Genotyping-by-sequencing reveals the effects of riverscape, climate and interspecific introgression on the genetic diversity and local adaptation of the endangered Mexican golden trout (Oncorhynchus chrysogaster)

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Abstract

How environmental and anthropogenic factors influence genetic variation and local adaptation is a central issue in evolutionary biology. The Mexican golden trout (*Oncorhynchus chrysogaster*), one of the southernmost native salmonid species in the world, is susceptible to climate change, habitat perturbations and the competition and hybridization with exotic rainbow trout (*O. mykiss*). The present study aimed for the first time to use genotyping-by-sequencing to explore the effect of genetic hybridization with *O. mykiss* and of riverscape and climatic variables on the genetic variation among *O. chrysogaster* populations. Genotyping-by-sequencing (GBS) was applied to generate 9767 single nucleotide polymorphisms (SNPs), genotyping 272 *O. chrysogaster* and *O. mykiss*. Population genomics analyses were combined with landscape ecology approaches into a riverine context (riverscape genetics). The clustering analyses detected seven different genetic groups (six for *O. chrysogater* and one for aquaculture *O. mykiss*) and a small amount of admixture between aquaculture and native trout with only two native genetic clusters showing exotic introgression. Latitude and precipitation of the driest month had a significant negative effect on genetic diversity and evidence of isolation by river resistance was detected, suggesting that the landscape heterogeneity was preventing trout dispersal, both for native and exotic individuals. Moreover, several outlier SNPs were identified as potentially implicated in local adaptation to local hydroclimatic variables. Overall, this study suggests that *O. chrysogater* may require conservation planning given i) exotic introgression from *O. mykiss* locally threatening *O. chrysogater* genetic integrity, and ii) putative local adaptation but low genetic diversity and hence probably reduced evolutionary potential especially in a climate change context.

Keywords: alien species, climate change, conservation, landscape genomics, riverscape genetics, river, salmonid, Sierra Madre Occidental.

1. INTRODUCTION

Natural and anthropogenic factors influence micro-evolutionary processes (i.e. migration, drift, and selection) generating spatial genetic patterns in wild populations. Disentangling the respective roles of environmental and anthropogenic variables in spatial genetic patterns has therefore become a central issue in evolutionary biology and conservation ecology (Baguette et al. 2013; Richardson et al. 2016).

Landscape genetics, and more specifically riverscape genetics (combination of population genetics with landscape ecology into a riverine context), can improve our understanding of the effects that riverine landscapes (riverscapes) and anthropogenic activities (e.g. aquaculture with exotic species) have on the microevolutionary

processes determining the spatial genetic structure and genetic diversity of populations, particularly in lotic environments (Manel and Holderegger 2013; Davis et al. 2018; Grummer et al. 2019). Microevolutionary processes are influenced by different alterations such as landscape fragmentation and water flow changes, which usually reduce habitat size, split environments and create barriers that isolate fish populations (Morita and Yamamoto 2002; Le Pichon et al. 2006). Gene flow and selection act on different ways (i.e. among basins, and between distant or nearby sites within basins). Therefore, it is necessary to account for those differences to analyse riverine environments, particularly in complex hydrological networks where the physical landscape structure, along with the reduced pathways, determines patterns of genetic variation (Chaput-Bardy et al. 2008; Kanno et al. 2011).

Both neutral and adaptive components of genetic diversity are the raw materials of evolution and should be considered in conservation studies (Kokko et al. 2017; Tikochinski et al. 2018). However, most of the already published riverscape genetics studies used only a small number of neutral molecular markers. Lately, some studies started to include large Next Generation Sequencing (NGS) datasets to assess gene-environment interactions (Bourret et al. 2013; Hecht et al. 2015; Brauer et al. 2016, 2018; Hand et al. 2016; Amish et al. 2019). The use of NGS datasets might be helpful for the genetic monitoring of endangered populations, as they provide information of local adaptation processes that is valuable for recovery programmes which could be apply in riverine environments (Faulks et al. 2017; Kleinman-Ruiz et al. 2017; Hunter et al. 2018; Fan et al. 2018).

