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RESEARCH ARTICLE

# Geographic distribution of genetic diversity in populations of Rio Grande Chub *Gila pandora*

René Galindo[1] · Wade D. Wilson[2] · Colleen A. Caldwell[3]

**Abstract** In the southwestern United States (US), the Rio Grande chub (*Gila pandora*) is state-listed as a fish species of greatest conservation need and federally listed as sensitive due to habitat alterations and competition with non-native fishes. Characterizing genetic diversity, genetic population structure, and effective number of breeders will assist with conservation efforts by providing a baseline of genetic metrics. Genetic relatedness within and among *G. pandora* populations throughout New Mexico was characterized using 11 microsatellite loci among 15 populations in three drainage basins (Rio Grande, Pecos, Canadian). Observed heterozygosity ($H_O$) ranged from 0.71–0.87 and was similar to expected heterozygosity (0.75–0.87). Rio Ojo Caliente (Rio Grande) had the highest allelic richness ($A_R = 15.09$), while Upper Rio Bonito (Pecos) had the lowest allelic richness ($A_R = 6.75$). Genetic differentiation existed among all populations with the lowest genetic variation occurring within the Pecos drainage. STRUCTURE analysis revealed seven genetic clusters. Populations of *G. pandora* within the upper Rio Grande drainage (Rio Ojo Caliente, Rio Vallecitos, Rio Pueblo de Taos) had high levels of admixture with Q-values ranging from 0.30–0.50. In contrast, populations within the Pecos drainage (Pecos River and Upper Rio Bonito) had low levels of admixture (Q = 0.94 and 0.87, respectively). Estimates of effective number of breeders ($N_b$) varied from 6.1 (Pecos: Upper Rio Bonito) to 109.7 (Rio Grande: Rio Peñasco) indicating that populations in the Pecos drainage are at risk of extirpation. In the event that management actions are deemed necessary to preserve or increase genetic diversity of *G. pandora*, consideration must be given as to which populations are selected for translocation.

**Keywords** Gila pandora · Southwestern United States · Native Fish · Arid lands · Connectivity

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## Introduction

The decline and eventual extirpation or extinction of native fishes can be attributed to degradation and fragmentation of habitat, introduction of non-native species, and conflict and competition for water. In the arid southwestern United States (US), population declines and risk of extinction can be exacerbated by drought and wildfire, which alter stream temperature, fish species composition, erosion patterns, water yield, and hydrologic processes (Bozek and Young 1994). Reduced flow, fragmented habitats, and the presence of non-native fishes have impeded re-colonization and persistence of native fishes shifting the fish community toward more thermally tolerant fish assemblages (Propst et al. 2008). Habitat connectivity within and among stream systems in the southwestern US is considered one of the more important factors for dispersal and re-colonization of extirpated fish populations (Fagan et al. 2002). As aquatic

habitat becomes fragmented, chances for re-colonization through dispersal processes become rare (Helfman et al. 2009). When populations become fragmented and isolated due to a lack of movement (Lande 1987), the strength of genetic drift increases due to a lack of gene flow. Depending on the demography of the population, genetic drift increases the rate at which genetic variation is lost and increases the risk of extinction (Lande 1988). This risk is offset by increasing or maintaining population connectivity to facilitate gene flow, which is essential for the long-term viability of a species (Segelbacher et al. 2010). Thus, understanding changes between historic and contemporary connectivity and gene flow is important for management and conservation of species to minimize the risk of extinction. As such, proactive planning and conservation goals should include genetic information to target species and community assemblages within ecological systems prior to landscape level alterations.

Of conservation interest in the southwestern US (southern Colorado and New Mexico) is a native fish assemblage that includes Rio Grande cutthroat trout (*Oncorhynchus clarkii virginalis*), Rio Grande sucker (*Catostomus plebeius*), and Rio Grande chub (*Gila pandora*) (Calamusso and Rinne 1996). Concerted management effort among state and federal agencies precluded listing of *O. c. virginalis* under the Endangered Species Act (U.S. Fish and Wildlife Service 2014). Similar efforts are underway with *C. plebeius*, in which a genetic analysis across the species range has identified factors that limit its distribution (McPhee et al. 2008). However, less is known about the distribution and genetic diversity throughout populations of *G. pandora*. The species is restricted to less than 25 % of its historical range (Rees and Miller 2005). Threats to *G. pandora* include introduction and range expansion of non-native fishes and overall degradation of habitat resulting in low recruitment (Calamusso 2005; Rees and Miller 2005). The distribution of *G. pandora* includes the Rio Grande drainage basin of southern Colorado, New Mexico, and Texas with the majority of the historical range in New Mexico (Biota Information System of New Mexico 2009). A single population resides as far north as Hot Creek in Conejos County (37°45′), Colorado, and as far south as Little Agua Creek in Jeff Davis County (30°45′), Texas (Sublette et al. 1990), neither of which were sampled in the current study. The status of the Texas population is uncertain, but presumed to be extant. Douglas and Douglas (2003, 2006) examined mitochondrial DNA diversity of *G. pandora* and reported between 1 and 12 haplotypes throughout populations in the Rio Grande drainage of southern Colorado and northern New Mexico. In New Mexico, the distribution of *G. pandora* includes the

tributaries within three drainage basins (Rio Grande, Pecos, Canadian). The species may have gained access to the Pecos River drainage through headwater exchanges during the Pleistocene (Smith and Miller 1986), but was recently listed as non-indigenous to the Canadian drainage basin (Fuller 2015). The State of New Mexico listed *G. pandora* as a species of greatest conservation need (New Mexico Department of Game and Fish 2006) and the US Forest Service listed the species as sensitive in New Mexico (U.S. Forest Service 2013).

