Recent Mollusca of Arkansas with annotations to systematics and zoogeography. Arkansas Academy of Science Proceedings 34: 58-62. https://libraries.uark.edu/aas/issues/1980v34/v34a16.pdf

Gordon ME (1982) Mollusca of the White River, Arkansas and Missouri. The Southwestern Naturalist 27(3): 347–352. http://www.jstor.org/stable/3670886

Grünwald NJ, Goodwin SB, Milgroom MG, Fry WE (2003) Analysis of genotypic diversity data for populations of microorganisms. Phytopathology 93(6): 738–746. https://doi.org/10.1094/PHYTO.2003.93.6.738

Haig SM, Mace TR, Mullins TD (2003) Parentage and relatedness in polyandrous comb-crested jacanas using ISSRs. Journal of Heredity 94(4): 302–309. https://doi.org/10.1093/jhered/esg072

Hale ML, Burg TM, Steeves TE (2012) Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. PLOS ONE 7: e45170. https://doi.org/10.1371/journal.pone.0045170

Hartl DL, Clark AG (1997) Principles of Population Genetics. Sinauer, Sunderland, 519 pp.

Hershler R, Liu HP, Frest TJ, Johannes EJ (2007) Extensive diversification of pebblesnails (Lithoglyphidae: *Fluminicola*) in the upper Sacramento River basin, northwestern USA. Zoological Journal of the Linnean Society 149(3): 371–422. https://doi.org/10.1111/j.1096-3642.2007.00243.x

Hoagstrom CW, Ung V, Taylor K (2014) Miocene rivers and taxon cycles clarify the comparative biogeography of North American highland fishes. Journal of Biogeography 41: 644–658. https://doi.org/10.1111/jbi.12244

Inoue K, Hayes DM, Harris JL, Christian AD (2013) Phylogenetic and morphometric analyses reveal ecophenotypic plasticity in freshwater mussels *Obovaria jacksoniana* and *Villosa arkansasensis* (Bivalvia: Unionidae). Ecology and Evolution 3(8): 2670–2683. https://dx.doi.org/10.1002/ece3.649

Johnson PD, Bogan AE, Brown KM, Burkhead NM, Cordeiro JR, Garner JT, Hartfield PD, Lepitzki DA, Mackie GL, Pip E, Tarpley TA, Tiemann JS, Whelan NV, Strong EE (2013) Conservation status of freshwater gastropods of Canada and the United States. Fisheries 38(6): 247–282. http://dx.doi.org/10.1080/03632415.2013.785396

Jones Jr W, Branson BA (1964) The radula, genital system, and external morphology in *Mudalia potosiensis* (Lea 1841) (Gastropoda: Prosobranchiata: Pleuroceridae) with life history notes. Transactions of the American Microscopical Society 83(1): 41–62. https://doi.org/10.2307/3224840

Kalinowski S (2005) Do polymorphic loci require large sample sizes to estimate genetic distances? Heredity 94: 33–36. https://doi.org/10.1038/sj.hdy.6800548

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589. https://doi.org/10.1038/nmeth.4285

Kamvar ZN, Brooks JS, Grünwald NJ (2015) Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. Frontiers in Genetics 6: 208. https://doi.org/10.3389/fgene.2015.00208

Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2: e281. https://doi.org/10.7717/peerj.281

Köhler F (2016) Rampant taxonomic incongruence in a mitochondrial phylogeny of *Semisulcospira* freshwater snails from Japan (Cerithioidea: Semisulcospiridae). Journal of Molluscan Studies 82(2): 268–281. https://doi.org/10.1093/mollus/eyv057

Köhler F, Deein G (2010) Hybridisation as potential source of incongruence in the morphological and mitochondrial diversity of a Thai freshwater gastropod (Pachychilidae, *Brotia* H. Adams, 1866). Zoosystematics and Evolution 86(2): 301–314. https://doi.org/10.1002/zoos.201000013

Leigh JW, Bryant D (2015) Popart: full-feature software for haplotype network construction. Methods in Ecology and Evolution 6(9):1110–1116. https://doi/org/10.1111/2041-210X.12410.

