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Disentangling Historical Biogeography and Anthropogenic Introductions: A Case Study of Red Shiner (*Cyprinella lutrensis*)

Tracy Diver

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**DISENTANGLING HISTORICAL BIOGEOGRAPHY AND
ANTHROPOGENIC INTRODUCTIONS: A CASE STUDY OF
RED SHINER (*CYPRINELLA LUTRENSIS*)**

BY

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ABSTRACT

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Results: MtDNA gene trees revealed deeply divergent lineages (Great Plains and Coastal) with *in situ* diversification within each geographic lineage between the Rio Grande and Pecos River. Nuclear DNA showed no support for assortative

mating between lineages. In concordance with nuclear DNA, results of morphological analyses indicated no phenotypic divergence with respect to geographic lineage.

Main conclusions: The presence of deeply divergent, co-occurring lineages of Red Shiner in New Mexico is the result of geologic events that separated and later reconnected the upper and lower Rio Grande Basins during the late Miocene, Pliocene, and Pleistocene. There is also evidence of recent anthropogenic movement of Red Shiner from the adjacent Arkansas Basin to the Rio Grande. This study demonstrates that multiple lines of evidence are required to accurately infer effects of historical and recent events that shape genetic diversity.

Keywords: Miocene, Pliocene, divergent lineage, Red Shiner, Rio Grande, Coastal, Great Plains

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PREFACE

Arid-land Rivers of the southwestern United States have unnaturally depauperate fish fauna because of anthropogenic alterations. Within New Mexico, the Pecos River supports a diverse community of fishes compared to other arid-land streams in the southwestern US. Relatively high alpha diversity results from an intact native fish fauna coupled with introductions of non-native species. We assessed nucleotide diversity for three native and two introduced fishes at the protein-encoding mtDNA ND4 gene to compare levels of genetic diversity amongst the Pecos fish community. Genetic theory predicts an introduced population will exhibit lower levels of genetic diversity relative to natives due to a founder effect decreasing variation in the introduced population. Contrary to expectation, diversity (as measured by gene diversity and theta) was higher for recently introduced species Arkansas River Shiner and Plains Minnow compared to native Speckled Chub, Rio Grande Shiner, and Pecos Bluntnose Shiner.

We tested whether increased levels of genetic diversity in non-natives resulted from multiple introductions from genetically divergent sources. Samples from the introduced Pecos River population of Arkansas River Shiner were compared to samples collected throughout the native range in New Mexico and Oklahoma. Nucleotide sequence data indicated that the Canadian River is the likely source population of the introduced population and that introduced individuals harbor nearly all genetic variation present at the source population. Similar results were found for introduced Plains Minnow, where the Pecos population maintained genetic diversity from the Red and Canadian Rivers. Increased genetic diversity may allow non-natives to persist and thrive despite presumed local adaptation and competitive advantage of natives.

From this study came an interesting discovery and the focus of my thesis research. Levels of genetic diversity in populations of native Red Shiner, *Cyprinella lutrensis*, had comparative levels of gene diversity with those of other Pecos native fishes. However, measures of theta (a measure of evolutionary divergence) were exceptionally high, with Red Shiner having the highest value of theta among Pecos River fishes. Upon comparing the phylogenies of the Pecos fishes, we noticed the presence of two deeply-divergent co-occurring lineages of Red Shiner with an estimated 18% sequence divergence. This explained elevated levels of theta compared to other fish species with less divergence. How these two lineages arose and have since persisted is unknown and formed the central question to my research. Given the history of introductions into the Pecos River we presumed an introduction of Red Shiner from neighboring drainages to be a likely explanation. Alternatively, drainage evolution in North America has been a long, dynamic process that has shaped the distribution and presence of numerous fishes, and could explain the observed pattern of diversity and divergence of Red Shiner in the Pecos River. The objective of my thesis is to disentangle the processes that have shaped patterns of diversity observed in Red Shiner populations within New Mexico.

INTRODUCTION

Understanding historical events, including evolutionary and colonization histories, that have produced the distributions of today's species is of intrinsic interest to the fields of biogeography, evolutionary biology, and conservation genetics. This knowledge can provide information on how to more effectively manage and preserve species. However, sampling strategies often employed in broad scale studies of phylogenetics and phylogeography may be inadequate to tease apart underlying mechanisms that shape the distribution of diversity

and for distinguishing the roles of historical (geology, ancient population expansions and contractions) and recent events. Many traditional biogeographical studies consider species (and populations) as homogenous entities and, for this reason, limit sampling to only one or a few individuals per sampling site or region. Moreover, there is often little attention given to the confounding role that recent (i.e., anthropogenic) introductions may have on the distribution of genetic diversity on the landscape.

Natural movements of freshwater fishes are constrained to wetted corridors; thus, large scale movements generally result from rare opportunities afforded by drainage rearrangements, transient hydrological connections (e.g., flooding events) between drainages, and accidental movements by terrestrial species (Banarescu 1990). Humans have facilitated the capacity of fish to breach natural barriers to movement by deliberate or inadvertent transport. In the United States, the number of fishes introduced from foreign sources or across watershed boundaries has increased dramatically with 67 species between 1851 and 1900, 140 species between 1901 and 1950, and 488 from 1951 to 1996 (Nico and Fuller 1999). Motives for, and mechanisms of fish transport include fish for food, the aquarium trade, recreation, and fisheries (Gozlan 2008). Thus, evolutionary biologists are now confronted with the task of reconciling historical biogeography of native species with emerging and rapidly expanding influences of recently translocated species (Olden et al. 2007).

Despite considerable appreciation of the harm that introduced taxa may cause to endemic species and ecosystems, the confounding role that they may exert in phylogenetic reconstructions is not widely acknowledged. Particularly problematic are translocations that occur across natural biogeographic barriers (e.g., between drainages) which occur within a

species native range. For example, non-native populations of Red Shiner (*Cyprinella lutrensis*) have been moved beyond (and likely within) their native range, through human-mediated transport by bait-bucket release, fish-farm/pond escape, stocking, and aquarium release (Hubbs and Lagler 1964; Holden and Stalnaker 1975; Moore et al. 1976; Wallace and Ramsey 1982; Jennings and Saiki 1990; Jenkins and Burkhead 1994; Moyle 2002; Herrington and DeVries 2008; Marsh-Matthews et al. 2011). Non-native Red Shiner populations now pose a major threat to aquatic biodiversity throughout the southwestern United States (Clarkson et al. 2013).

Our study focuses on Red Shiner at the edge of their native and introduced ranges in the southwestern US and seeks to understand natural and anthropogenic events that shaped patterns of diversity in this species. The region is well suited to examine what is necessary to disentangle contemporary and historical influences on phylogeographic patterns in general because geological processes that have shaped major drainage basins are relatively well known. Moreover, the native fish fauna has been highly altered with 25-50% of species considered non-native (Rahel 2000; Rahel 2007).

Previous studies of Red Shiner have played an important role in understanding the evolutionary history of freshwater fishes of North America (Mayden 1988; Buth et al., 1979; Kristmundsdottir and Gold 1996; Broughton and Gold 2000). This is because the species consists of a widespread complex of morphologically and genetically variable and non-monophyletic forms found across diverse watersheds (Matthews 1987; Broughton & Gold 2000; Schönhuth & Mayden, 2010). Deep evolutionary splits are apparent between major drainage basins in the south-central and southwestern United States. For example, Broughton and Gold (2000) identified roughly 10.5% sequence divergence between

paraphyletic lineages distributed across major river basins within the United States.

Schönhuth and Mayden (2010) presented a more detailed analysis that confirmed paraphyly and further suggested Red Shiner consisted of two geographic clusters: (1) a “Coastal Group” that included Pecos River, Conchos River and other small southern Rio Grande tributary populations and (2) a “Great Plains Group” composed of individuals from the upper Rio Grande drainages and Mississippi River tributaries (i.e., Canadian, Arkansas, Red Rivers). Their results were in agreement with regional morphological variation observed by Matthews (1987), who identified a deeper-bodied Great Plains group, a slender Coastal group, and a morphologically intermediate group found in Texas and the lower Red and Mississippi drainages.

Although Red Shiner is native to the Pecos River, the system’s ichthyofauna has been supplemented by inadvertent introduction of non-natives (Moyer et al. 2005; Osborne et al. 2013). Red Shiner is a successful invader in 12 states where it has been introduced (Hubbs and Lagler 1964; Minckley 1973; Holden and Stalnaker 1975; Moore et al. 1976; Wallace and Ramsey 1982; Jennings and Saiki 1990; Jenkins and Burkhead 1994; Moyle 2002; Herrington and DeVries 2008; Marsh-Matthews et al. 2011). In addition to translocation to introduced habitats, Red Shiner has very likely been moved within its native range. Previous phylogenetic studies of Red Shiner have not adequately considered recent introductions within the species native range as a possible mechanism for patterns of diversity seen in this species (described above). Here we use Red Shiner as a case study to examine whether we can disentangle the relative roles of recent, anthropogenic alterations and historical mechanisms for shaping observed diversity.

It is not trivial to identify and discriminate the roles of recent and historical phenomena. To untangle the influence of these events requires (i) dense sampling (large sample sizes and multiple collection localities within a region), (ii) a good understanding of geological history and broad-scale phylogeny of the species, (iii) comparative data from other taxa and museum records (that can be used to document anthropogenic introductions), (iv) genetic data (nuclear and mitochondrial) and (v) morphological and ecological data. Using this information, explicit consideration of alternative hypotheses is feasible. Here we employ mitochondrial and nuclear gene sequences, morphometric, geometric morphometric and meristic data, and existing broad scale phylogenies of Red Shiner to accomplish this task.

MATERIALS AND METHODS

Sample collection

Red Shiner specimens (N=222) were collected from eight sites along the Pecos River, New Mexico, between Sumner Dam and Brantley Reservoir using a 4' x 6' (1.2x1.8 m) seine net. Whole fish that were greater than or equal to 30 mm in standard length (SL) or greater were retained for further processing. After capture, fish were anesthetized in MS-222 and caudal fin clips were removed and placed in 95% ethanol for genetic analysis. After fin clips were taken, specimens were fixed in 10% formalin for morphological and morphometric analyses. Fin clips from were also collected from fish in the Rio Grande, Canadian and Delaware Rivers for inclusion in the molecular data set to provide geographical context for the Pecos River results (Table 1), but morphological analysis was not conducted on these specimens

Molecular methods

Total genomic DNA was isolated from ethanol preserved tissue using standard phenol-chloroform extraction (Sambrook et al. 1989). Double-stranded polymerase chain reactions (PCR) were performed for mitochondrial ND4 (322 base pairs [bp]) for most specimens (n = 221). Following initial screening and identification of two major lineages via ND4, a subset of individuals (Table 1) from each lineage were selected for sequencing for the mitochondrial cytochrome b (cytb - 1141 bp) gene and the nuclear recombination-activating gene intron 1 (RAG-1, 359 bp).

Mitochondrial ND4 (mt-ND4) was amplified and sequenced following procedures outlined in Alò & Turner (2005). A similar protocol was employed for cytochrome b except we used an annealing temperature of 48° C (rather than 50° C for ND4). RAG-1 (359 bps) was sequenced using primers designed specifically for Red Shiner RAG-1 F (5' TGGCAGCCGGCTTTAAAAACG 3') and RAG-1 R (5' AAACATGAGGCACAAAGGTCT 3') with the following reaction mixture: template DNA, 1x reaction buffer, 2mM MgCl₂, 125µM dNTPs, 0.5 µM forward and reverse primers, and 0.375 units Promega Taq. Touchdown PCR was employed that consisted of initial denaturation at 95° C for 3 minutes (min), followed by 20 cycles of denaturation at 95° C for 1 min, annealing at 60° C for 40 seconds (sec), and then extension at 72° C for 40 sec. This was followed immediately by a second round of PCR with 20 cycles of denaturation at 95° C for 1 min, annealing at 48° C for 40 sec, extension at 72° C for 40 sec, and a final extension at 72° C for 10 min. All PCR products were prepared for sequencing using Omega filter purification for cleanup (following manufacturer's instructions), and then sequenced using

Big Dye Terminator cycle sequencing chemistry and an ABI3130 automated DNA sequencer (Applied Biosystems, Inc.).

Morphological Methods

We used geometric morphometric analysis, meristic characters, and morphometric measurements to test whether morphology was concordant with observed molecular divergence. Two-dimensional images of each preserved fish were taken for geometric morphometric analysis using a Sony Cyber Shot camera and copy stand. Shape data were processed for 13 landmarks using the TPS software series (available at: life.bio.sunysb.edu/morph). Homologous landmarks were selected based on easily identified features such as fin origins, snout, and center of the eye. Generalized Procrustes Analysis (GPA) was performed to remove variation in landmark configurations due to differences in size, position, and orientation (Rohlf and Slice, 1990). Shape data consisting of partial warps and two uniform scores were calculated and used for statistical analysis.

Meristic characters and morphological measures were collected from a subset of 23 individuals selected from each distinct mtDNA lineage, for a total of 46 individuals measured. Counts and measurements were made on formalin-fixed and ethanol-preserved specimens following protocols described by Hubbs and Lagler (1964). A total of 11 morphometric characters were collected: standard length, body depth, depth of caudal peduncal, length of caudal peduncle, predorsal length, head length, eye width, snout length, and pelvic and pectoral fin length. Morphometric characters were measured to the nearest 0.01 mm using digital slide calipers. Seven meristic measures were obtained, including counts of dorsal fin rays, anal fin rays, pelvic fin rays, pectoral fin rays, lateral line scales,

scales above lateral line, and scales below lateral line. Fin rays and lateral line scales were enumerated under a dissecting scope. To ensure all measures were collected properly, all individuals were measured multiple to ensure quality control.