At present, the genetic diversity and population structure of *G. pandora* are unknown in New Mexico. An understanding of genetic diversity, population structure, and effective population size within and among populations throughout New Mexico will be critical in the management of the species to avoid risk of extinction. Conservation goals should include identifying areas that retain genetic diversity with the potential for long-term viability (Neely et al. 2001); and, should the need arise, these goals would prioritize which populations could serve as source populations for translocation in New Mexico. To aid in restoration and recovery efforts of *G. pandora*, we examined genetic diversity, population structure, and effective number of breeders throughout the species range among three drainage basins in New Mexico.

## Materials and methods

### Study areas and sample collection

A comprehensive genetic assessment for only New Mexico populations of *G. pandora* was considered for this study. In 2010, 15 populations were non-randomly selected from the Rio Grande, Pecos, and Canadian drainages (Fig. 1). Populations (Table 1) were identified throughout New Mexico from prior field studies (Calamusso 1992) and agency records (personal communications with G. Gustina, US Bureau of Land Management; R. Hansen and E. Frey, New Mexico Department of Game and Fish). Using a backpack electrofisher (Smith-Root LR-24), fish were collected from slow moving runs, pools, and undercut banks. Non-lethal tissue samples were collected on site by clipping the anal fin and placing the tissue into a 2.0 ml Nalgene™ cryogenic tube filled with 95 % ethanol for preservation (McPhee and Turner 2004). Fish were released at the capture location immediately after tissue collection. Populations in the Rio Hondo and Rio Ruidoso of the Pecos drainage are presumed extirpated (personal observations of the authors), thus the genetic structure within these watersheds could not be examined.

**Fig. 1** Sampling locations of 15 populations of *G. pandora* within watersheds *dark gray shaded areas*. Populations numbers, drainage areas, and watersheds are listed in Table 1
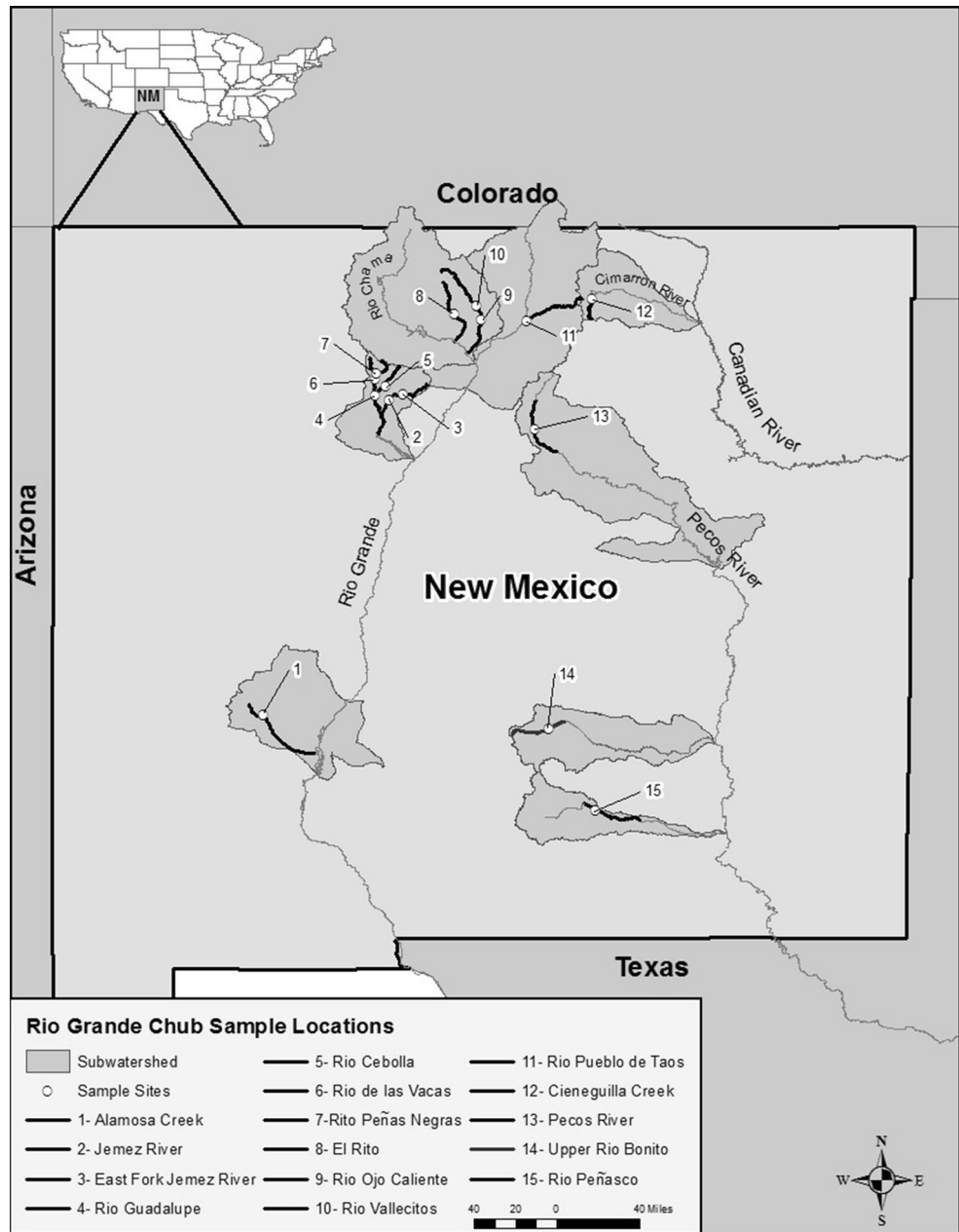