For riverine species the introduction of exotic species for recreational and aquaculture purposes, might have a detrimental effect on the native populations due to predation, competition, the introduction of diseases and genetic introgression (Perrier et al. 2011; Penaluna et al. 2016). When the exotic species is phylogenetically close to the native species it can cause genetic introgression, which in turn can have extremely harmful effects such as homogenizing biodiversity (Allendorf et al. 2001). Moreover, the modification of the native genetic pool might result in the loss of selective values (fitness) affecting survival skills in changing environments (Milián-García et al. 2015). Recent climatic variations have been associated with the spread of genetic introgression in riverine habitats, leading into irreversible evolutionary consequences for endangered species. The negative impact of genetic introgression in reverine habitats have been widely documented in salmonids due to the extensive use of exotic species for aquaculture (Perrier et al. 2013; Muhlfeld et al. 2017).

Climate conditions such as temperature and precipitation might be drivers of adaptive processes in different salmonid species because they are associated with migration, reproduction, and mortality. In this sense, upstream salmonids are extremely vulnerable to the detrimental effects arising from climate change, as they have

a much smaller habitat than marine and terrestrial environments (Parmesan 2006; Muhlfeld et al. 2017); but also, because of their specific habitat requirements (Ruiz-Luna et al. 2017).

Such is the case of the Mexican golden trout (MGT, *Oncorhynchus chrysogaster*; Needham and Gard, 1964), one of the southernmost native salmonid species, inhabiting three different basins (Río Fuerte, Río Sinaloa, and Río Culiacán) in the highest parts of the Sierra Madre Occidental (SMO) mountains in north-western Mexico (Hendrickson et al. 2002). The most broadly accepted hypothesis to explain the origin of this species is the colonization process which occurred at the end of the Pleistocene (~ 12 000 years ago), when a group of steelhead trout migrated from the Gulf of California to the upper parts of SMO rivers (Behnke et al. 2002). Subsequently, these trout were isolated in different streams and basins experiencing genetic drift, reduced gene flow, and potentially specific selective pressure (García-De León et al. 2020). Like all salmonids, this species is susceptible to climate change, habitat perturbations, and the introduction of non-native species (i.e. rainbow trout for aquaculture purposes; Hendrickson et al. 2006). *O. chrysogaster* is an endangered species listed in the Mexican official norm of threatened species (NOM 059) and the International Union for Conservation of Nature (IUCN) red list. Many aspects of *O. chrysogaster*'s biology including its foraging habits, homing and reproductive behaviour, and dispersal are still unknown (but see Ruiz-Luna and García-De León, 2016). Biological studies of this species have mainly been limited due to the difficulty accessing its habitat because of both the lack of road infrastructure, and the prevalence of drug trafficking. Existing genetic studies of this species have suggested that *O. chrysogaster* is genetically structured by hydrological basins (e.g.; Camarena-Rosales et al. 2008; Escalante et al. 2014) and a recent works described up to four distinct genetic clusters (Escalante et al. 2016; García-De León et al. 2020). However, these studies were conducted with neutral markers, a small number of sample sites, and sample designs that were not adapted to account for riverscape complexity. This means that microevolutionary processes and their association with the adjacent riverscape are not yet properly understood, and consequently there is a lack of management plans to protect this endangered species.

Some studies for this species have revealed a genetic substructure and introgression processes in locations near aquaculture facilities (Escalante et al. 2014; Abadía-Cardoso et al. 2015). Moreover, a recent simulation study of *O. chrysogaster* suggested that the riverscape acts as a barrier against exotic introgression and that riverscape resistance (the combined effect of riverine distance, topographic slope, altitude gradient, stream order changes and temperature increase) is the main factor fragmenting populations within basins (Escalante et al. 2018). Strong spatial genetic structure and local adaptation processes in *O. chrysogaster* arising from riverscape factors are

89 therefore to be expected, with the consequence that this complex riverscape will prevent introgression processes
90 in populations not in proximity to aquaculture farms.

91 This study focuses on the effect of anthropogenic factors as well as riverscape and climatic variables on
92 the genetic diversity, population structure and local adaptation of the endangered endemic salmonid *O.*
93 *chrysogaster* in order to obtain technical data for developing management and conservation strategies.
94 Specifically, it addresses the following questions: 1) What is the impact of *O. mykiss* aquaculture escapes on the
95 genetic structure of the endemic *O. chrysogaster*? 2) Which riverscape features (i.e. hydroclimatic and topographic
96 variables) affect the extent and distribution of genetic diversity? 3) Are there genomic footprints of local adaptation
97 to heterogeneous hydroclimatic features? In order to answer these questions, this study explored the genetic
98 structure of *O. chrysogaster* populations and aquaculture trout (237 wild trout, 7 aquaculture *O. chrysogaster*, 24
99 aquaculture *O. mykiss*, and 4 lab hybrids), collected from 28 sites in three basins in north-western Mexico or
100 provided by federal agencies and genotyped for 9767 Single Nucleotide Polymorphisms (SNPs). The results were
101 discussed in the context of conservation strategies for endangered riverine species, considering both ecological
102 and evolutionary criteria.
103