Lisek A, Rozpara E (2010) Identification of pear cultivars with RAPD and ISSR markers. Journal of Fruit and Ornamental Plant Research 18(2): 17–22. http://www.insad.pl/files/journal_pdf/journal_2010_2/full2%202010_2_.pdf

Lydeard C, Yoder JH, Holznagel WE, Thompson FG, Hartfield P (1998). Phylogenetic utility of the 5'-half of mitochondrial 16S rDNA gene sequences for inferring relationships of *Elimia* (Cerithioidea: Pleuroceridae). Malacologia 39(1-2): 183–193. http://biodiversitylibrary.org/page/13106892

Machkour-M'Rabet S, Hénaut Y, Dor A, Pérez-Lachaud G, Pélissier C, Gers C, Legal L (2009) ISSR (Inter Simple Sequence Repeats) as molecular markers to study genetic diversity in tarantulas (Araneae, Mygalomorphae). Journal of Arachnology 37(1): 10–14. https://doi.org/10.1636/A08-27.1

Maruyama T, Fuerst PA (1985) Population bottlenecks and non-equilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics 111(3): 675–689. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1202664/

Matthews WJ, Robison HW (1998) Influence of drainage connectivity, drainage area and regional species richness on fishes of the interior highlands in Arkansas. American Midland Naturalist 139(1): 1–19. https://doi.org/10.1674/0003-0031(1998)139[0001:IODCDA]2.0.CO;2

Mayden RL (1985) Biogeography of Ouachita Highland fishes. Southwestern Naturalist 30(2): 195–211. https://doi.org/10.2307/3670734

Mayden RL (1988) Vicariance biogeography, parsimony, and evolution in North American freshwater fishes. Systematic Zoology 37(4): 329–355. https://doi.org/10.2307/2992197

Minh BQ, Nguyen MAT, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. Molecular Biology and Evolution 30(5): 1188–1195. https://doi.org/10.1093/molbev/mst024

Minton RL, Lydeard C (2003) Phylogeny, taxonomy, genetics and global heritage ranks of an imperiled, freshwater snail genus *Lithasia* (Pleuroceridae). Molecular Ecology 12(1): 75–87. https://doi.org/10.1046/j.1365-294X.2003.01719.x

Minton RL, Savarese Jr SP (2005) Consideration of genetic relationships in management decisions for the endangered Anthony's riv-

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ersnail, *Leptoxis crassa anthonyi* (Redfield, 1854) (Gastropoda: Pleuroceridae). Nautilus 119(1): 11–14. http://biodiversitylibrary.org/page/34681916

Minton RL (2013) A new species of *Lithasia* (Gastropoda: Pleuroceridae) from the Buffalo River, Tennessee, USA. Nautilus 127(3): 119–124. http://www.biodiversitylibrary.org/item/203167

Minton RL, Lewis EM, Netherland B, Hayes DM (2011) Large differences over small distances: plasticity in the shells of *Elimia potosiensis* (Gastropoda: Pleuroceridae). International Journal of Biology 3(1): 23–32. http://dx.doi.org/10.5539/ijb.v3n1p23

Miura O, Köhler F, Lee T, Li J, Ó Foighil D (2013) Rare, divergent Korean *Semisulcospira* spp. mitochondrial haplotypes have Japanese sister lineages. Journal of Molluscan Studies 79(1): 86–89. https://doi.org/10.1093/mollus/eys036

NatureServe (2017) NatureServe Explorer: an online encyclopedia of life [web application]. Version 7.1. Retrieved 4 June 2017 from http://explorer.natureserve.org.

Nei M (1973) Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences 70(12): 3321–3323. https://doi.org/10.1073/pnas.70.12.3321

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2015) Iq-tree: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Palumbi SR (1996) Nucleic Acids II: the polymerase chain reaction. In: Hills DM, Moritz C, Mable BK (Eds) Molecular Systematics. Sinauer, Sunderland, 205–247.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6(1): 288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Pruett CL, Winker K (2008) The effects of sample size on population genetic diversity estimates in song sparrows *Melospiza melodia*. Journal of Avian Biology 39(2): 252–256. https://doi.org/10.1111/j.0908-8857.2008.04094.x

Ruzzante DE (1998) A comparison of several measures of genetic distance and population structure with microsatellite data: Bias and sampling variance. Canadian Journal of Fisheries and Aquatic Sciences 55(1): 1–14. https://doi.org/10.1139/f97-203