## DNA isolation and screening of microsatellite markers

Total genomic DNA was extracted from fin clips using a DNeasy Blood and Tissue Kit (Qiagen 2006). Eleven microsatellite loci were screened (Table S1) from microsatellites developed for four cyprinid species: bonytail (*Gila elegans*; Keeler-Foster et al. 2004), Sacramento splittail (*Pogonichthys macrolepidotus*, Baerwald and May 2004), Sacramento pikeminnow (*Ptychocheilus grandis*, Baerwald and May 2004), and tui chub (*G. bicolor*; Baerwald and May 2004). The mix for the polymerase chain reaction (PCR) consisted of 3.5 µl ddH$_2$O, 1× GeneAmp®10× PCR Buffer, 2.5 mM MgCl$_2$, 1.5 mM dNTPs, 0.875 U AmpliTaq Gold® DNA polymerase, 0.5 µl each of forward and reverse primers. All PCR primers were purchased from Applied Biosystems (Foster City, CA). Amplification protocol used a touchdown thermal profile (annealing temperature decreased by 0.2 °C at each cycle) on a GeneAmp PCR System 9700 thermal cycler. The profile started with a 9 min initial denaturing step at 95 °C (activation of AmpliTaq Gold® DNA polymerase), 33 cycles at 95 °C for 45 s, 56 °C for 1 min, and 72 °C for 1 min with a final extension of 30 min at 72 °C. PCR

**Table 1** *Gila pandora* sample collection including drainage, watershed, population, land ownership

| Drainage/watershed | (Map number) population | Land ownership | Total number | Collection date | UTM (E) | UTM (N) |
|---|---|---|---|---|---|---|
| Rio Grande | | | | | | |
|   Elephant Butte Reservoir | (1) Alamosa Creek | State | 30 | 2/14/2011 | 261152 | 3717396 |
|   Jemez River | (2) Jemez River | USFS-SFNF | 30 | 6/10/2010 | 347507 | 3961478 |
| | (3) East Fork Jemez River | USFS-SFNF | 30 | 8/18/2010 | 356527 | 3965771 |
| | (4) Rio Guadalupe | USFS-SFNF | 30 | 8/25/2010 | 338560 | 3965077 |
| | (5) Rio Cebolla | USFS-SFNF | 30 | 8/18/2010 | 345507 | 3971767 |
| | (6) Rio de las Vacas | USFS-SFNF | 30 | 7/15/2010 | 338464 | 3977630 |
| | (7) Rito Peñas Negras | USFS-SFNF | 30 | 7/15/2010 | 339664 | 3982014 |
|   Rio Chama | (8) El Rito | USFS-CNF | 30 | 5/31/2010 | 389424 | 4027618 |
| | (9) Rio Ojo Caliente | BLM | 30 | 9/20/2010 | 406630 | 4022484 |
| | (10) Rio Vallecitos | USFS-CNF | 30 | 9/19 2010 | 403895 | 4033935 |
|   Upper Rio Grande | (11) Rio Pueblo de Taos | BLM | 30 | 9/13/2010 | 434728 | 4021645 |
| Canadian | | | | | | |
|   Cimarron River | (12) Cieneguilla Creek | State | 30 | 5/23/2010 | 476304 | 4038428 |
| Pecos | | | | | | |
|   Pecos Headwaters | (13) Pecos River | State | 30 | 9/25/2010 | 439327 | 3937179 |
|   Rio Hondo | (14) Upper Rio Bonito | BLM | 30 | 10/11/2010 | 446819 | 3703745 |
|   Rio Peñasco | (15) Rio Peñasco | Private | 25 | 4/9/2011 | 477332 | 3640086 |

Total number of individuals collected, date collected, and UTM coordinates (Zone 13 N)

*USFS* United States Forest Service, *SFNF* Santa Fe National Forest, *CNF* Carson National Forest, *BLM* Bureau of Land Management, *State* State of New Mexico owned public land, *Private* privately owned land

products were processed using an ABI 3130xl genetic analyzer and GeneScan™ 500 LIZ® size standard (Applied Biosystems, Foster City, CA). GeneMapper 4.0 (Applied Biosystems, Foster City, CA) was used to score and edit genotypes.