Data Analysis

Nucleotide sequences were aligned using Sequencher Version 4.9 (Genecodes) and verified by eye. For RAG-1 sequence data, the program PHASE (as implemented in DNAsp v5; Librado, P. and Rozas, J. 2009) was used to determine allelic identities of heterozygous individuals. Preliminary analyses were run in MEGA 5.05 (Tamura et al. 2011) using maximum likelihood, parsimony, and distance methods. Likelihood ratio tests and Bayesian Information Criterion analysis (conducted in MEGA) were used to select the most appropriate model of sequence evolution for each gene (Posada 2008). Phylogenetic trees were constructed in GARLI v. 0.951 (Zwickl, D. J. 2006). GARLI searches used on the GTR+ Γ +I model (six general time-reversible substitution rates, assuming gamma rate heterogeneity and a proportion of invariable sites), with model parameters estimated over the duration of specified runs. 500 bootstrap replicates were used. A median-joining network was constructed from ND4 and RAG-1 sequence data using R version 2.13.1 (Bandelt et al. 1999; 2011 The R Foundation for Statistical Computing).

Morphological data were also analyzed using R. Relative warps for geometric morphometric data and combined morphometric and meristic data were analyzed using principal components analysis (PCA). Multivariate analysis of covariance (MANCOVA) was used to test the hypothesis of no difference between monophyletic groups revealed by molecular analysis.

To put our results into broader systematic context, we determined mtDNA cytb sequences from select individuals (Table 1) and aligned these to a previously aligned Genbank dataset (Popset number: 283147685) and additional sequences reported in Broughton et al. (2011). Aligned sequence data were analyzed in MEGA using the Maximum Likelihood (ML) model described by Schönhuth and Mayden (2010). For all phylogenetic analyses, we assessed support for branches by bootstrapping with 1000 pseudo-replicates. We tested for monophyly of two deeply divergent lineages of Red Shiner by including two sister species (*C. garmani* and *C. forlonensis*) to the data set. Species from a closely related genus, *Notropis girardi* and *Notropis rubellus*, were selected as outgroup taxa to root the tree. Why did we select these taxa?

Divergence dates (in millions of years) were estimated from genetic distance measures obtained from the model of evolution selected to be most appropriate for our sequence data (TN93+G). For each major node (Coastal-Great Plains, Great Plains Rio Grande-Pecos, and Coastal Rio Grande-Pecos) minimum and maximum distance measures were calculated to estimate relative rates of divergence. A substitution rate of 2% per million years (Dowling et al. 2002) was applied to estimate the time to most recent common ancestry (t_{MRCA}) for each of three major divergence events identified in the study. The substitution rate we employed has been observed for other small-bodied minnows in desert streams (Spencer et al. 2008). Estimated t_{MRCA} was interpreted in the context of Galloway et al. (2011) to infer the nature of geological events that have shaped genetic diversity in Red Shiner.

RESULTS

Of the four rivers studied, the Pecos River was the most thoroughly sampled with a total of 221 individuals collected throughout the system. Two major mtDNA lineages were identified in the Pecos River and the number of individuals within the identified lineages was highly skewed with 198 individuals sharing common ancestry in one lineage (Coastal) and 23 in the other (Great Plains). For this reason, a subset of 23 individuals from the Pecos River were selected at random from the Coastal lineage to create equal sample sizes for the final morphometric, meristic, and nuc-RAG-1 data sets (n=46). The final ND4 sequence data set included all Pecos River sequences plus individuals from the Rio Grande (n=69), Delaware River (n=39), and Canadian River (n=16) (Table 1).

ND4 –

Thirty-three unique haplotypes were identified from the 309 ND4 sequences that were successfully amplified. The TN93+G model of substitution was selected based on LRT and BIC criteria. Sequence divergence ranged from 15.4%-22.1% between the two most deeply divergent lineages. The presence of deeply divergent geographic lineages observed in New Mexico is consistent with diversity patterns described in Matthews (1987) and Schönhuth and Mayden (2010). Network analysis (Figure 1) of the data set revealed both lineages are present in the Rio Grande and Pecos River. However, individuals from the Delaware River only exhibited the Coastal lineage, and individuals from the Canadian River only exhibited the Great Plains lineage. Within the Coastal lineage, three unique haplotypes were identified in the Rio Grande, three in the Delaware, nine in the Pecos. One haplotype was common to the Pecos and Delaware Rivers. Within the Great Plains lineage, three unique haplotypes were found in the Rio Grande, two in the Pecos, eight in the Canadian, one was shared between the Rio Grande and Canadian River, and two between the Pecos and

Canadian River. Within each major lineage, two monophyletic clades were identified with respect to the Rio Grande and Pecos River. The range of pairwise haplotype divergence between the Rio Grande and Pecos River within the Coastal lineage was 2.6-3.7%.

Comparatively, the range of haplotype divergence within the Great Plains lineage was much larger at 13.1-15.8% between the Rio Grande and Pecos River.

Cytonuclear disequilibrium (RAG-1) –

A subset of individuals (n=62) from the Pecos, Rio Grande, and Canadian River were sequenced for the RAG-1 intron (Table 1) with equal sample sizes from the identified Coastal lineage (n=32) and Great Plains lineage (n=32). Network analysis (Figure 2) revealed 18 unique haplotypes. Of these, seven haplotypes were shared between the two major identified mitochondrial lineages. Seven unique haplotypes were found in the Great Plains lineage and four in the Coastal lineage. Haplotypes were randomly distributed in the RAG-1 network with respect to mitochondrial lineages. A chi-square test of independence revealed no cytonuclear disequilibrium ($P > 0.05$), suggesting no assortative mating with respect to ND4 lineage.

Morphological diversification –

Multivariate Analysis of Covariance (MANCOVA) of geometric morphometric data revealed substantial overlap of body-shape dimensions with no significant difference in shape between mitochondrial ND4 lineages ($P = 0.45$). Visual inspection of PCA plots from the weighted matrix of landmarked points (Figure 3) support MANCOVA results with significant overlap in shape between the two major lineages. Likewise, MANCOVA results of morphometric measures and meristic characters revealed no support for a difference between ND4 lineages ($P=0.10$).

Broader Phylogenetic Context –

Mitochondrial-cytb sequences were obtained to investigate whether the mt-ND4 pattern observed in Red Shiner from New Mexico was consistent with divergent lineages observed by Schönhuth and Mayden (2010) and Broughton (2000). A subset of sequenced cytb individuals identified within each deeply divergent ND4 lineage (Table 1) were placed in a broader geographic context to determine origin of lineage in order to understand what events allowed for the co-occurrence of each lineage within New Mexico (Figure 4) (Matthews, 1987; Schönhuth and Mayden, 2010; Broughton et al. 2011). Individuals from the Rio Grande and Pecos River labeled as “Coastal Lineage” fell into a clade with sequences from the Pecos, Conchos, and other small Rio Grande tributaries. Whereas, individuals from the Canadian, Rio Grande, and Pecos River labeled as “Great Plains Lineage” fell into a monophyletic clade with individuals from the Rio Grande and Mississippi drainage. Results from ML analysis show the identified mt-ND4 lineages are consistent with the lineages previously discovered across the species’ geographic distribution (Fig. 4), which could be consistent with a recent introduction or historic colonization.

Estimated divergence dates –

Estimated dates of divergence calculated from Tamura-Nei (93) distances using a substitution rate of 2% per million years provided estimates of time to most recent common ancestry ($t_{MRC A}$) for each lineage. The estimated split between the Great Plains and Coastal lineage was calculated from minimum and maximum TN93 distances and ranges from 5.675-2.375 MY. Within each of the two geographic lineages (Great Plains and Coastal) we observed a high degree of sequence divergence between the Rio Grande and Pecos River. Estimated dates from $t_{MRC A}$ between the Rio Grande and Pecos River within the Great Plains

lineage ranges between 4.125-2.825 MY. Lastly, we observed a more recent divergence event between the Rio Grande and Pecos River within the Coastal lineage with estimated dates ranging from 1.1-0.575 MY.

DISCUSSION

Identifying evolutionary and ecological processes that promote and maintain diversity is an important, but difficult task. Historically, intraspecific diversity was shaped through population expansions and contractions and allopatric processes caused by geologic events (vicariance) and environmental variables (climate). Humans have also altered how genetic diversity is distributed on the landscape by deliberately or inadvertently transporting species across natural barriers. In our study we evaluated Red Shiner, a broadly distributed, morphologically diverse, and genetically divergent species (Baird and Girard 1853, C.L. Hubbs and Ortenburger, 1929; Gibbs, 1957; Contreras-Balderas 1975; C. Hubbs, 1977; Matthews 1987; Broughton and Gold 2005; Schönhuth and Mayden, 2010) that has undergone extensive anthropogenic movement (Marsh-Matthews et al. 2011), to assess whether effects of modern and historic events can be distinguished. We used (i) dense sampling (ii) geological data and broad-scale phylogenies, (iii) comparative data, (iv) multiple molecular markers and (v) morphological data to infer what processes have shaped patterns of diversity observed in populations of Red Shiner in New Mexico.

Patterns of genetic diversity observed in Red Shiner populations in New Mexico appear to be the result of both historical processes and modern human-mediated movement. MtDNA results show the presence of two deeply divergent co-occurring lineages in the Rio Grande and Pecos River. When placed in the context of *cytb* sequences from previous biogeographic studies (Broughton et al. 2011; Schönhuth and Mayden 2010), individuals

from each lineage fell into two geographic clades. Individuals belonging to the Great Plains Lineage share ancestry with individuals from eastern drainages including the Mississippi, whereas individuals in the Coastal Lineage belong to an ancestral southern Rio Grande population (Figure 4). We determined if the co-occurrence of these two lineages within New Mexico came to co-exist through historical or modern (introduction) events by using nuDNA to test for origin of diversity.

Discerning Recent and Historical Patterns

The introduction of small bodied minnows (Plains minnow and Arkansas River Shiner) into the Pecos River (New Mexico) was first documented in the late 1960's and early 1970's (Bestgen et al. 1989). Since Red Shiner is native to the Rio Grande drainage it is difficult to determine if and when one of the lineages was introduced but it is probable that movement has occurred and likely within a similar timeframe as documented for these other species. Under such a scenario a high degree of assortment between nuclear and mitochondrial haplotypes with evidence for cytonuclear disequilibrium would be predicted (Scribner et al. 2001). Instead, we found no support for assortive mating within Great Plains and Coastal lineages, which suggests individuals from each lineage have been randomly mating for a period much longer than a recent introduction. Morphological results are in agreement with nuDNA and show no evidence for phenotypic divergence between lineages. Matthews (1987) showed morphology of Red Shiner varied by geographical region and described a slender Coastal group and a deep bodied Great Plains Group. For this reason, recent introduction of one of these lineages may have resulted in morphological divergence coinciding with the two mtDNA lineages (1987) yet there is no evidence of this pattern.

Within both the Great Plains and Coastal lineages we observed a high degree of genetic diversification between the Rio Grande and Pecos River suggesting significant *in situ* divergence. Lineage diversification within each geographic lineage suggests ancestral vicariance and/or multiple colonization events of Red Shiner within the Rio Grande basin rather than recent human mediated movement.

The tempo and mode of diversification suggests that vicariance caused by geological events and natural colonization explains the presence of deeply divergent sympatric lineages of Red Shiner in New Mexico. MtDNA patterns observed in Red Shiner suggest three major events have shaped its evolutionary history. Time to most recent common ancestry of Coastal and Great Plains lineage occurred during the late Miocene/early Pliocene. Geologic evidence from this period indicates that the lower Rio Grande and the upper Rio Grande and historic Pecos system were not contiguous waterways. Until the late Pleistocene, the upper Rio Grande and Pecos diverted east and connected to major Midwestern basins such as the Mississippi (Galloway et al. 2011). The lower Rio Grande, however, flowed through southern basins before emptying into the western portion of the Gulf of Mexico (Galloway et al. 2011). It is likely the divergence between Great Plains and Coastal lineages is a reflection of geologic isolation of the lower Rio Grande from the upper Rio Grande and Pecos. We propose that the Coastal lineage is an evolutionary descendant of the ancestral lower Rio Grande population and the Great Plains lineage represents an ancestral connection of the upper Rio Grande and Pecos populations to eastern drainage populations.

Populations of Red Shiner are divergent between the Rio Grande and Pecos River in both Coastal and Great Plains lineages, suggesting further *in situ* diversification as a consequence of drainage isolation. The oldest of these events involved the Great Plains

lineage and occurred during the Pliocene. Divergence of Rio Grande and Pecos River populations probably resulted from drainage isolation of Rio Grande and Pecos systems, or ancestral recolonization of eastern populations to one of the two systems by means of interdrainage connections. Population divergence within the Coastal lineage is much more recent and likely occurred during the Pleistocene. Throughout the Miocene and Pliocene, subsidence of the Rio Grande rift inhibited drainage of the lower Rio Grande to the Gulf of Mexico. Pleistocene glaciation caused an increased rate of sediment supply to drainages and basins. Consequently, sediment deposition in the Rio Grande rift finally exceeded subsidence allowing connection of the Rio Grande and Pecos in western Texas before reaching the Gulf of Mexico (Galloway et al. 2011). Connection of the Pecos and Rio Grande would have allowed ancestral lower Rio Grande populations to move into the Pecos and upper Rio Grande.