104 ## 2. MATERIALS AND METHODS

105 ### 2.1. Study system and data collection

106 The survey was conducted in the Río Fuerte, Río Sinaloa, and Río Culiacán basins, at altitudes ranging from 1 965
107 to 2 730 m (Fig. 1). The study area covers approximately 27 500 km^2 with an estimated length of the river network
108 of 12,700 km. This zone has a complex topography and hydrology with sharp slopes and stream orders mostly
109 corresponding to 1, 2 and 3. The annual mean temperature and annual mean precipitation for the zone are 14.4 °C
110 and 911 mm respectively (Escalante et al. 2018). The heterogeneous riverscape of this area offers a diversity of
111 terrestrial and aquatic habitats providing high endemism and biodiversity, with *O. chrysogaster* as one of the main
112 aquatic predators (Hendrickson et al. 2006). For these river basins the dams are present downstream at altitudes of
113 less than 500 meters, and at altitudes above 1000 meters there is no evidence of other anthropogenic undertakings,
114 except for some rural activities (e.g. aquaculture and artisanal agriculture; author's field observations). However,
115 the introduction of rainbow trout for aquaculture purposes has been reported since the 1860s, and was strongly
116 supported by federal agencies during the 1980s and 1990s (Escalante et al. 2014).

117 Six variables characterizing the riverscape and with a strong influence on *O. chrysogaster* occurrence and
118 genetic divergence were obtained from a previous study (Escalante et al. 2018): precipitation of the driest month,

119 temperature of the warmest quarter, river length (distance from the lowermost to the uppermost part of the river
120 above 1 500 m), slope, altitude, and stream order. They were generated and/or processed from data available on
121 the worldclim (http://www.worldclim.org/) and the Japanese Space System
122 (http://www.jspacesystems.or.jp/ersdac/GDEM/E/4.html) websites with 1 km and 30 meter resolution,
123 respectively. Worldclim variables were geometrically corrected to 30 meter resolution (for further details see
124 Escalante et al. 2018). The effect of latitude and longitude, recorded using GPS, was also tested.

125 Wild trout were sampled by electrofishing at 25 sample sites in the Río Fuerte (10 sites), Río Sinaloa (10
126 sites), and Río Culiacán (5 sites) basins during the winter and spring seasons of 2013, 2014, and 2015 (collection
127 permit numbers SGPA/DGVS/02485/13 and SGPA/DGVS/05052/15). The sampling was conducted from both
128 the core and edges of the *O. chrysogaster* ecological niche defined in a previous study (Escalante et al. 2018), and
129 at multiple locations with high environmental heterogeneity (topographical and hydrological divergence) avoiding
130 spatial autocorrelation (Fig. 1). Rainbow trout samples were obtained from two aquaculture farms in the Río
131 Sinaloa basin. Additionally, seven farmed *O. chrysogaster* samples and four lab hybrids from *O. chrysogaster* and
132 *O. mykiss* from Guachochi Aquculture Center in Maderas Mexico donated by the Mexican Institute of Fisheries
133 (INAPESCA), were included in the study for further genotyping. Small portions of tissue (either fin or muscle)
134 were clipped from these samples and preserved in 95% ethanol for later analysis.
135

136 **2.2. Genotyping by sequencing**

137 Genomic DNA was extracted for each individual following the Qiagen DNeasy Blood & Tissue Kit protocol
138 (Qiagen, Hilden, Germany, http://www1.qiagen.com). DNA quality was checked using agarose gel electrophoresis
139 and quantified using a NanoDrop spectrophotometer (Thermo Scientific) and the Quant-iT PicoGreen dsDNA
140 Assay Kit (Invitrogen). DNA libraries were then generated with GBS methods (Elshire et al. 2011) at Cornell
141 University in Ithaca, New York, using the ECOT221 enzyme. Finally, single-read 100-bp sequencing was
142 performed on an Illumina HiSeq2500.