## Statistical analysis

Departures from Hardy–Weinberg (HW) expectations and genotypic equilibrium were tested using GENEPOP'007 (Rousset 2008). Deviations from HW due to stuttering, null alleles, and large allele dropout were tested using MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004). Allelic richness ($A_R$) and inbreeding coefficient ($F_{IS}$) were calculated using FSTAT 2.9.3 (Goudet 2001). Allelic richness is the mean number of alleles per locus adjusted for sample size using rarefaction methods (Leberg 2002). Observed ($H_O$) and expected ($H_E$) heterozygosity, allele frequencies, and private alleles were estimated using GENALEX 6 (Peakall and Smouse 2006). Tests of both HW and genotypic disequilibrium were evaluated after sequential Bonferroni correction (Holm 1979; Rice 1989).

Partitioning of genetic variation among samples was assessed using a hierarchical analysis of molecular variance (AMOVA) in ARLEQUIN v3.5 (Excoffier et al. 1992, 2005) for three different levels (within populations, among populations within a basin, and among basins) using Weir and Cockerham (1984). Values for $F_{ST}$ were compared using Wright's guidelines (Wright 1978) and $F_{SC}$ was used to compare populations within a basin. A Bayesian clustering approach implemented in STRUCTURE v2.3.3 identified genetic clusters of *G. pandora* and assigned individuals to ancestry clusters (K) (Pritchard et al. 2000) using the admixture model. A 200,000 burn-in period was followed by 20 iterations of 500,000 Markov Chain Monte Carlo replicates per K (1–17). The maximum number of populations (K) was determined by using the largest ΔK value (Evanno et al. 2005) as implemented in STRUCTURE HARVESTER (Earl and von Holdt 2012). CLUMPAK (Kopelman et al. 2015) was used to create optimal alignment plots of the STRUCTURE results.

A Discriminate Analysis of Principal Components (DAPC) was performed to visualize genetic differentiation and the relationship among population using the R package (R DevelopmentCore Team 2015) adegenet (Jombart 2008; Jombart and Ahmed 2011). This multivariate approach does not require linkage or HW equilibrium assumptions about an underlying genetic model (Jombart et al. 2008). Populations for the analysis were determined by sampling location. Cross-validation was performed to determine the number of principal components to retain based on an accumulated variance explained by the eigenvalues of the

principal component analysis. A discriminant analysis of the retained principal components was then performed on the loadings to visualize between-group variance and within-group variance. In addition, the optimal number of genetic clusters that best described the data was explored using the function *find.clusters*. The function runs the K-means sequentially with increasing values of K and compares models using Bayesian Information Criterion and plots these against increasing values of K (see Fig. S1).

Estimation of effective numbers of breeders ($N_b$) utilized the single sample linkage-disequilibrium method proposed by Hill (1981). The modified method corrects for bias due to sample size (see England et al. 2006) and is implemented in the program NeESTIMATOR v2 (Do et al. 2014). Alleles with frequencies less than 0.02 ($P_{crit} = 0.02$) were removed when estimating $N_b$ (Waples and Do 2008; Do et al. 2014).

# Results

Eleven loci were used to genotype 445 individuals of *G. pandora* (Table S1). Analysis of HW demonstrated that four loci were statistically significant (*Gel*222, *Cyp*G26, *Cyp*G5, *Gel*257) indicating departure from HW due to the presence of null alleles; however, these four loci were not removed since HW departures were randomly distributed among populations indicating departures were at the population level and not at the locus level. A test for linkage disequilibrium revealed two loci (*Gel*323 and *Gel*257) deviated from random association. Results did not change after reanalyzing the data without *Gel*323 and *Gel*257; therefore, these loci were retained and 11 loci were used for final analyses.

For 11 loci, total number of alleles ($N_A$) per locus (Table S2) ranged from 2 to 27 across populations with an average number of alleles from as low as 7 in the Pecos drainage (Upper Rio Bonito) to as high as 16 in the Rio Grande drainage (Rio Ojo Caliente). Allelic richness was lower than the total number of alleles due to an adjustment for sample size from 30 to 25 individuals across all populations. Allelic richness was lowest in the Upper Rio Bonito ($A_R = 6.75$) within the Pecos drainage while the Rio Ojo Caliente within the Rio Grande drainage exhibited the highest allelic richness ($A_R = 15.09$). Observed heterozygosity varied from 0.708 (Upper Rio Bonito) to 0.872 (Rio Ojo Caliente) while $H_E$ varied from 0.747 (Upper Rio Bonito) to 0.857 (Rio Pueblo de Taos). The inbreeding coefficient within populations ($F_{IS}$) across all loci ranged from −0.033 (East Fork Jemez River) to 0.070 (Upper Rio Bonito) (Table S2).

The AMOVA revealed moderate genetic differentiation among all 15 populations of *G. pandora* ($F_{ST} = 0.080$, P < 0.001; Table 2). Genetic differences among drainage basins were low with an $F_{CT} = 0.021$ (P < 0.001; Table 2). However, the main source of variation was from individuals within populations (91.9 %) and not among populations within a basin (5.9 %) or among basins (2.1 %). Population pairwise $F_{ST}$ values varied from little genetic differentiation among populations within the Rio Grande drainage (Rio Guadalupe: Rio de las Vacas: Rito Peñas Negras) to high genetic differentiation between two populations in the Rio Grande drainage and Pecos drainage (Upper Rio Cebolla: Upper Rio Bonito, $F_{ST} = 0.171$, P < 0.001; Table 2). Overall, the Pecos River had the highest pairwise $F_{ST}$ values. The STRUCTURE analysis and optimal K suggested seven genetic clusters (Fig. 2). The Upper Rio Bonito displayed the least amount of admixture compared to all other populations within the Pecos drainage. Populations of *G. pandora* within the upper Rio Grande drainage (Rio Ojo Caliente, Rio Vallecitos, Rio Pueblo de Taos) had high levels of admixture with Q-values ranging from 0.30 to 0.50. In contrast, populations within the Pecos drainage (Pecos River and Upper Rio Bonito) had low levels of admixture (Q = 0.94 and 0.87, respectively; Fig. 2).