Sada DW, Vinyard GL (2002) Anthropogenic changes in biogeography of Great Basin aquatic biota. In Hershler R, Madsen DB, Currey DR (Eds) Great Basin aquatic systems history. Smithsonian Contributions to the Earth Sciences 33: 277–293. https://repository.si.edu/bitstream/handle/10088/826/SCES-0033.pdf?sequence=1&isAllowed=y

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard R (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences USA 81(24): 8014–8018. https://doi.org/10.1073/pnas.81.24.8014

Serb JM, Barnhart MC (2008) Congruence and conflict between molecular and reproductive characters when assessing biological diversity in the western fanshell *Cyprogenia aberti* (Bivalvia, Unionidae). Annals of the Missouri Botanical Garden 95(2): 248–261. https://doi.org/10.3417/2006103

Shimodaira H (2002) An approximately unbiased test of phylogenetic tree selection. Systematic Biology 51(3): 492–508. https://doi.org/10.1080/10635150290069913

Simonsen KL, Churchill GA, Aquadro CF (1995) Properties of statistical tests of neutrality for DNA polymorphism data. Genetics 141(1): 413–429. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1206737/

Snegin E (2014) Analysis of genetic variability in populations of a terrestrial snail *Chondrula tridens* Müll. (Gastropoda, Pulmonata), based on the RAPD and ISSR markers. Russian Journal of Genetics: Applied Research 4(5): 444–454. https://doi.org/10.1134/S207905971405013X

Stein CB, Stansberry DH (1984) A systematic study of the morphological forms of ellipstomid snails in the Duck River, Tennessee, using electrophoretic analysis. Unpublished report submitted to the U.S. Fish and Wildlife Service, U.S. Department of the Interior, Washington D.C. http://applcc.org/projects/trb/resources/PDF_Files_%20Neves_Library/SPA-STE/Stein%20Stansbery%201984.pdf/at_download/file

Stift G, Pachner M, Lelley T (2003) Comparison of RAPD fragment separation in agarose and polyacrylamide gel by studying *Cucurbita* species. Cucurbit Genetics Cooperative Report 26: 62–65. http://cuke.hort.ncsu.edu/cgc/cgc26/cgc26-19.pdf

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123(3): 585–595. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1203831/

Tiemann JS, Cummings KS (2007) Newly recognized distribution records for two pleurocerids (Gastropoda) in Kansas. Transactions of the Kansas Academy of Science 110(3–4): 268–271. https://doi.org/10.1660/0022-8443(2007)110[268:NRDRFT]2.0.CO;2

Tsumura Y, Ohba K, Strauss S (1996) Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas fir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). Theoretical and Applied Genetics 92(1): 40–45. https://doi.org/10.1007/BF00222949

Turner TF, Trexler JC, Kuhn DN, Robison HW (1996) Life-history variation and comparative phylogeography of darters (Pieces: Percidae) from the North American Central Highlands. Evolution 50(5): 2023–2036. http://www.jstor.org/stable/2410760

Vijayan K, Anuradha H, Nair C, Pradeep A, Awasthi A, Saratchandra B, Rahman S, Singh K, Chakraborti R, Urs SR (2006) Genetic diversity and differentiation among populations of the Indian eri silkworm *Samia cynthia ricini*, revealed by ISSR markers. Journal of Insect Science 6: 1–11. https://doi.org/10.1673/2006_6_30.1

Walker D, Avise JC (1998) Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. Annual Review of Ecology and Systematics 29: 23–58. https://doi.org/10.1146/annurev.ecolsys.29.1.23

Whelan NV, Strong EE (2016) Morphology, molecules and taxonomy: Extreme incongruence in pleurocerids (Gastropoda, Cerithioidea, Pleuroceridae). Zoologica Scripta 45(1): 62–87. https://doi.org/10.1111/zsc.12139

Wilmer JW, Murray L, Elkin C, Wilcox D, Niejalke D, Possingham H (2011) Catastrophic floods may pave the way for increased genetic diversity in endemic artesian spring snail populations. PLOS ONE 6: e28645. https://doi.org/10.1371/journal.pone.0028645

Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chainw reaction amplification. Genomics 20(2): 176–183. https://doi.org/10.1006/geno.1994.1151

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