Modern Movement of Red Shiner

Superimposed upon the ancient events described above is the signature of recent movement of Red Shiner within its native range, specifically, movement of individuals from the Canadian River into both the Rio Grande and Pecos River (Figure 1). The presence of shared identical haplotypes in the Canadian River mirrors results seen in other fishes including Arkansas River Shiner and Plains minnow that were recently introduced from neighboring drainages (Bestgen et al. 1989., Moyer et al. 2005; Osborne et al. 2013). Historically, the Canadian River maintained drainage connections to eastern drainages (Galloway et al. 2011), hence Red Shiner haplotypes from the Canadian should have affinities with the Great Plains Lineage. This idea is supported from previous studies (Schönhuth and Mayden 2010; Broughton et al. 2011) that have grouped individuals

collected from the Canadian River into the Great Plains Lineage (citations are required). If individuals were introduced from the Rio Grande and Pecos to the Canadian, by chance we would expect to see individuals that belong to the Coastal Lineage present in the Canadian. In addition, nuclear haplotypes from the Rio Grande and Pecos River that are identical to Canadian River haplotypes show now evidence for cytonuclear disequilibrium, supporting the idea of a recent introduction. We conclude Red Shiner was introduced from the Canadian into the Pecos and Rio Grande and not vice versa because all individuals from the Canadian River fall into the Great Plains Lineage.

Conclusions

Contemporary geographic distributions of populations are a reflection of a species' history. Various ecological and evolutionary processes, such as vicariance and recent introductions, can shape present-day patterns of diversity. Herein, we presented a case study where multiple evolutionary hypotheses regarding the origin and maintenance of diversity in Red Shiner were tested. We thoroughly sampled populations of Red Shiner in New Mexico and placed them in the context of previous biogeographic studies in order to determine if observed patterns of divergence were the consequence of modern or historical events. We have shown that both ancient evolutionary events and modern anthropogenic alterations have shaped the evolution of Red Shiner population in New Mexico through thorough examination of alternative hypotheses. The co-occurrence of deeply divergent lineages within New Mexico has been shaped for millions of years by geologic processes; however, humans have augmented native populations through translocation of individuals to neighboring drainages. Disentangling these events is not a trivial task, but fundamentally important in further

understanding biogeography, evolutionary biology, and conservation biology to more effectively manage and preserve species.

Our study has provided valuable insight to understanding how to determine various processes that can shape a species' diversity. The southwestern United States has a long, dynamic geologic history involving tectonic uplift, basin subsidence, and sediment accumulation that have all greatly influenced drainage evolution over the course of millions of years. The genetic diversity seen in populations of Red Shiner in New Mexico is a remnant of such processes; however, humans have made their imprint through translocation of individuals across barriers that would normally be impassable to aquatic organisms. We have provided a method for determine whether historic and/or modern anthropogenic processes have shaped diversity within a population.

Through this study we have observed other historic footprints that can be observed in Red Shiner. In comparison to most fishes found in New Mexico, Red Shiner is often the most common and abundant species present. Interestingly, we observe surprising low levels of genetic diversity (haplotype diversity) in populations found in the Rio Grande Basin. This suggests the species has undergone a historic bottleneck that resulted in the loss of variation. Factors that have contributed to this loss need further investigation beyond this study, but could prove invaluable for understanding additional processes (disease, habitat degradation/alteration) that have shaped genetic diversity within extant populations.

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FIGURES & TABLES

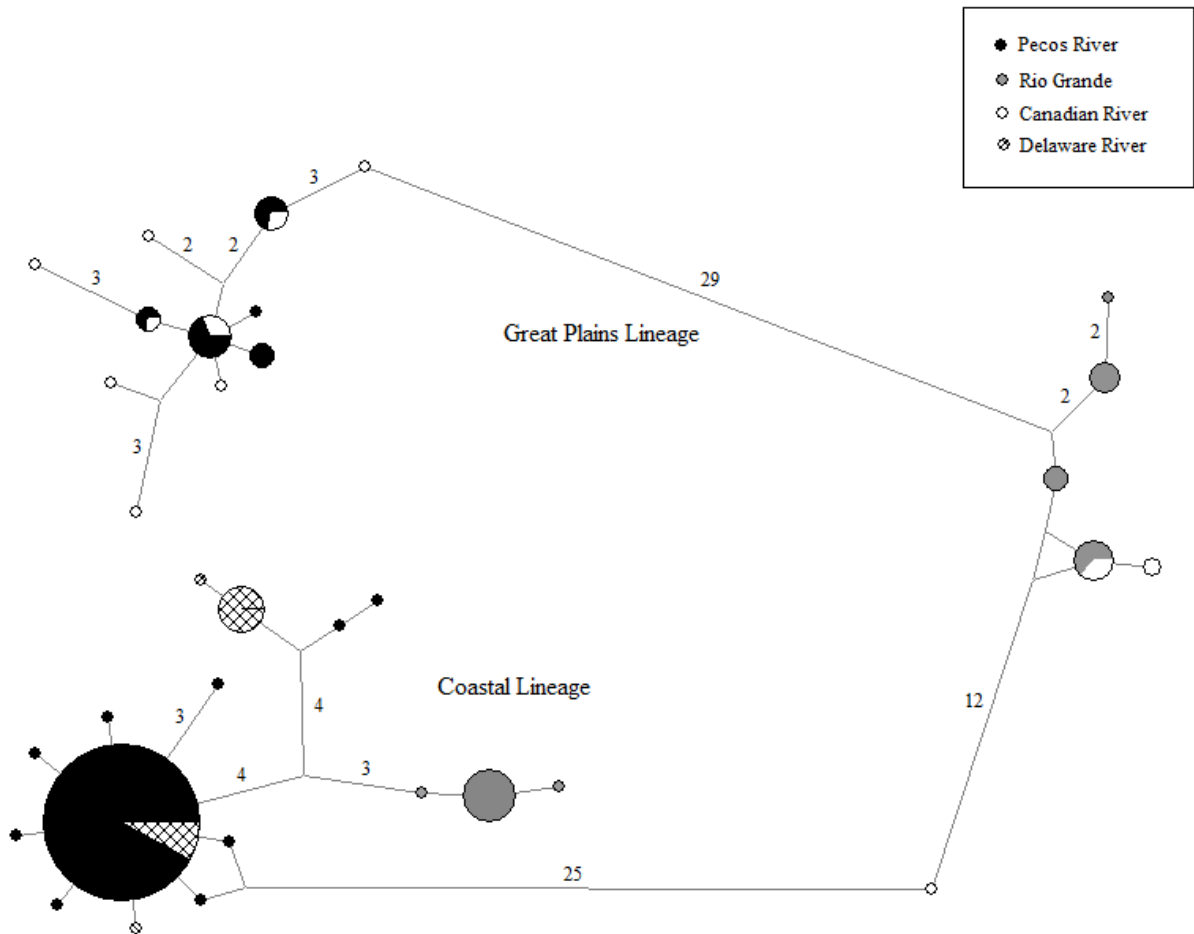


Figure 1. Median-joining haplotype network based on ND4 sequence data. Size of circles is proportional to haplotype frequencies. Shading distinguishes distinct drainages: Pecos River in black, Rio Grande in gray, Canadian in white and Delaware with diagonal grids. Numeric values near a branch indicates length > 1 bp change between haplotypes.

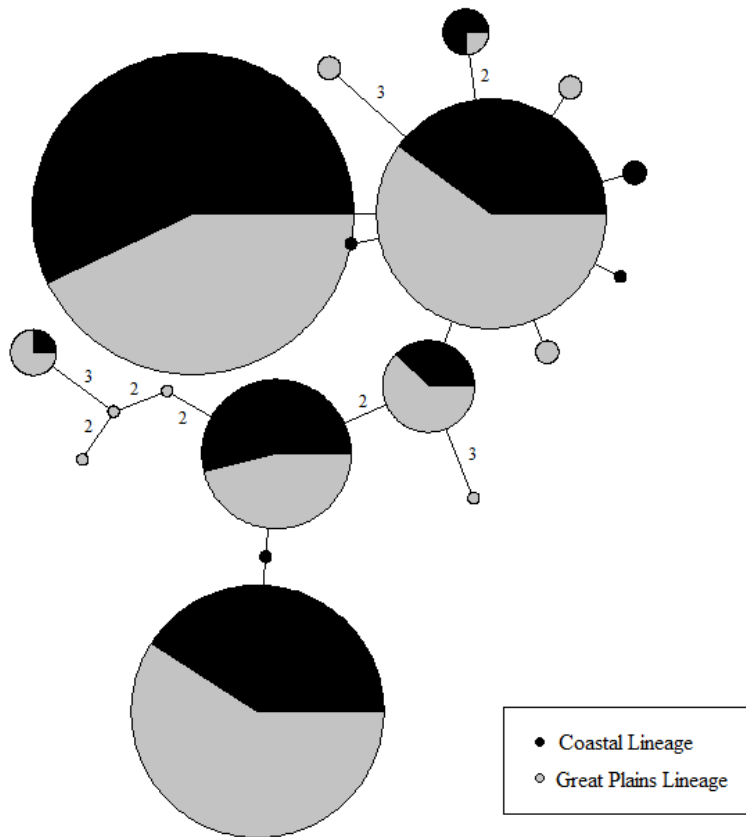


Figure 2. Median-joining network for RAG-1 sequences. MtDNA lineages are superimposed on the RAG-1 network; the Coastal Lineage is in black and the Great Plains lineage is in gray. Size of circles is proportional to mtDNA haplotype frequency. Changes greater than one base pair are indicated by numeric values near a branch.

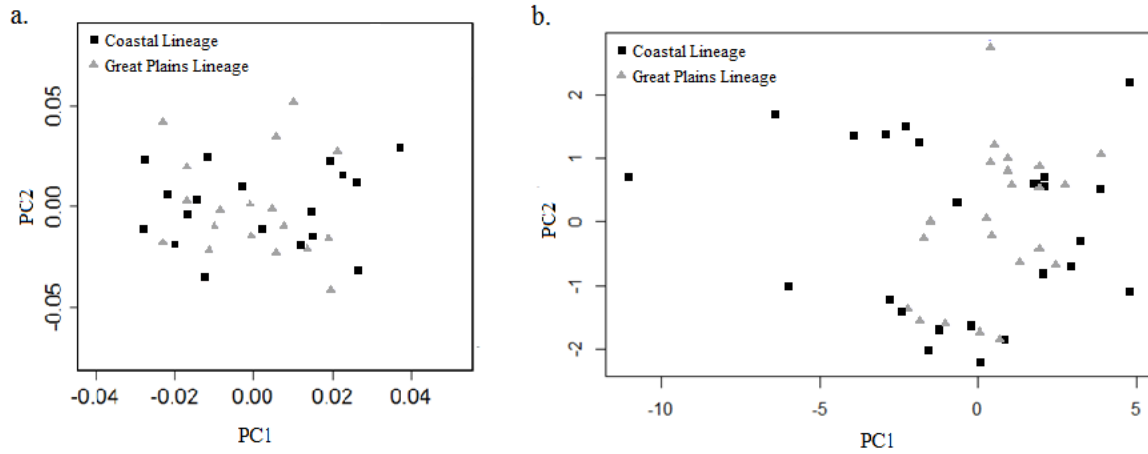


Figure 3. Principal components analysis of (a) geometric weight-matrix and (b) combined morphometric and meristic data. The Coastal Lineage is represented in black squares and the Great Plains Lineage is represented by gray triangles