A total of 150 principal components and 14 discriminant analysis axes were retained In the DAPC analysis. The proportion of conserved variance was 96 %. Similar to the STRUCTURE results, the plot of Bayesian Information Criterion against K suggested 7–10 clusters would be most descriptive of the *G. pandora* data (Fig. S1). The majority of the clusters overlapped, including Alamosa Creek and El Rito, which indicates a genetic link between the northern and southern tributaries in the Rio Grande (Fig. 3a). Upper Rio Bonito did not overlap with other clusters. Likewise Upper Rio Cebolla was a distinct cluster, however, its elliptical space trended towards other clusters represented by sample locations within the Jemez River (Fig. 3a). When four clusters from the southern drainage basins (Alamosa Creek, Upper Rio Bonito, Rio Peñasco, Pecos River) were removed from the DAPC analysis, the final groupings are consistent with the STRUCTURE analysis (Fig. 3b).

Effective number of breeders ($N_b$) were lowest for *G. pandora* in Upper Rio Bonito ($N_b = 6.1$) and highest in Rio Peñasco ($N_b = 109.7$), both within the Pecos drainage (Table 3). Within the Rio Grande drainage, estimates of $N_b$ ranged from 19.1 (Rio Vallecitos) to 89.1 (Rio Pueblo de Taos). The upper bounds for confidence intervals were infinite in six of the 15 populations while all the lower bound estimates were positive.

**Table 2** Pairwise $F_{ST}$ values for 15 New Mexico populations of *G. pandora*

| Rio Grande | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | * | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 2 | 0.080 | * | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3 | 0.082 | 0.027 | * | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 4 | 0.076 | 0.026 | 0.040 | * | 0.0000 | 0.0991 | 0.7748 | 0.0000 |
| 5 | 0.104 | 0.063 | 0.073 | 0.068 | * | 0.0000 | 0.0000 | 0.0000 |
| 6 | 0.075 | 0.036 | 0.038 | **0.004** | 0.064 | * | 0.03604 | 0.0000 |
| 7 | 0.080 | 0.025 | 0.037 | **-0.002** | 0.065 | **0.007** | * | 0.0000 |
| 8 | 0.095 | 0.070 | 0.063 | 0.068 | 0.088 | 0.059 | 0.068 | * |
| 9 | 0.069 | 0.031 | 0.026 | 0.031 | 0.053 | 0.019 | 0.034 | 0.040 |
| 10 | 0.071 | 0.050 | 0.037 | 0.045 | 0.078 | 0.031 | 0.051 | 0.057 |
| 11 | 0.077 | 0.035 | 0.043 | 0.039 | 0.050 | 0.029 | 0.037 | 0.060 |
| 13 | 0.074 | 0.053 | 0.057 | 0.046 | 0.073 | 0.036 | 0.040 | 0.065 |
| 14 | 0.146 | 0.138 | 0.152 | 0.147 | 0.171 | 0.141 | 0.143 | 0.164 |
| 15 | 0.131 | 0.083 | 0.097 | 0.098 | 0.108 | 0.090 | 0.094 | 0.095 |
| 12 | 0.081 | 0.055 | 0.052 | 0.052 | 0.081 | 0.046 | 0.055 | 0.055 |

| Rio Grande | | | | Pecos | | | Canadian |
|---|---|---|---|---|---|---|---|
| | 9 | 10 | 11 | 13 | 14 | 15 | 12 |
| 1 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 2 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 4 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 5 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 6 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 7 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 8 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 9 | * | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 10 | 0.013 | * | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 11 | 0.029 | 0.041 | * | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 13 | 0.032 | 0.051 | 0.050 | * | 0.0000 | 0.0000 | 0.0000 |
| 14 | 0.127 | 0.156 | 0.145 | 0.099 | * | 0.0000 | 0.0000 |
| 15 | 0.058 | 0.100 | 0.077 | 0.070 | 0.165 | * | 0.0000 |
| 12 | 0.038 | 0.050 | 0.047 | 0.058 | 0.164 | 0.101 | * |

Bolded $F_{ST}$ values represent non-significant comparisons after Bonferroni corrections ($\alpha < 0.0004$). Rio Grande Drainage: Alamosa Creek (1); East Fork Jemez River (2); Jemez River (3); Rio Guadalupe (4); Rio Cebolla (5); Rio de las Vacas (6); Rito Peñas Negras (7); El Rito (8); Rio Ojo Caliente (9); Rio Vallecitos (10); Rio Pueblo de Taos (11). Canadian Drainage: Cieneguilla Creek (12). Pecos River Drainage: Pecos River (13); Upper Rio Bonito (14); Rio Peñasco (15). Numbers above diagonal are P values, and numbers below the diagonal are $F_{ST}$ values

## Discussion

Our analysis revealed seven genetic clusters of *G. pandora* throughout the 15 sampling localities in New Mexico. Three of these genetic clusters represent unique isolated populations of *G. pandora* in southern New Mexico (Alamosa Creek, Rio Bonito, Rio Peñasco). This genetic differentiation is most likely attributed to the combined effects of isolation during the last glacial period (Schmidly

1977) and recent fragmentation preventing gene flow (Turner et al. 2015). *Gila pandora* is a member of an assemblage of Rio Grande fishes (*O. c. virginalis* + *C. plebeius* + *G. pandora*) that likely established residence in the southern portion of the Rio Grande in the late Pleistocene during the last glacial period (Wisconsin) when the region was cooler and mesic (Schmidly 1977). An evaluation of distribution patterns of co-occurring species can assist with understanding genetic data (De Luca et al.
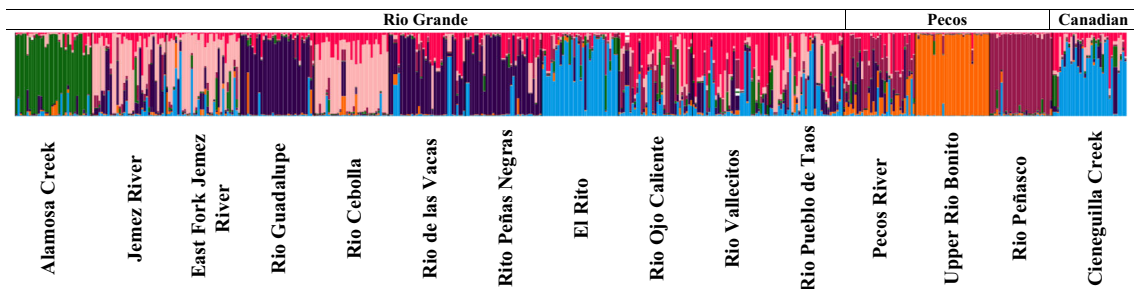
**Fig. 2** *CLUMPAK* plot of *STRUCTURE* assignment results (K = 7)

**Fig. 3** Discriminate analysis of principal components (DAPC) scatterplots of *G. pandora* microsatellite data showing the first two principal components of the DAPC. Sampling locality was used as a prior. *Dots* represent individual fish and *ellipses* represent different sampling localities. **a** Plot of all 15 sample locations. **b** Plot of the eleven northern sample locations with southern sample locations 1, 14, and 15 removed, and sample location 13 from the Pecos drainage removed. The number of each sample locality is referenced in Fig. 1
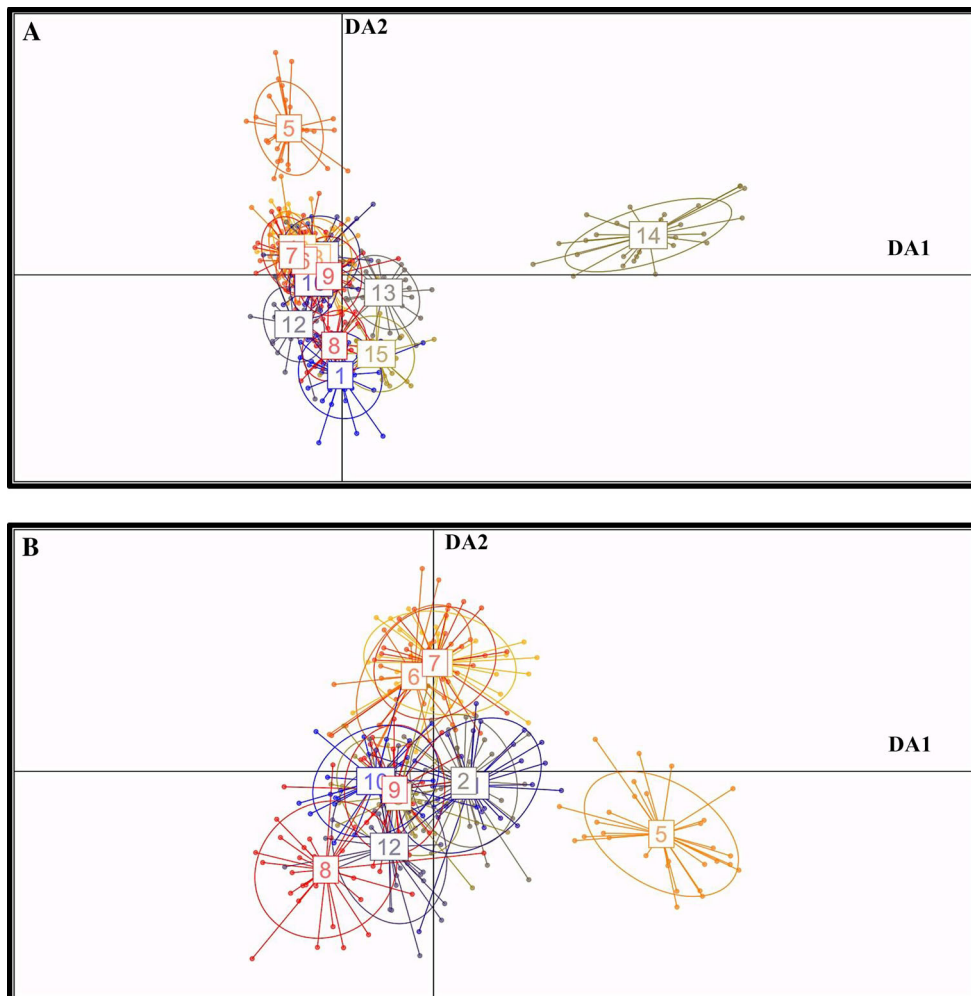