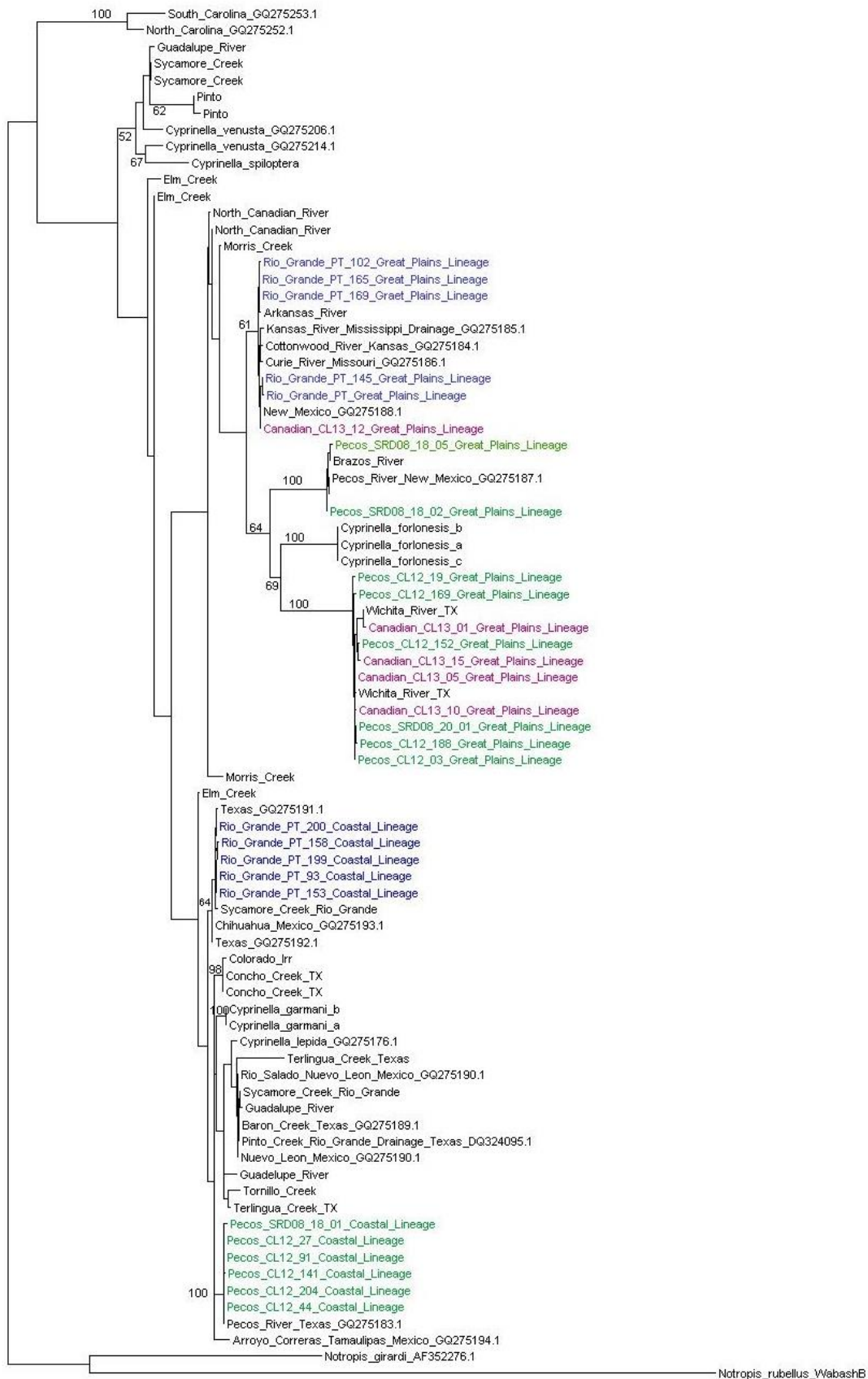


Figure 4. MtDNA cytb sequences from Red Shiners from the Rio Grande (blue), Pecos (green), and Canadian River (pink) aligned with data from previous studies (Schonhuth and Mayden 2010, Broughton 2011) (black). Individuals for this analysis were selected based on ND4 haplotype sequences and thereby identified as either Great Plains Lineage or Coastal Lineage.

Table 1. Number of individuals from each river sequenced for genetic and morphological analyses.

River	ND4	RAG-1	cytb	Meristics	Geomorph
Pecos	221	46	10	46	46
Delaware	31	0	0	0	0
Rio Grande	41	10	10	0	0
Canadian	16	6	5	0	0
Total	309	62	25	46	46

APPENDIX

Supplemental Table 1. ND4 haplotype frequencies followed by sequences associated with each respective haplotype.

Haplotype	Pecos	Rio Grande	Delaware	Canadian	Total
A	172	0	15	0	187
B	0	0	1	0	1
C	0	1	0	0	1
D	0	1	0	0	1
E	0	19	0	0	19
F	0	0	1	0	1
G	0	0	15	0	15
H	0	7	0	4	11
I	0	0	0	2	2
J	0	1	0	0	1
L	0	4	0	0	4
M	0	6	0	0	6
N	0	0	0	1	1
O	0	0	0	1	1
P	0	0	0	1	1
Q	5	0	0	2	7
R	0	0	0	1	1
S	0	0	0	1	1
T	0	0	0	1	1
U	1	0	0	0	1
V	0	0	0	1	1
W	4	0	0	0	4
X	3	0	0	1	4
Y	9	0	0	4	13
Z	1	0	0	0	1
AA	1	0	0	0	1
BB	1	0	0	0	1
CC	1	0	0	0	1
DD	1	0	0	0	1
EE	1	0	0	0	1
FF	1	0	0	0	1
GG	1	0	0	0	1

#A

ATTATGAACAACAACCGCCACTCATGGACTCTTAATTGCCTTAACAAGCCTCGCATGATTTAGCTG
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ATTGGTTCTAACGTGTTGACTTCTTCCCTTAATAATCTTGGCTAGCCAGAATCACATCAACCCTGAG
CCCATTATCCGCCAGCGGCTTTACATTACGCTTCTCACCTCACTCCAGACTTTCTCATCATAGCTT
TCGGCGCCACAGAAATCATTATGTTCTACATCATGTTTGAAGCCACTCTCATCCCC

#B

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ATTGGTTCTAACGTGTTGACTTCTTCCCTTAATAATCTTGGCTAGCCAGAATCACATCAACCCTGAG
CCCATTATCCGCCAGCGGCTTTACATTACGCTTCTCACCTCACTCCAGGCTTTCTCATCATAGCTT
TCGGCGCCACAGAAATCATTATGTTCTACATCATGTTTGAAGCCACTCTCATCCCC

#C

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GCCATTATCCGCCAGCGGCTTTACATTACGCTTCTCACCTCACTCCAAACTTTCTCATCATAGCT
TTCGGCGCTACAGAAATCATTATGTTCTACATCATGTTTGAAGCCACTCTCATCCCC

#D

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#E

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#F

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#G

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#H

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#I

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#J

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#K

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#L

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TCGGCGCCACCGAAATCATTATGTTCTATATCATATTTNAAGCCACTCTCATCCCC

#T

ACTATGAACAGCAACCGCCGCTCATGGACTCTTGATTGCCTTAGCAAGCCTCACATGGTTTGCCTG
AACCTCAGAAACCGGGTGGACTTCATCCAGCACCTATTTGGCCACTGACCCCTTATCCACACCCCT
CTTAGTCCTGACATGTTGGCTCCTTCCCTTAATAATCTTAGCTAGCCAAAACCACATCAACCCTGA
GCCCGTCGTCCGTCAACGGCTTTACATTACTCTTCTTACTTCATTACAGACTTTCCTGATTATGGCT
TTCGGCGCCACCGAAATCATTATGTTCTATATCATATTTGAAGCCACTCTCATCCCC

#U

ACTATGAGCAGCAACCGCCGCTCATGGACTCTTGATTGCCTTAGCAAGCCTCACATGGTTTGCCTG
AACCTCAGAAACCGGGTGGACTTCATCCAGCACCTATTTGGCCACTGACCCCTTATCCACACCCCT
CTTAGTCCTGACATGGTGGCTCCTTCCCTTAATAATCTTAGCTAGCCAAAACCACATCAACCCTGA
GCCCGTCGtCCGTCAACGGCTTTACATTACTCTTCTTACTTCATTACAGACTTTCCTGATTATGGCTT
TCGGCGCCACCGAAATCATTATGTTCTATATCATATTTGAAGCCACTCTCATCCCC

#V

ACTATGAGCAGCAaCCGCCGCTCATGGACTCTTGATTGCCTTAGCAAGCCTCACATGGTTTGCCTG
AACCTCAGAAACCGGGTGGACTTCATCCAGCACCTATTTGGCCACTGACCCCTTATCCACGCCCT
CTTAGTCCTGACATGTTGGCTCCTTCCCTTAATAATCTTAGCTAGCCAAAACCACATCAACCCTGA
GCCCGTCGTCCGTCAACGGCTTTACATTACTCTTCTTACTTCATTACAGACTTTCCTGATTATGGCT
TTCGGCGCCACCGAAATCATTATGTTCTATATCATATTTGAAGCCACTCTCATCCCC

#W

ACTATGAGCAGCAACCGCCGCTCATGGACTCTTGATTGCCTTGGCAAGCCTCACATGGTTTGCCTG
AACCTCAGAAACCGGGTGGACTTCATCCAGCACCTATTTGGCCACTGACCCCTTATCCACACCCCT
CTTAGTCCTGACATGTTGGCTCCTTCCCTTAATAATCTTAGCTAGCCAAAACCACATCAACCCTGA

GCCCGTCGTCCGTCAACGGCTTTACATTACTCTTCTTACTTCATTACAGACTTTCCTGATTATGGCT
TTCGGCGCCACCGAAATCATTATGTTCTATATCATATTTGAAGCCACTCTCATCCCC

#X

ACTATGAGCAGCAACCGCCGCTCATGGACTCTTGATTGCCTTAGCAAGCCTCACATGGTTTGCCTG
AACCTCAGAAACCGGGTGGACTTCATCCAGCACCTATTTGGCCACTGACCCCTTATCCACACCCCT
CTTAGTCCTGACATGTTGGCTCCTTCCCTTAATAATCTTAGCTAGCCAAAACCACATCAACCCTGA
GCCCGTCGTCCGTCAACGGCTTTACATTACTCTTCTTACTTCATTACAGACTTTCCTGATTATGGCT
TTCGGCGCCACCGAAATCATTATGTTCTATATCATATTTGAAGCCACTCTCATCCCC

#Y

ATTATGAACAACAACCGCCACTCATGGACTCTTAATTGCCTTAACAAGCCTCGCATGATTTAGCTG
AACCTCAGAGGCTGGATGAGGTTTCATCCAGCATCTACTTAGCCACCGATCCCTTATCAACACCCCT
ATTGGTTCTAACGTGTTGACTTCTTCCCTTAATAATCTTGGCTAGCCAGAATCACATCAACCCTGAG
CCCATTATCCGCCAGCGGCTTTACATTACGCTTCTCACCTCACTCCAGACTTTCCTCATCATAGCTT
TCGGCACACAGAAATCATTATGTTCTACATCATGTTTGAAGCCACTCTCATCCCC

#Z

ATTATGAACAACAACCGCCACTCATGGACTCTTAATTGCCTTAACAAGCCTCGCATGATTTAGCTG
AACCTCAGAGGCTGGATGAGGTTTCATCCAGCATCTACATAGCCACCGATCCCTTATCAACACCCCT
ATTGGTTCTAACGTGTTGACTTCTTCCCTTAATAATCTTGGCTAGCCAGAATCACATCAACCCTGAG
CCCATTATCCGCCAGCGGCTTTACATTACGCTTCTCACCTCACTCCAGACTTTCCTCATCATAGCTT
TCGGCGCCACAGAAATCATTATGTTCTACATCATGTTTGAAGCCACTCTCATCCCC

#AA

ATTATGAACAACAACCGCCACTCATGGACTCTTAATTGCCTTAACAAGCCTCGCATGATTTAGCTG
AACCTCAGAGGCTGGATGAGGTTTCATCCAGCATCTACTTAGCCACCGATCCCTTATCAACACCCCT
ATTGGTTCTAACGTGTTGACTTCTTCCCTTAATAATCTTGGCTAGCCAGAATCACATCAACCCTGAG
CCCATTATCCGCCAGCGGCTTTACATTACGCTTCTCACCTCACTCCAGACTTTCCTCATCATAGCTT
TCGGCGCCACAGAAATCATTATGTTCTACATCATGTTTGAAGCCACTCTCATCCCC

#BB

ATTATGAACAACAACCGCCACTCATGGACTCTTAATTGCCTTAACAAGCCTCGCATGATTTAGCTG
AACCTCAGAGGCTGGATGAGGTTTCATCCAGCATCTACTTAGCCACCGATCCCCTATCAACACCCCT
ATTGGTTCTAACGTGTTGACTTCTTCCCTTAATAATCTTGGCTAGCCAGAATCACATCAACCCTGAG
CCCATTATCCGCCAGCGGCTTTACATTACGCTTCTCACCTCACTCCAGACTTTCCTCATCATAGCTT
TCGGCGCCACAGAAATCATTATGTTCTACATCATGTTTGAAGCCACTTCTCATCCCC

#CC

ATTATGAACAACAACCGCCACTCATGGACTCTTAATTGCCTTAACAAGCCTCACATGATTTAGCTG
AACCTCAGAGGCTGGATGAGGTTTCATCCAGCATCTACTTAGCCACCGATCCCCTTATCAACACCCCT
ATTGGTTCTAACGTGTTGACTTCTTCCCTTAATAATCTTGGCTAGCCAGAATCACATCAACCCTGAG
CCCATTATCCGCCAGCGGCTTTACATTACGCTTCTCACCTCACTCCAGACTTTCCTCATCATAGCTT
TCGGCGCCACAGAAATCATTATGTTCTACATCATGTTTGAAGCCACTTCTCATCCCC

#DD

GTTATGAACAACAACCGCCACTCATGGACTCTTAATTGCCTTAACAAGCCTCGCATGATTTAGCTG
AACCTCAGAGGCTGGATGAGGTTTCATCCAGCATCTACTTAGCCACCGATCCCCTTATCAACACCCCT
ATTGGTTCTAACGTGTTGACTTCTTCCCTTAATAATCTTGGCTAGCCAGAATCACATCAACCCTGAG
CCCATTATCCGCCAGCGGCTTTACATTACGCTTCTCACCTCACTCCAGACTTTCCTCATCATAGCTT
TCGGCGCCACAGAAATCATTATGTTCTACATCATGTTTGAAGCCACTTCTCATCCCC

#EE

ATTATGAACAACAACCGCCACTCATGGACTCTTAATTGCCTTAACAAGCCTCGCATGATTTAGCTG
AACCTCAGAGGCTGGATGAGGTTTCATCCAGCATCTACTTAGCCACCGATCCCCTTATCAACACCCCT
ATTGGTTCTAACGTGTTGACTTCTTCCCTTAATGATCTTGGCTAGCCAGAATCACATCAACCCTGAG
CCCATTATCCGCCAGCGGCTTTACATTACGCTTCTCACCTCACTCCAGACTTTCCTCATTATGGCTT
TCGGCGCCACAGAAATCATTATGTTCTACATCATGTTTGAAGCCACTTCTCATCCCC

#FF

ATTATGAACAACAACCGCCACTCATGGACTCTTAATTGCCTTGACAAGCCTCGCATGATTTAGCTG
AACCTCAGAGGCTGGATGAAGTTCATCCAGCATCTACTTAGCCACCGATCCCCTTATCAACACCCCT
GTTGGTTCTAACATGTTGACTCCTTCCCTTAATAATCTTGGCTAGCCAAAATCACATCAACCCTGA

GCCATTGTCCGCCAGCGGCTTTACATTACGCTTCTCACCTCACTCCAAACTTTCTCATCATAGCT
 TTCGGCGCCACAGAAATCATTATGTTCTACATCATGTTTGAAGCCACTCTCATCCCC

#GG

ATTATGAACGACAACCGCCACTCATGGACTCTTAATTGCCTTGACAAGCCTCGCATGATTTAGCTG
 AACCTCAGAGGCTGGATGAAGTTCATCCAGCATCTACTTAGCCACCGATCCCTTATCAACACCCCT
 GTTGGTTCTAACATGTTGACTCCTTCCCTTAATAATCTTGGCTAGCCAAAATCACATCAACCCTGA
 GCCATTGTCCGCCAGCGGCTTTACATTACGCTTCTCACCTCACTCCAAACTTTCTCATCATAGCT
 TTCGGCGCCACAGAAATCATTATGTTCTACATCATGTTTGAAGCCACTCTCATCCCC

Supplemental Table 2. RAG-1 haplotype frequencies based on mtDNA lineage followed by haplotype sequences.