2014); likewise, just as multiple independent genetic markers can reinforce observed patterns, comparison of genetic information among co-occurring species can strengthen genetic patterns (Avise et al. 1987). To this end, *G. pandora* is presumed to be native to Alamosa Creek because native populations of *C. plebeius* and *O. c. virginalis* (Rio Grande native fish assemblage) co-occur throughout these southern tributaries in the Rio Grande drainage. Similarly, McPhee et al. (2008) found that *C.*

*plebeius* in Palomas Creek (an adjacent southwestern tributary to the lower Rio Grande) was genetically similar to *C. plebeius* populations throughout the northern Rio Grande drainage. In addition, *O. c. virginalis* was documented in a small tributary to the Rio Grande (Las Animas Creek) and represents the most southern distribution of the subspecies and that of all cutthroat trout (Behnke 1992). Thus, these documented historical distributions and genetic relatedness of the Rio Grande fish assemblage indicates a

**Table 3** Effective number of *G. pandora* as breeders ($N_b$)

| Drainage | Population | $N_b$ | 95 % CI |
|---|---|---|---|
| Rio Grande | Alamosa Creek | 37.1 | 15.8–428.1 |
| | East Fork Jemez River | 43.3 | 20.3–286.8 |
| | Jemez River | 41.5 | 20.4–183.0 |
| | Rio Guadalupe | 63.1 | 24.9–∞ |
| | Rio Cebolla | 34.5 | 17.0–135.1 |
| | Rio de las Vacas | 42.1 | 21.0–177.7 |
| | Rito Peñas Negras | 50.8 | 21.7–3003.9 |
| | El Rito | 73.3 | 30.0–∞ |
| | Rio Ojo Caliente | 65.3 | 27.7–∞ |
| | Rio Vallecitos | 19.1 | 11.5–36.4 |
| | Rio Pueblo de Taos | 89.1 | 31.9–∞ |
| Canadian | Cieneguilla Creek | 49.8 | 19.5–∞ |
| Pecos | Pecos River | 64.7 | 29.5–1251.2 |
| | Upper Rio Bonito | 6.1 | 3.1–9.8 |
| | Rio Peñasco | 109.7 | 42.0–∞ |
| | Harmonic Mean | 32.4 | |

Estimates were calculated using the linkage-disequilibrium method as implemented in NeEstimator2 ($P_{CRIT} = 0.02$), 95 % CI from the Jackknife method

∞ represents infinity

historical connection between the southern and northern *G. pandora* populations.

*Gila pandora* is presumably native to the southern Pecos tributaries. Sublette (1975) described *G. pandora* in the Rio Ruidoso, Rio Felix, and Rio Peñasco (see map in Minckley 1980). The species presumably established populations throughout southern New Mexico during the Wisconsin. As the region became warmer and drier, *G. pandora* was relegated to the cooler tributaries of the Rio Hondo, Rio Bonito, and Rio Peñasco within the Pecos drainage. Although data are not available from McPhee et al. (2008) for cross species comparison, Sublette (1975) documented *C. plebeius* in the Rio Bonito and Behnke (1992) documented a resident population of *O. c. virginalis* in the Rio Bonito. *Gila pandora* in the Rio Bonito represents a unique population with low genetic diversity, a single private allele, a critically low estimate of effective number of breeders, and few low-frequency alleles. The reduced genetic diversity of the population in the Rio Bonito is likely due to drought and multiple water diversions that began in the early twentieth century. A major water diversion was created with the installation of a dam to form Bonito Lake in 1931 that was used by the Southern Pacific Railroad for steam engines (URH-WRAS 2004). Presumably, this diversion prevented movement of *G. pandora* among the tributaries and thereby increasing loss of genetic diversity through genetic drift (Haag et al. 2010).

Unlike the southern-most populations of *G. pandora*, population structure throughout the northern Rio Grande drainage (Jemez River, Rio Chama, Upper Rio Grande) revealed gene flow. Within the Jemez River watershed, populations from Rio Guadalupe, Rio de las Vacas, and Rito Peñas Negras exhibited very low $F_{ST}$ values indicating connectivity and gene flow. *Gila pandora* from the Rio Cebolla, which flows into the Rio Guadalupe just below the confluence of the Rio de las Vacas and Rio Guadalupe, were genetically similar to both the Jemez River and East Fork Jemez River but not the Rio Guadalupe. We surmise that *G. pandora* reached the Rio Cebolla via stream capture or physical translocation from the Jemez River. The scenario of stream capture is plausible given that the upper portion of Rio Cebolla (ca. 2582 m elevation) runs parallel to San Antonio Creek (ca. 2552 m elevation) which is a tributary to the Jemez River. San Antonio Creek was once a lake (40 km$^2$) until the late Pleistocene. The Rio Cebolla population may have originated from San Antonio lake which had lacustrine deposits as high as 2650 m elevation and post-lake stream terrace at least 2700 m in elevation (Reneau et al. 2007). Thus, the low allelic diversity of the Rio Cebolla population could be linked to a founder event from stream capture between San Antonio Creek and the Rio Cebolla. If the Rio Cebolla population originated from stream capture, a biological or environmental barrier to fish movement into the Rio Guadalupe would have prevented gene flow between the two populations (gene flow between these two populations was not evident). Low allelic diversity of the Rio Cebolla population may be exacerbated by contemporary habitat loss because the stream currently contains little pool habitat and overall poor water quality (Simino and Bassett 2003).