Haplotype	Coastal	Great Plains	Total
A	16	12	28
B	8	12	20
C	1	0	1
D	3	5	8
E	0	2	2
F	3	1	4
G	0	1	1
H	0	1	1
I	7	6	13
J	1	0	1
K	9	13	22
L	1	3	4
M	1	0	1
N	2	0	2
O	0	2	2
P	0	2	2
Q	0	1	1
R	0	1	1

#A

GGACAACCTTTGTTGAGGATGTCCCTGCGGATAACCATTTCCAGACGATTCCGCTACGACGTGGCGC
 TGGTTTCTGCATTAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA

TGACAGCATTTCACCTCTGGCTTCACTGTGGTGGTGAAAGAATCTTGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCCGTGAGGTTTTCTTCACAATCA
TGTCAGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGAGCCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#B

GGACAACCTTTGTTGAGGATGTCCCTGCGGATACCATTTCCAGACGATTCCGCTACGACGTGGCGC
TGGTTTCTGCATTA AAAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA
TGACAGCATTTCACCTCTGGCTTCACTGTGGTGGTGAAAGAATCATGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCCGTGAGGTTTTCTTCACAATCA
TGTCAGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGAGCCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#C

GGACAACCTTTGTTGAGGATGTCCCTGCGGATACCATTTCCAGACGATTCCGCTACGACGTGGCAC
TGGTTTCTGCATTA AAAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA
TGACAGCATTTCACCTCTGGCTTCACTGTGGTGGTGAAAGAATCATGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCCGTGAGGTTTTCTTCACAATCA
TGTCAGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGAGCCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#D

GGACAACCTTTGTTGAGGATGTCCCTGCGGATACCATTTCCAGACGATTCCGCTACGACGTGGCGC
TGGTTTCTGCATTA AAAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA
TGACAGCATTTCACGTCTGGCTTCACTGTGGTGGTGAAAGAATCATGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCCGTGAGGTTTTCTTCACAATCA
TGTCAGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGAGCCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#E

GGACAACCTTTGTTGAGGATGTCCCTGCGGATACCATTTCCAGACGATTCCGCTACGACGTGGCGC
TGGTTTCTGCATTA AAAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA

TGACAGCATTTGCACATCTGGCTTCACTGTGGTGGTGAAAGAATCATGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCCGTGAGGTTTTCTTCACAATCA
TGTCAGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGAGCCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#F

GGACAACCTTTGTTGAGGATGTCCCTGCGGATAACCATTTCCAGACGATTCCGCTACGACGTGGCGC
TGGTTTCTGCATTA AAAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA
TGACAGCATTTGCACCTCTGGCTTCACTGTGGTGGTGAAAGAATCATGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAAGCCGTGAGGTTTTCTTCACAATCA
TGTCGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGAGCCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#G

GGACAACCTTTGTTGAGGATGTCCCTGCGGATAACCATTTCCAGACGATTCCGCTACGACGTGGCGC
TGGTTTCTGCATTA AAAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA
TGACAGCATTTGCACCTCTGGCTTCACTGTGGTGGTGAAAGAATCATGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCCGTTAGGTTTTCTTCACAATCA
TGTCGGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGAACCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#H

GGACAACCTTTGTTGAGGATGTCCCTGCGGATAACCATTTCCAGACGATTCCGCTACGACGTGGCGC
TGGTTTCTGCATTA AAAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA
TGACAGCATTTGCACGTCTGGCTTCACTGTGGTAGTGAAAGAATCATGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAAGCCGTGAGGTTTTCTTCACAATCA
TGTCGGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGAGCCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#I

GGACAACCTTTGTTGAGGATGTCCCTGCGGATAACCATTTCCAGACGATTCCGCTACGACGTGGCGC
TGGTTTCTGCATTA AAAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA

TGACAGCATTTCACGTCTGGCTTCACTGTGGTGGTCAAAGAATCATGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCCGTGAGGTTTTCTTCACAATCA
TGTCCGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGAACCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#J

GGACAACCTTTGTTGAGGATGTCCCTGCGGATAACCATTTCAGACGATTCCGCTACGACGTGGCGC
TGGTTTCTGCATTAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA
TGACAGCATTTCACGTCTGGCTTCACTGTGGTGGTCAAAGAATCATGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCCGTGAGGTTTTCTTCACAATCA
TGTCCGTCTCCATCCGAGTTGAGGGCAAGGACGATGGCATCACCATCTTCCAAGAACCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#K

GGACAACCTTTGTTGAGGATGTCCCTGCGGATAACCATTTCAGACGATTCCGCTACGACGTGGCGC
TGGTTTCTGCATTAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA
TGACAGCATTTCACGTCTGGCTTCACTGTGGTGGTCAAAGAATCATGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCCGTGAGGTTTTCTTCACAATCA
TGTCCGTCTCCATCCGAGTTGAGGGCAAGGACGATGGCATCACCATCTTCCAAGAGCCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#L

GGACAACCTTTGTTGAGGATGTCCCTGCGGATAACCATTTCAGACGATTCCGCTACGACGTGGCAC
TGGTTTCTGCATTAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA
TGACAGCATTTCACCTCTGGCTTCACTGTGGTGGTCAAAGAATCTTGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCAGTTCTGAGAAGGCAGTTAGGTTTTCTTCACAATCA
TGTCCGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGAACCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#M

GGACAACCTTTGTTGAGGATGTCCCTGCGGATAACCATTTCAGACGATTCCGCTACGACGTGGCGC
TGGTTTCTGCATTAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA

TGACAGCATTTGCACCTCTGGCTTCACTGTGGTGGTGAAAGAATCATGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCCGTGAGGTTTTCTTCACAATCA
TGTCAGTCTCCATCCGAGTTGAGGGCAAGGACGATGGCATCACCATCTTCCAAGAGCCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#N

GGACAACCTTTGTTGAGGATGTCCCTGCGGATACCATTTCCAGACGATTCCGCTACGACGTGGCGC
TGGTTTCTGCATTA AAAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA
TGACAGCATTTGCACCTCTGGCTTCACTGTGGTGGTGAAAGAATCATGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCCGTGAGGTTTTCTTCACAATCA
TGTCAGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGGGCCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#O

GGACAACCTTTGTTGAGGATGTCCCTGCGGATACCATTTCCAGACGATTCCGCTACGACGTGGCGC
TGGTTTCTGCATTA AAAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA
TGACAGCATTTGCACCTCTGGCTTCACTGTGGTGGTGAAAGAATCATGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCCGTGAGGTTTTCTTCACAATCA
TGTCAGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGAGCCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#P

GGACAACCTTTGTTGAGGATGTCCCTCACCATAACCATTTCCAGACGATTCCGCTACGACGTGGCGC
TGGTTTCTGCATTA AAAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA
TGACAGCATTTGCACCTCTGGCTTCACTGTGGTGGTGAAAGAATCATGTGATGGTATGGGAGATGT
CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCCGTGAGGTTTTCTTCACAATCA
TGTCAGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGAGCCAAAGCCA
AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#Q

GGACAACCTTTGTTGAGGATGTCCCTGCGGATACCATTTCCAGACGATTCCGCTACGACGTGGCGC
TGGTTTCTGCATTA AAAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA

TGACAGCATTTGCACCTCTGGCTTCACTGTGGTGGTGAAAGAATCTTGTGATGGTATGGGAGATGT
 CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCAGTTAGGTTTTCTTCACAATCA
 TGTCCGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGAACCAAAGCCA
 AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

#R

GGACAACCTTTGTTGAGGATGTCCCTGCGGATAACCATTTCCAGACGATTCCGCTACGACGTGGCAC
 TGGTTTCTGCATTAAGACCTGGAGGAGGACATAATGGAAGGACTAAGAGAGAGGGGGCTAGA
 TGACAGCATTTGCACCTCTGGCTTCACTGTGGTGGTGAAAGAATCTTGTGATGGTATGGGAGATGT
 CAGCGAGAAGCATGGAAATGGTCCAGCGGTTCTGAGAAGGCAGTTAGGTTTTCTTCACAATCA
 TGTCCGTCTCCATCCGAGTTGAGGGCGAGGACGATGGCATCACCATCTTCCAAGAGCCAAAGCCA
 AACTCTGAGCTCTCTTGTAGACCTTTGTGCCTC

Supplemental Table 3. Cytb haplotype frequency and sequences from individuals collected from the Rio Grande Basin for this study.

Haplotype	Pecos	Rio Grande	Canadian	Total
A	0	3	0	3
B	0	2		2
C	0	0	1	1
D	1	0	0	1
E	1	0	0	1
F	1	0	0	1
G	0	0	1	1
H	1	0	1	2
I	0	0	1	1
J	0	0	1	1
K	2	0	0	2
L	1	0	0	1
M	0	1	0	1
N	0	2	0	2
O	0	2	0	2
P	6	0	0	6

#A

CTCCCAACACCCCTCGAATATCTCCGCACTGTGGAATTTTGGTTCCCTATTAGGATTGTGTTTAATTA
CTCAAATCCTGACCGGATTATTTCTGGCAATACATTACACCTCCGACATCTCAACCGCATTTCATC
CGTAACTCACATTTGCCGTGACGTTAACTACGGCTGGCTTATCCGTAACATACATGCCAACGGAGC
ATCATTTTTCTTCATCTGTATTTATATACATATCGCCCGGGCCTCTACTACGGATCATATCTCTAT
AAGGAGACCTGGAATATCGGAGTCGTCCTACTTCTCCTCGTCATAATAACGGCCTTTGTAGGCTAC
GTGCTCCCGTGAGGTCAAATGTCTTTCTGAGGTGCTACAGTTATTACAAACCTTCTCTCAGCAGTA
CCCTATATGGGGGATACCTTGGTCCAATGAATTTGGGGAGGTTTCTCAGTGGACAACGCAACACTG
ACCCGATTCTTTGCCTTCCACTTCTTATTCCCCTTCGTAATCGCTGGTGCAACTGTTCTCCACCTAC
TTTTCTGCACGAGACAGGATCGAATAATCCCGCCGGAATAACTCCGACGCAGATAAAATCTCCT
TCCACCCCTACTTTTCTACAAGGACCTTCTTGGCTTCGTTCTAATATTGTTAGCTCTCACATCCCT
AACCTTATTCTCCCCTACCCTACTCGGTGACCCAGAGAACTTCACCCCTGCTAATCCCCTGGTCAC
CCCCCACACATCCAGCCTGAGTGATATTTCTTATTTCGCTACGCCATTCTACgGTCTATCCCAAAC
AAATTAGGAGGTGTACTAGCACTCTTGTTTAGCATTCTAGTACTATTAGTGGTCCCGATTCTACAC
ACCTCAAACAACGAGGACTAACCTTCCGACCTATTACCCAATTCTTATTCTGAACCTTAGTTGCG
GATATACTTATCCTGACATGAATCGGAGCTATACCTGTAGAACATCCATATATCAiCATTGGCCAA
GTCGCCTCCGTCCTGTACTTCGCA

#B

CTCCCAACACCNTCGAATATCTCTGCACTGTGGAATTTTGGTTCCCTACTAGGATTGTGTTTAATTA
CTCAAATCCTGACCGGATTATTTCTAGCAATACATTACACCTCCGACATCTCAACCGCATTTCATC
CGTAACTCACATTTGCCGTGACGTTAACTACGGCTGACTiATCCGTAACATACATGCcAACGGAGCA
TCATTTTTCTTCATCTGTATTTATATACATATCGCCCGGGTCTCTACTACGGATCATATCTCTATA
AGGAGACCTGGAATATCGGAGTCATCCTACTTCTCCTCGTCATAATAACGGCCTTTGTAGGCTACG
TGCTCCCGTGAGGTCAAATGTCTTTCTGAGGTGCTACAGTTATTACAAACCTTCTCTCAGCAGTAC
CCTATATGGGGGATACCTTGGTTCAATGAATTTGGGGAGGTTTCTCAGTGGACAACGCAACACTGA
CCCGATTCTTTGCCTTCCACTTCTTATTCCCCTTCGTAATCGCTGGTGCAACTGTTCTCCACCTACTT
TTCTGCACGAGACAGGGTCAATAATCCCGCCGATTAACTCCGACGCAGATAAAATCTCCTT
CCACCCCTACTTTTCTACAAGGACCTTCTTGGCTTCGTTCTAATATTATTAGCTCTCACATCCCTA

ACCTTATTCTCCCCTACCCTACTCGGTGACCCAGAGAACTTCACCCCTGCTAATCCCCTGGTCACCC
CCCCACACATCCAGCCTGAGTGATATTTCTTATTTCGCCTACGCCATTCTACGGTCTATCCCGAACA
AATTAGGAGGTGTACTAGCACTCTTGTTTAGCATTCTAGTACTATTAGTGGTCCCGATTCTACACA
CCTCAAAACAACGAGGACTAACCTTCCGACCTATTACCCAATTCTTATTCTGAACCTTAGTTGCGG
ATATACTTATCCTGACATGAATCGGAGGTATACCTGTAGAACATCCATATATTATCATTGGCCAAAG
TCGCCTCCGTCTGTACTTCGCA

#C

CTCCCAACACCCTCGAATATCTCTGCACTGTGGAATTTTGGTTCCCTACTAGGATTGTGTTTAATTA
CTCAAATCCTGACCGGATTATTTCTGGCAATACATTACACCTCCGACATCTCAACCGCATTTCATC
CGTAACTCACATTTGCCGTGACGTTAACTACGGCTGACTTATCCGTAACATACATGCCAACGGAGC
ATCATTTTTCTTCATCTGTATTTATATACATATCGCCCGGGTCTCTACTACGGATCATATCTCTAT
AAGGAGACCtGGAATATCGGAGTCATCCTACTTCTCCTCGTCATAATAACGGCCTTTGTAGGCTAC
GTGCTCCCGTGAGGTCAAATGTCTTTCTGAGGTGCTACAGTTATTACAAACCTTCTCTCAGCAGTA
CCCTATATGGGGGATACCTTGGTCCAATGAATTTGGGGAGGTTTCTCAGTGGACAACGCAACACTG
ACCCGATTCTTTGCCTTCCACTTCTTATTCCCCTTCGTAATCGCTGGTGCAACTGTTCTCCACCTAC
TTTTCTGCACGAGACAGGATCGAATAATCCCGCCGGATTAACTCCGACGCAGATAAAATCTCCT
TCCACCCCTACTTTTTCTACAAGGACCTTCTTGGCTTCGTTCTAATATTATTAGCTCTCACATCCCT
AACCTTATTCTCCCCTACCCTACTCGGTGACCCAGAGAACTTCACCCCTGCTAATCCCCTGGTCAC
CCCCCACACATCCAGCCTGAGTGATATTTCTTATTTCGCCTACGCCATTCTACGGTCTATCCCGAAC
AAATTAGGAGGTGTACTAGCACTCTTGTTTAGCATTCTAGTACTATTAGTGGTCCCGATTCTACAC
ACCTCAAAACAACGAGGACTAACCTTCCGACCTATTACCCAATTCTTATTCTGAACCTTAGTTGCG
GATATACTTATCCTGACATGAATCGGAGGTATACCTGTAGAACATCCATATATTATCATTGGCCAA
GTCGCCTCCGTCTGTACTTCGCG

#D

CTCCCAACACCCTCAAATATCTCTGCGCTGTGAAATTTTGGTTCCCTATTAGGGTTATGTTTAATTA
CTCAAATTCTGACCGGACTATTTCTAGCAATACATTACACCTCTGATATCTCAACCGCATTTCGTC
CGTAACTCACATTTGCCGTGACGTTAACTACGGCTGACTTATCCGTAACATACATGCCAACGGAGC
ATCATTTTTCTTCATCTGCATTTATATACATATCGCCCGGGCCTCTACTACGGATCATATCTCTAC

AAAGAGACCTGAAATATCGGGGTCGTCCTACTTCTCCTCGTCATGATGACAGCCTTTGTAGGCTAC
GTACTCCCCTGAGGTCAAATATCTTTCTGGGGTGCTACAGTTATTACGAACCTTCTCTCAGCAGTCC
CTTATATGGGAGATACCTTGGTCCAATGAATTTGAGGGGGTTTTTCGGTAGATAACGCAACGCTGA
CCCGATTCTTTGCCTTCCACTTCTTATTCCCCTTTGTCATCGCTGGTGCGACTGTTCTTCACCTACTT
TTCCTACACGAAACAGGATCAAATAACCCTGCCGGACTAAACTCCGACGCAGATAAAAATCTCCTT
CCACCCCTACTTTTCTACAAAGACCTCCTTGGCTTCGTTCTAATATTGTTGGCCCTCACATCGCTC
ACCTTATTCTCCCCACCCTGCTAGGAGACCCAGAGAACTTCACCCCTGCAAACCCTTTAGTCACC
CCGCCGCACATTCAACCCGAATGGTATTTCTTGTTCGCCTACGCCATTCTACGATCTATCCCAAAC
AAATTAGGAGGGGTGCTAGCGCTCTATTTAGCATTCTAGTACTACTAGTAGTCCCGATTCTACAC
ACTTCAAAGCAACGAGGACTAACCTTCCGACCTATCACCCAGTTCTTATTCTGAACCTTAGTTGCC
GATATACTCATCCTGACATGAATCGGAGGCATACCTGTGGAGCATCCGTATATCATCATCGGCCAA
GTCGCCTCCGTCTGTACTTCGCA

#E

CTCCCAACACCATCAAACATTTCTGCACTATGGAATTTGGTTCCCTCCTAGGATTATGTTTAATTA
CTCAGATTTTAACCGGACTATTTCTGGCAATACATTACACCTCTGATATTTCAACCGCCTTCTCATC
CGTGACTCACATTTGCCGGGATGTTAACTACGGCTGACTTATTCGTAACATGCATGCCAACGGAGC
ATCATTTTTCTTCATCTGTATTTATATACATATCGCCCGGGCCTATACTACGGATCATACTCTAT
AAAGAAACCTGAAATATTGGAGTAGTCCTACTTCTCCTCGTCATAATAACAGCCTTTGTGGGCTAC
GTGCTCCCCTGAGGTGAGATGTCTTTCTGGGGCGCCACAGTTATTACGAACCTTCTCTCAGCAGTA
CCTTATATGGGAGATACCTTAGTCCAATGGATTTGGGGAGGTTTCTCAGTAGATAACGCCACGCTC
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TTTTCTACACGAGACAGGGTCAAACAACCCCGCCGGACTAAACTCCGACGCGGATAAAAATCTCC
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#F

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#G

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#I

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#J

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#K

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#L

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#M

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#N

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#0

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#P

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Supplemental Table 4. Raw morphometric measures from individuals collected from the Pecos River, NM. The heading indicates the following measures: SL = standard length, BD = body depth, DCP = depth of caudal peduncle, LCP = length of caudal peduncle, PDL = predorsal length, HL = head length E = eye width, and S = snout length.

INDIVIDUAL	LINEAGE	SL	BD	DCP	LCP	PDL	HL	E	S
CL11-08	Great Plains	32.99	7.62	3.62	8.12	17.39	8.95	2.93	2.22
CL11-10	Great Plains	32.83	7.11	3.4	7.86	17.81	8.83	2.81	2.11
CL11-14	Coastal	24.4	5.32	2.77	6.76	12.9	6.66	2.37	1.49
CL11-17	Coastal	27.1	5.54	2.9	8.07	13.69	7.16	2.48	1.62
CL11-24	Great Plains	26.8	5.28	2.7	7.42	13.65	7.14	2.57	1.82
CL11-54	Coastal	25.55	4.76	2.54	7.72	13.39	6.73	2.54	1.48
CL12-003	Great Plains	34.01	9.71	3.93	7.98	18.74	9.69	3	2.41
CL12-027	Coastal	28.33	7.08	2.85	7.52	15.1	7.73	2.6	1.81
CL12-031	Coastal	33.31	8.39	3.92	7.69	17.39	9.66	2.86	2.33
CL12-044	Coastal	29.31	7.06	3.16	7.65	15.02	7.74	2.57	1.96
CL12-050	Great Plains	30.09	7.63	3.29	7.66	16	8.34	2.78	1.95
CL12-051	Great Plains	30.61	6.51	2.94	8.73	15.91	8.16	2.58	2.22
CL12-067	Coastal	30.14	6.69	2.72	7.54	16.11	8.39	2.67	2.11
CL12-072	Coastal	33.06	8.78	3.68	7.61	17.97	9.91	3.16	2.4
CL12-079	Great Plains	32.93	8.04	3.08	8.22	18.11	9.5	3.14	2.23
CL12-085	Coastal	31.46	8.25	3.25	6.9	16.56	8.65	2.93	1.92
CL12-091	Coastal	30.24	8.06	2.74	7.63	16.49	8.47	2.55	1.96
CL12-093	Coastal	30.53	7.1	3.09	7.41	16.21	8.12	2.74	1.81
CL12-100	Great Plains	31.77	7.9	3.4	8.24	17.05	8.75	2.93	2.24
CL12-109	Coastal	51.28	15.9	6.8	12.47	27.74	14.44	3.62	4.51
CL12-110	Coastal	43.16	13.2	5.46	9.41	23.07	12.5	3.1	3.2
CL12-113	Coastal	38.68	10.46	4.62	8.87	19.64	10.24	3.02	2.78
CL12-118	Coastal	32.96	8.86	3.55	8.41	17.12	8.92	2.7	2.18
CL12-127	Coastal	39.29	11.35	4.88	9.48	20.03	11.11	2.94	2.83
CL12-134	Coastal	28.22	6.38	3.02	7.49	14.41	7.52	2.22	1.99
CL12-137	Coastal	47.45	13.52	5.4	10.96	24.65	12.85	3.34	3.09
CL12-141	Coastal	40.5	11.85	4.77	9.42	21.2	11.61	3.07	2.79
CL12-150	Great Plains	33.79	9.04	3.52	7.9	17.4	8.52	2.68	2.39
CL12-152	Great Plains	38.05	9.34	3.88	8.5	19.89	9.58	2.98	2.53
CL12-157	Great Plains	32.99	7.95	3.61	6.88	17.46	8.51	2.98	2.44
CL12-158	Great Plains	34.29	9.44	3.99	8.73	18.12	9.25	2.86	2.39
CL12-167	Coastal	36.36	10.63	4.47	8.37	18.71	10.27	3.16	2.15
CL12-169	Great Plains	34.94	10.04	4.19	8.37	17.9	10.08	3.17	2.76
CL12-173	Great Plains	35.77	9.66	4.33	7.98	18.29	9.48	3.19	2.71
CL12-176	Great Plains	31.48	7.94	3.42	7.59	16.01	8.22	2.69	2.39
CL12-178	Coastal	35.22	9.06	3.71	8.76	17.69	9.2	2.86	2.52
CL12-185	Great Plains	33.12	8.9	3.56	7.05	16.68	8.35	2.73	2.25
CL12-188	Great Plains	30.09	7.67	3.12	7	16.33	8.01	2.68	2.07
CL12-193	Great Plains	29.69	7.73	2.92	7.53	15.65	7.77	2.39	1.98
CL12-203	Great Plains	33.31	8.83	3.59	7.98	17.44	8.86	2.94	2.15
CL12-204	Coastal	37.14	10.81	4.23	8.63	19.69	10.35	3.1	2.81

CL12-207	Great Plains	32.87	8.21	3.56	7.75	17.54	9.11	3.22	2.28
CL12-211	Coastal	37.66	9.26	3.9	9.38	20.15	10.52	3.38	2.62
CL12-221	Great Plains	30.15	7.35	2.8	7.42	15.44	7.77	2.63	1.99
CL12-226	Great Plains	30.96	6.74	2.99	7.16	16.09	8.3	2.89	2.01

Supplemental Table 5. Raw meristic counts from individuals collected from the Pecos River, NM. The heading indicates the following characters: P1 = pectoral fin rays, P2 = pelvic fin rays, DF = dorsal fin rays, AF = anal fin rays, LL = lateral line scales, SALL = scales above lateral line, SBLL = scales below lateral line.