*Gila pandora* populations within the Rio Chama and upper Rio Grande watersheds exhibited the overall greatest genetic diversity. With the exception of El Rito, tributaries within these watersheds were not dominated by a single genetic cluster. Rio Ojo Caliente, Rio Vallecitos, and Rio Pueblo de Taos contained ancestry from multiple genetic clusters indicating minimal drift and lineage sorting. With the exception of Rio Vallecitos ($N_b = 19.1$), estimated effective number of breeders for the two most northern watersheds were the highest ($N_b$ range 65.3–89.1). The low $N_b$ of Rio Vallecitos caused an increase in linkage disequilibrium as four locus pairs were out of equilibrium. These same loci were in equilibrium in Rio Chama and other upper Rio Grande populations. However, *G. pandora* in the Rio Vallecitos, tributary to the Rio Chama, may have linkage disequilibrium due to a bottleneck. The Rio Vallecitos is impaired due to high turbidity, high levels of aluminum, and high temperatures (RC-WRAS 2005). In contrast, neither Rio Ojo Caliente nor El Rito experience water quality impairment.

*Gila pandora* from Cieneguilla Creek, a tributary within the Canadian drainage basin in eastern New Mexico,

shared a unique genetic cluster with the population in El Rito (a tributary in the upper Rio Grande basin). Historical accounts of *G. pandora* in the Canadian River basin are few and often lack details. Sublette (1975) reported *G. pandora* in the Mora River, a tributary of the Canadian River; however, collection details were not provided. Minckley (1980) and Rees and Miller (2005) indicated that *G. pandora* may have been introduced into tributaries of the Canadian River, but again, details or citations were not provided. Evidence of distributions from the other two species of the native fish assemblage (*O. c. virginalis* and *C. plebeius*) is either conflicting or lacking (Pritchard et al. 2008).

The effective number of breeders ($N_b$) estimates for *G. pandora* were low, but in some cases similar to *G. nigra* (Headwater Chub; $N_{eD} = 61.0–112.0$) from the Gila River in New Mexico (Pilger et al. 2015) and *G. nigrescens* (Chihuahua Chub; $N_{eD} = 54.4–226.0$) from the Mimbres River, also in New Mexico (Osborne et al. 2012). In species with overlapping generations, these raw $N_b$ estimates are likely biased downward due to the Wahlund effect with the magnitude of the bias affected by the number of cohorts within the sample (Waples 2005; Waples et al. 2014; see also Robinson and Moyer 2013). This bias could be corrected and used to estimate generational effective size ($N_e$) using several life history traits including adult lifespan and age at maturity (Waples et al. 2014); however, values of these traits are unknown for *G. pandora*. Relating the current $N_b$ estimates to the $N_e$ 50–500 rule (see Frankham et al. 2013; Jamieson and Allendorf 2012, 2013) is unwarranted at this time because $N_b \neq N_e$ (see Waples et al. 2014). That being said, an $N_b$ of 6.1 (Upper Rio Bonito) is likely below an $N_e = 50$. This population is in danger of extirpation due to increased inbreeding (Palstra and Ruzzante 2008).

## Conservation implications

Habitat loss was listed as the leading cause of decline in *G. pandora* (WildEarth Guardians 2013). Habitat loss can fragment and thus reduce genetic exchange among populations as well as increase susceptibility to extirpation from catastrophic events. We characterized genetic variation and population structure throughout the extant range of *G. pandora* in New Mexico to aid as reference points in conservation planning and recovery. We recommend incorporating genetic monitoring into conservation planning of *G. pandora* to determine how physical barriers of connectivity as well as biological barriers of non-native fishes affect genetic variation and number of breeders. Conservation planning should also take into consideration the impact that a changing climate will have on biological diversity (Comte et al. 2013). For example, Zeigler et al.

(2012) reported an increase in air temperature (0.29 °C decade$^{-1}$), earlier onset of snowmelt (2.3 days decade$^{-1}$), and a decrease in snow-water equivalent (5.3 % decade$^{-1}$) throughout southern Colorado and New Mexico. Not only will these climate patterns affect the distribution and continued persistence of cool-water fishes such as *G. pandora*, but also such conditions increase the frequency and intensity of wildfires (Rieman and Isaak 2010). An example was the 2012 Whitewater-Baldy fire in the Gila National Forest of New Mexico where approximately 121,410 ha burned (Tillery et al. 2012). The fire eliminated several populations of the federally endangered Gila trout (*O. gilae*). Prior knowledge of genetic diversity resulted in a multi-agency response to rescue relict populations for preservation of extant genetic diversity. In the event that populations of *G. pandora* are subject to extirpation, then populations can be targeted for translocations to preserve genetic diversity. Proactive planning such as this would guide rescue and conservation efforts of *G. pandora* to ensure continued persistence in an arid landscape.

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