INDIVIDUAL	LINEAGE	P1	P2	DF	AF	LL	SALL	SBLL
CL11-08	Great Plains	6.99	5.12	8	9	35	6	3
CL11-10	Great Plains	7.61	5.11	8	10	35	7	3
CL11-14	Coastal	5.06	3.9	8	9	34	7	3
CL11-17	Coastal	5.73	3.79	8	9	34	6	3
CL11-24	Great Plains	5.27	3.6	8	10	35	6	3
CL11-54	Coastal	4.88	4.01	7	9	35	6	3
CL12-003	Great Plains	8.45	6.1	8	9	36	7	4
CL12-027	Coastal	5.46	4.33	8	9	34	7	3
CL12-031	Coastal	6.93	5.46	8	9	34	7	4
CL12-044	Coastal	6.98	4.87	8	9	35	7	3
CL12-050	Great Plains	6.8	4.98	8	9	35	7	3
CL12-051	Great Plains	6.19	4.63	8	9	34	6	3
CL12-067	Coastal	6.26	4.68	8	9	35	6	3
CL12-072	Coastal	7.38	5.31	8	10	34	6	4
CL12-079	Great Plains	6.61	4.81	8	9	34	6	3
CL12-085	Coastal	7.02	5.16	8	9	35	7	4
CL12-091	Coastal	6.34	4.9	8	9	34	6	3
CL12-093	Coastal	6.42	5	8	9	34	6	3
CL12-100	Great Plains	6.06	4.99	8	9	34	6	3
CL12-109	Coastal	10.09	7.75	8	10	35	7	4
CL12-110	Coastal	9.17	7.24	8	9	35	7	4
CL12-113	Coastal	7.83	6.14	8	9	34	7	4
CL12-118	Coastal	7.8	5.61	8	8	34	7	4
CL12-127	Coastal	7.6	5.86	8	9	34	6	3
CL12-134	Coastal	6.35	4.76	8	8	35	6	3
CL12-137	Coastal	9.93	7.61	8	9	35	6	3
CL12-141	Coastal	9.01	6.74	8	9	35	6	3
CL12-150	Great Plains	6.97	5.48	8	9	34	7	4
CL12-152	Great Plains	7.69	5.37	8	9	35	6	4
CL12-157	Great Plains	6.66	4.95	8	9	34	6	3
CL12-158	Great Plains	7.61	6.09	8	9	34	7	4

CL12-167	Coastal	8.54	6.31	8	9	34	7	4
CL12-169	Great Plains	8.38	6.08	8	9	35	7	4
CL12-173	Great Plains	8.15	5.83	8	9	34	7	3
CL12-176	Great Plains	7.54	5.42	8	9	34	6	3
CL12-178	Coastal	7.48	5.81	8	9	34	7	4
CL12-185	Great Plains	6.61	4.84	8	9	35	7	4
CL12-188	Great Plains	6.38	4.47	8	9	35	6	3
CL12-193	Great Plains	6.12	4.39	8	9	34	6	4
CL12-203	Great Plains	6.23	4.92	8	9	34	6	4
CL12-204	Coastal	7.06	5.86	8	9	34	6	3
CL12-207	Great Plains	7.87	4.95	7	9	34	6	3
CL12-211	Coastal	7.78	5.93	8	9	35	6	3
CL12-221	Great Plains	5.93	4.25	8	9	34	6	3
CL12-226	Great Plains	6.14	4.41	8	9	35	6	4

Supplemental Table 6. Geometric morphometric weight-matrix raw data for 13 homologous landmarks (X,Y) from Pecos River individuals.

Individual	Lineage	Centroid Size	1X	1Y	2X	2Y	3X	3Y
CL12-109	Coastal	7.62E+02	2.16E-03	1.66E-03	1.83E-03	2.32E-03	-1.11E-02	1.21E-02
CL12-110	Coastal	7.22E+02	2.36E-03	1.17E-03	1.65E-03	2.40E-03	1.47E-03	3.07E-03
CL12-113	Coastal	7.48E+02	-1.36E-03	-7.15E-04	3.20E-04	-1.28E-03	5.65E-03	6.29E-03
CL12-118	Coastal	7.09E+02	2.78E-03	-4.74E-03	-3.02E-04	6.52E-05	-1.43E-02	3.65E-03
CL12-127	Coastal	7.61E+02	-4.09E-04	2.77E-03	5.90E-04	-6.09E-04	-3.84E-03	-1.85E-03
CL12-134	Coastal	6.40E+02	-2.00E-04	3.46E-03	3.61E-03	-1.81E-03	7.48E-03	-3.02E-03
CL12-137	Coastal	7.26E+02	-8.00E-04	-1.87E-03	1.31E-03	1.20E-03	-8.31E-03	5.42E-03
CL12-141	Coastal	7.20E+02	2.40E-06	-8.65E-04	9.65E-04	1.94E-03	-6.24E-03	3.75E-03
CL12-167	Coastal	7.41E+02	-2.48E-03	3.46E-04	1.14E-03	1.99E-03	-1.00E-02	4.19E-03
CL12-178	Coastal	7.34E+02	4.42E-04	-6.50E-04	-8.70E-04	2.33E-03	-7.87E-03	3.39E-03
CL12-204	Coastal	7.90E+02	2.13E-03	6.19E-03	5.36E-04	5.00E-04	-5.42E-03	4.24E-03
CL12-211	Coastal	7.92E+02	2.54E-04	2.97E-05	-3.97E-03	3.24E-05	-5.90E-04	-6.13E-03
CL12-027	Coastal	6.42E+02	9.72E-04	-4.03E-03	-3.59E-03	-1.51E-03	8.50E-03	-1.28E-03
CL12-031	Coastal	7.13E+02	-9.60E-04	2.77E-03	-3.24E-03	-2.55E-03	-3.02E-04	-5.36E-03
CL12-072	Coastal	7.66E+02	-1.12E-03	-6.78E-03	2.53E-03	1.69E-03	-8.77E-04	3.23E-03
CL12-085	Coastal	7.20E+02	-2.95E-03	-8.44E-03	4.80E-04	-4.92E-04	5.77E-03	3.76E-03
CL12-091	Coastal	6.49E+02	2.83E-03	-5.44E-03	7.11E-04	-1.50E-03	2.41E-03	2.84E-03
CL12-093	Coastal	6.96E+02	3.99E-03	-1.69E-03	3.10E-03	-9.68E-04	9.59E-03	-5.09E-04
CL12-003	Great Plains	7.28E+02	-1.69E-03	-1.15E-03	1.39E-02	6.25E-04	1.03E-02	-5.30E-03
CL12-100	Great Plains	6.63E+02	3.46E-04	-4.62E-03	-4.93E-03	9.28E-04	7.93E-03	9.61E-04
CL12-150	Great Plains	7.27E+02	4.45E-04	1.17E-03	-4.86E-03	1.74E-04	2.87E-03	8.23E-04
CL12-152	Great Plains	8.20E+02	-3.04E-04	1.55E-03	5.67E-04	-4.69E-04	-5.65E-03	-4.37E-04
CL12-157	Great Plains	7.10E+02	-1.84E-04	3.32E-03	3.70E-03	1.26E-03	-5.27E-03	5.38E-03

CL12-158	Great Plains	7.28E+02	-1.87E-03	3.36E-03	2.19E-03	-2.17E-03	-2.03E-03	4.75E-03
CL12-169	Great Plains	7.07E+02	-5.25E-03	-2.62E-03	-1.18E-03	-1.55E-04	-3.69E-03	5.61E-04
CL12-173	Great Plains	7.61E+02	-2.06E-03	3.61E-03	3.77E-03	9.14E-05	-4.44E-03	-1.03E-02
CL12-176	Great Plains	6.65E+02	2.79E-03	1.25E-03	-1.14E-03	-1.19E-03	6.18E-03	-2.93E-03
CL12-185	Great Plains	7.19E+02	3.83E-03	-1.01E-04	-2.36E-03	-7.81E-04	-1.40E-03	-2.55E-03
CL12-188	Great Plains	6.41E+02	-3.42E-03	5.11E-04	-4.63E-03	-2.46E-03	5.06E-04	-8.86E-06
CL12-193	Great Plains	7.08E+02	-7.15E-04	1.60E-04	-3.50E-03	5.62E-04	5.89E-03	-1.04E-03
CL12-203	Great Plains	7.27E+02	1.52E-04	3.92E-03	-3.40E-03	3.37E-03	3.21E-03	-1.23E-03
CL12-207	Great Plains	7.16E+02	-6.39E-04	4.52E-03	-2.35E-04	-2.86E-03	-5.55E-03	-4.91E-03
CL12-221	Great Plains	6.92E+02	8.78E-04	1.49E-03	2.06E-04	1.25E-03	3.57E-03	1.44E-04
CL12-226	Great Plains	6.76E+02	2.25E-03	2.50E-03	-1.73E-03	-1.84E-03	-3.36E-03	-9.01E-03
CL12-050	Great Plains	6.58E+02	-1.22E-03	-3.69E-03	-4.81E-03	-3.57E-04	1.62E-02	-1.68E-03
CL12-051	Great Plains	7.09E+02	-7.68E-04	-4.11E-03	-1.01E-03	2.50E-04	6.21E-03	-3.86E-03
CL12-079	Great Plains	6.69E+02	-2.10E-04	5.75E-03	2.66E-03	2.12E-05	-3.45E-03	-7.15E-03

Individual	Lineage	4X	4Y	5X	5Y	6X	6Y	7X	7Y
CL12-109	Coastal	1.66E-03	5.29E-03	-3.38E-03	-9.46E-04	-2.29E-03	4.38E-03	2.81E-03	3.26E-03
CL12-110	Coastal	1.55E-03	-3.80E-03	-3.52E-04	-3.64E-03	5.50E-03	4.47E-03	9.96E-03	3.37E-03
CL12-113	Coastal	3.18E-03	8.95E-03	4.20E-04	-2.44E-03	3.67E-04	1.50E-03	9.24E-04	3.53E-03
CL12-118	Coastal	7.49E-03	-7.47E-04	7.04E-04	-1.00E-02	4.88E-03	3.09E-03	-1.90E-03	3.99E-03
CL12-127	Coastal	-2.43E-03	6.70E-03	4.86E-04	3.44E-03	-3.16E-03	2.26E-04	-5.49E-03	-1.17E-02
CL12-134	Coastal	3.30E-04	8.75E-03	-5.18E-04	5.60E-03	-6.06E-03	1.13E-03	-2.26E-03	-5.40E-03
CL12-137	Coastal	4.48E-03	-8.29E-03	-2.02E-03	-5.50E-03	-1.56E-03	-2.84E-03	5.50E-03	1.08E-02
CL12-141	Coastal	2.08E-03	-6.70E-03	-7.70E-03	-8.16E-03	-4.89E-03	-3.05E-03	2.63E-03	4.88E-04
CL12-167	Coastal	2.45E-03	3.80E-04	1.57E-03	8.86E-04	-1.02E-03	2.35E-03	7.22E-03	9.41E-03
CL12-178	Coastal	9.77E-03	-4.39E-03	1.20E-03	4.19E-04	-3.52E-03	7.12E-04	6.56E-03	3.78E-03
CL12-204	Coastal	1.09E-02	2.03E-03	-4.43E-03	-5.05E-03	7.92E-04	-2.28E-03	-5.18E-03	-5.41E-03
CL12-211	Coastal	-4.77E-04	3.47E-03	-1.79E-03	4.41E-03	-2.85E-03	-2.26E-03	-4.31E-03	-5.43E-03
CL12-027	Coastal	-9.26E-03	-2.67E-03	6.48E-03	-5.73E-03	-4.28E-03	-8.37E-03	4.22E-03	-3.26E-03
CL12-031	Coastal	-1.43E-04	4.45E-03	-1.17E-02	-8.31E-05	6.13E-04	-2.30E-03	7.03E-03	2.47E-03
CL12-072	Coastal	-7.25E-03	-8.14E-03	3.91E-03	-2.21E-03	-1.09E-03	-4.02E-03	-1.38E-02	1.00E-02
CL12-085	Coastal	-6.33E-03	-1.07E-02	7.90E-03	2.85E-04	-9.58E-04	-2.28E-04	1.01E-03	6.00E-05
CL12-091	Coastal	-1.24E-02	-7.83E-03	8.47E-03	-6.78E-03	-1.81E-03	5.01E-03	3.69E-03	2.48E-03
CL12-093	Coastal	-8.65E-03	-7.98E-03	5.47E-03	-2.27E-03	2.40E-03	5.94E-03	5.70E-03	3.98E-03
CL12-003	Great Plains	-7.96E-03	-3.16E-03	-4.80E-03	3.02E-03	3.68E-04	4.28E-04	1.88E-02	1.05E-03
CL12-100	Great Plains	-9.93E-03	-3.86E-03	2.32E-03	-2.27E-03	-7.76E-03	-1.33E-04	2.88E-03	-2.44E-03
CL12-150	Great Plains	5.75E-03	1.14E-02	1.50E-03	2.10E-03	-1.20E-04	7.88E-06	-5.59E-03	-2.36E-03
CL12-152	Great Plains	2.36E-03	6.47E-04	5.66E-03	2.43E-03	6.02E-03	1.58E-03	-2.98E-03	-1.84E-03
CL12-157	Great Plains	3.97E-03	8.74E-03	7.55E-04	-1.04E-03	1.03E-02	-1.69E-03	-1.27E-02	-3.63E-03
CL12-158	Great Plains	5.74E-03	-2.14E-03	-5.32E-03	-3.34E-03	4.24E-03	3.87E-03	4.37E-03	8.15E-03
CL12-169	Great Plains	5.29E-03	-1.04E-02	-5.72E-03	-8.69E-03	-1.17E-03	-2.24E-03	6.05E-03	7.71E-03
CL12-173	Great Plains	2.82E-03	1.45E-03	2.14E-03	9.00E-03	8.80E-03	2.42E-03	-3.19E-04	-1.32E-03

CL12-176	Great Plains	1.11E-03	4.54E-05	2.92E-04	5.03E-03	4.50E-03	2.01E-03	6.07E-03	-4.13E-03
CL12-185	Great Plains	-2.04E-03	3.71E-04	2.97E-03	-2.62E-03	1.42E-03	6.44E-03	-7.02E-03	-9.33E-04
CL12-188	Great Plains	1.50E-03	8.41E-03	-3.23E-03	3.96E-03	4.00E-03	3.51E-03	-3.66E-03	-9.16E-03
CL12-193	Great Plains	9.23E-04	6.20E-03	8.51E-03	9.62E-03	-5.46E-03	-1.17E-03	-7.65E-03	-4.55E-03
CL12-203	Great Plains	8.35E-03	-1.46E-04	-8.09E-03	2.07E-03	-7.33E-04	-2.34E-03	3.43E-05	3.29E-03
CL12-207	Great Plains	4.38E-03	6.23E-03	-8.34E-03	6.45E-03	7.23E-03	-1.25E-03	-7.77E-03	-1.80E-03
CL12-221	Great Plains	-3.48E-03	1.02E-02	1.20E-04	3.95E-03	-3.92E-04	-2.14E-03	-5.05E-04	4.29E-04
CL12-226	Great Plains	-1.18E-03	3.21E-03	1.03E-03	3.21E-03	-7.87E-03	-4.44E-03	-1.20E-02	-1.08E-02
CL12-050	Great Plains	9.42E-04	-5.14E-03	4.42E-03	1.13E-04	-9.30E-04	-2.86E-03	3.96E-03	4.42E-03
CL12-051	Great Plains	-3.11E-03	-5.68E-03	2.06E-03	-2.12E-03	-1.35E-03	-5.25E-03	-2.40E-03	-3.50E-03
CL12-079	Great Plains	-1.24E-02	-5.18E-03	-9.74E-04	6.91E-03	-2.17E-03	-1.97E-04	-3.86E-03	-4.97E-03

Individual	Lineage	8X	8Y	9X	9Y	10X	10Y	11X	11Y
CL12-109	Coastal	-1.08E-02	-1.86E-03	7.71E-03	4.88E-03	-4.10E-03	1.19E-02	4.08E-04	-6.95E-03
CL12-110	Coastal	-1.03E-02	-6.79E-04	-8.08E-03	2.15E-03	1.97E-03	2.28E-03	-3.48E-03	-3.48E-03
CL12-113	Coastal	3.36E-04	-2.29E-03	-6.89E-03	-8.18E-05	1.99E-03	1.79E-04	-6.96E-03	-8.10E-03
CL12-118	Coastal	-1.14E-02	-2.03E-03	-6.12E-03	1.34E-04	3.85E-03	4.32E-03	-4.44E-03	-3.96E-04
CL12-127	Coastal	3.52E-03	-1.78E-03	-1.72E-03	3.32E-03	1.25E-02	-6.24E-03	-3.64E-04	-8.30E-03
CL12-134	Coastal	5.24E-03	-3.83E-03	-1.40E-03	-4.53E-03	5.82E-03	-4.04E-04	9.97E-03	1.81E-04
CL12-137	Coastal	-1.84E-02	-1.84E-03	4.61E-03	2.26E-04	-6.91E-03	2.66E-03	-5.38E-03	-6.16E-03
CL12-141	Coastal	-1.83E-02	5.43E-04	-7.54E-03	9.23E-07	2.34E-03	-3.19E-03	-7.59E-03	-1.78E-03
CL12-167	Coastal	-1.91E-02	1.50E-03	4.01E-04	4.37E-03	-7.33E-03	4.19E-03	-7.29E-03	-8.21E-03
CL12-178	Coastal	-1.02E-02	4.24E-03	-1.17E-02	-3.27E-04	-1.10E-02	5.84E-03	-6.71E-03	-2.02E-03
CL12-204	Coastal	-4.92E-03	7.03E-04	-9.81E-03	-9.19E-05	3.02E-04	-8.92E-03	-2.52E-03	5.79E-03
CL12-211	Coastal	-1.73E-03	1.35E-03	1.06E-02	-5.15E-04	1.18E-03	2.88E-03	8.15E-03	1.66E-03
CL12-027	Coastal	8.55E-03	2.77E-03	4.73E-03	-1.19E-03	2.19E-02	-1.37E-02	1.31E-02	1.24E-03
CL12-031	Coastal	6.88E-04	8.50E-04	-9.76E-03	-1.40E-03	-7.17E-03	3.05E-03	6.40E-03	3.61E-03
CL12-072	Coastal	1.73E-02	3.24E-03	1.23E-02	-9.26E-04	-4.55E-03	-1.05E-02	6.86E-03	2.70E-03
CL12-085	Coastal	-4.40E-03	1.39E-03	2.27E-03	-3.46E-03	-9.99E-04	6.14E-03	-1.74E-03	4.70E-03
CL12-091	Coastal	1.40E-02	-2.59E-03	-8.25E-04	-9.66E-05	8.17E-04	-6.43E-03	1.12E-02	1.20E-02
CL12-093	Coastal	7.95E-03	-3.02E-03	4.04E-03	-1.87E-03	-2.52E-03	3.05E-03	-2.35E-03	-1.73E-03
CL12-003	Great Plains	1.22E-03	3.54E-03	-1.93E-03	3.69E-03	-2.75E-02	-7.28E-03	-8.15E-03	1.25E-03
CL12-100	Great Plains	2.25E-03	-3.06E-03	3.89E-03	-2.81E-03	3.25E-03	2.72E-03	-1.04E-02	5.31E-03
CL12-150	Great Plains	1.16E-04	-3.64E-03	5.83E-03	3.73E-03	5.36E-03	3.84E-03	-4.15E-03	9.03E-04
CL12-152	Great Plains	-1.43E-03	3.09E-04	5.10E-03	-2.17E-03	-4.31E-03	1.86E-03	3.00E-03	2.66E-03
CL12-157	Great Plains	1.32E-02	1.22E-03	4.79E-03	-1.55E-03	-1.27E-02	2.99E-03	5.23E-03	-2.29E-03
CL12-158	Great Plains	-4.99E-03	2.08E-05	-5.64E-03	-1.32E-03	-2.06E-03	4.97E-03	-1.83E-03	-2.08E-03
CL12-169	Great Plains	-5.31E-03	-1.87E-03	7.92E-04	1.66E-03	-9.05E-03	1.30E-03	3.80E-03	-4.43E-03
CL12-173	Great Plains	-6.42E-03	-6.45E-04	-9.60E-04	4.21E-04	3.77E-04	3.73E-03	8.16E-03	-6.86E-03
CL12-176	Great Plains	7.77E-03	-3.21E-03	4.77E-03	1.97E-04	-6.23E-03	1.53E-04	-2.42E-03	-5.54E-03
CL12-185	Great Plains	-8.56E-05	1.45E-03	-2.32E-03	3.72E-03	-7.44E-04	-8.25E-03	1.54E-02	-7.13E-03
CL12-188	Great Plains	3.28E-03	-6.58E-03	-1.04E-02	-2.51E-03	1.06E-02	1.50E-03	-7.74E-03	5.08E-03

CL12-193	Great Plains	2.52E-03	2.16E-03	4.01E-03	-3.42E-04	3.75E-03	-1.50E-03	1.73E-02	1.22E-04
CL12-203	Great Plains	6.75E-03	1.81E-03	-5.60E-03	1.69E-03	4.47E-03	-3.03E-03	1.00E-02	-2.66E-03
CL12-207	Great Plains	6.01E-03	3.67E-03	3.69E-03	-4.44E-03	1.42E-02	6.67E-03	1.57E-03	4.52E-03
CL12-221	Great Plains	3.12E-03	2.37E-03	6.06E-03	-3.96E-04	1.65E-03	1.47E-03	1.37E-02	8.34E-03
CL12-226	Great Plains	5.31E-03	3.12E-03	-4.71E-04	-1.92E-03	1.08E-02	-5.04E-03	-8.57E-03	4.27E-03
CL12-050	Great Plains	1.07E-02	-1.04E-03	-5.33E-04	1.31E-04	1.92E-03	1.44E-03	-7.43E-03	1.36E-05
CL12-051	Great Plains	8.32E-03	-6.98E-04	4.11E-03	-3.51E-03	6.14E-03	9.50E-05	-5.31E-03	4.43E-03
CL12-079	Great Plains	-2.88E-04	4.44E-03	2.09E-03	5.14E-03	-8.02E-03	-4.70E-03	-2.95E-02	9.29E-03

Individual	Lineage	12X	12Y	13X	13Y	UniX	UniY
CL12-109	Coastal	1.23E-02	1.02E-02	-1.11E-02	1.43E-04	-2.47E-02	6.52E-03
CL12-110	Coastal	1.00E-02	1.51E-03	-5.09E-03	-3.69E-02	-1.11E-02	-6.90E-03
CL12-113	Coastal	1.43E-02	1.17E-03	-2.61E-04	-2.87E-02	3.34E-03	6.93E-03
CL12-118	Coastal	-8.89E-03	1.19E-02	-8.42E-03	-3.19E-03	5.79E-04	-3.07E-03
CL12-127	Coastal	-2.88E-03	3.35E-03	6.89E-03	-1.12E-02	-1.53E-02	6.31E-04
CL12-134	Coastal	-1.14E-02	1.49E-02	1.74E-02	1.89E-02	8.23E-03	7.78E-03
CL12-137	Coastal	-5.76E-03	-4.08E-03	7.09E-03	4.05E-02	6.65E-03	1.38E-02
CL12-141	Coastal	-1.06E-02	6.56E-03	-5.25E-03	-1.58E-02	-7.09E-03	2.56E-03
CL12-167	Coastal	8.91E-03	-1.19E-02	1.62E-02	3.62E-03	1.32E-03	1.68E-02
CL12-178	Coastal	-5.50E-03	3.77E-03	-7.69E-03	1.37E-02	1.24E-02	1.46E-03
CL12-204	Coastal	8.23E-06	1.30E-02	-1.62E-02	-1.71E-03	-1.44E-02	-6.43E-03
CL12-211	Coastal	-3.35E-03	-1.31E-03	1.79E-03	2.11E-02	-1.54E-03	4.51E-03
CL12-027	Coastal	9.48E-03	5.73E-05	-1.34E-02	-6.61E-03	1.27E-03	-9.70E-03
CL12-031	Coastal	-2.20E-02	4.96E-03	-8.13E-03	2.49E-02	-2.65E-02	-6.55E-03
CL12-072	Coastal	5.17E-03	-2.84E-02	4.23E-03	1.17E-03	5.76E-03	-3.59E-03
CL12-085	Coastal	-2.90E-03	-1.31E-02	1.25E-02	-3.24E-03	1.44E-02	1.29E-02
CL12-091	Coastal	1.77E-02	-5.04E-03	-2.40E-03	-6.83E-03	2.83E-02	-1.43E-02
CL12-093	Coastal	8.05E-03	-2.09E-02	1.86E-02	-6.05E-03	3.28E-02	4.79E-03
CL12-003	Great Plains	2.50E-03	-1.09E-02	-7.74E-03	1.12E-02	-2.26E-02	-8.09E-04
CL12-100	Great Plains	-1.20E-04	-4.53E-03	6.05E-03	-2.46E-02	1.73E-02	6.72E-03
CL12-150	Great Plains	-6.70E-03	5.93E-04	1.78E-03	-1.67E-03	-9.53E-03	-2.71E-03
CL12-152	Great Plains	2.45E-02	-3.36E-04	-1.83E-02	2.43E-02	3.34E-04	-3.01E-03
CL12-157	Great Plains	7.75E-03	8.22E-03	4.35E-03	-4.95E-03	2.30E-03	-2.75E-03
CL12-158	Great Plains	-3.03E-03	1.10E-02	-5.51E-03	-1.20E-02	-1.47E-03	-1.93E-03
CL12-169	Great Plains	4.91E-03	-5.88E-03	1.17E-02	1.73E-02	-1.58E-02	1.70E-02
CL12-173	Great Plains	8.90E-03	1.23E-02	6.95E-04	6.87E-03	-2.13E-02	1.45E-03
CL12-176	Great Plains	-1.46E-02	-4.44E-03	1.59E-02	1.62E-03	-1.06E-02	-1.69E-03
CL12-185	Great Plains	3.77E-02	8.22E-03	-6.95E-04	4.09E-02	-6.48E-03	6.50E-03
CL12-188	Great Plains	-1.53E-02	1.77E-02	-4.68E-04	-7.05E-03	3.18E-04	9.21E-03
CL12-193	Great Plains	-2.29E-02	1.39E-03	5.53E-03	9.96E-03	6.06E-04	-2.40E-03
CL12-203	Great Plains	-9.56E-03	3.94E-04	-7.86E-03	3.86E-03	-8.68E-03	-1.09E-02
CL12-207	Great Plains	-5.17E-03	1.45E-02	-3.01E-02	1.30E-02	-2.98E-03	-1.85E-02

CL12-221	Great Plains	-1.27E-02	-3.13E-03	7.85E-03	2.26E-02	8.03E-03	-6.28E-03
CL12-226	Great Plains	-4.32E-03	1.00E-02	-8.73E-03	-1.81E-02	1.05E-02	-7.18E-03
CL12-050	Great Plains	-1.68E-03	-1.97E-02	1.55E-02	-3.84E-02	2.02E-02	-4.98E-03
CL12-051	Great Plains	-3.38E-03	-1.00E-02	1.97E-02	-9.29E-03	2.16E-02	-3.54E-04
CL12-079	Great Plains	6.49E-04	-1.20E-02	-1.66E-02	-3.92E-02	3.81E-03	-5.58E-03