143 The quality of the raw sequences was controlled via investigating per base averaged quality, presence of
144 adapters and over-represented sequences were detected using FastQC (Andrews 2010). Reads were processed with
145 Cutadapt (Martin 2011) to remove potential fragments of Illumina adapters, allowing a 10% mismatch rate in the
146 adapter sequence. During this process, reads were trimmed to 70 bp. The bioinformatics software/pipeline Stacks
147 1.32 (Catchen et al. 2011; Catchen 2013) was hence used to demultiplex reads, identify restriction site-associated
148 DNA (RAD) loci and call SNPs. Reads were demultiplexed and trimmed to 64 bp using the process_radtags

module, with which, one potential mismatch in the barcode sequence was allowed. The ustacks module was used, with a minimum stack depth of 4x and a maximum distance allowed between stacks of 4 (6 for secondary reads). The catalogue of loci was built using the cstacks module with n = 4. With the sstacks module, samples were matched against the catalogue of loci. Finally, individuals were genotyped using the population module, with at least 70% of individuals being genotyped, and a minimum read depth of 5x for each locus and individual. Genotypes were exported in VCF format for further filtering. The SNP dataset was filtered using VCFtools (Danecek et al. 2011), achieving a minimum average read depth ranging from 8x to 40x across genotypes and a minor allele frequency of 1%, to limit any potential low quality data or paralogous loci. Based on three populations with large sampling sizes that were exempt of stocking (FVE, FLQ and SBA) a blacklist of loci deviating from Hardy-Weinberg equilibrium (HWE, p-value ≤ 0.05) was constituted. Thus, loci deviating from HWE in at least one of the three populations considered were removed.

Subsequently, five datasets were created including all loci after SNP calling at four different spatial scales: Dataset A for population genetics analyses including all the genotyped individuals; Dataset B for gene-environment associations (landscape genomics analyses) across the entire study area without significantly introgressed native trout (individuals with ancestry coefficients $\geq 20\%$ from aquaculture were removed); Dataset C for landscape genetics analyses with spatially continuous native populations (removing the isolated populations in the south of Río Culiacán to prevent biases in the landscape genetics analyses) without significant introgression; Dataset D for landscape genetics analyses of native trout without significant introgression in Río Fuerte (removing sampling sites isolated by long riverine distance at the east of Río Fuerte); and Dataset E for landscape genetics analyses of native trout without significant introgression in Río Sinaloa. Further information about the datasets is included in Online Resource 1.

2.3. Population genomics analyses

The genetic diversity of trout (Dataset A) was estimated from expected heterozygosity (H_E), and observed heterozygosity (H_O) using the adegenet R package (Jombart, 2008). Effective population size (N_E) was calculated by applying a molecular co-ancestry method (Nomura 2008) implemented in NeEstimator (Do et al. 2014).

The genetic differentiation between all sample sites (Dataset A) was assessed considering three different approaches. Firstly, pairwise genetic differentiation coefficients (F_{ST}) were calculated between all sample sites in Genodive 3.0 (Meirmans and Van Tienderen 2004); this method uses an analysis of molecular variance, performed between each population pair. Secondly, a phenogram was built in adegenet from all individuals using the

neighbour-joining algorithm (Saitou and Nei 1987) based on Nei's genetic distance (Tamura and Nei 1993). Bootstrap confidence intervals were estimated from 10 000 permutations.

Thirdly, an unsupervised Bayesian clustering approach implemented in the package fastStructure (Raj et al. 2014) was used to assess the genetic structure of *O. chrysogaster* and genetic admixture with aquaculture trout. This approach infers population genetic structure for a large number of SNP datasets without assuming predefined populations. Thus, fastStructure was run using Dataset A including all SNPs from all the genotyped individuals and Dataset B including all SNPs from individuals without significant exotic introgression. Based on an approximation of the number of sample sites (total number of sampling sites + 1), $K = 30$ was considered the maximum value to avoid underestimating the number of clusters.

2.4. Riverscape genetics analyses

Only Dataset C was considered when exploring the riverscape's influence on genetic diversity, due to the reduced number of sampling sites for the datasets at intra-basin scale (i.e. datasets D and E). Initially, the collinearity between seven predictors was tested using the R package corrplot (Taiyun Wei 2017): latitude, longitude, precipitation of the driest month, temperature of the warmest quarter, river length, altitude, and stream order. Afterwards, to test the effect of both climate and geographical distance, only latitude and precipitation of the driest month were retained because of their low collinearity (-0.48). Finally, the effect of latitude and precipitation of the driest month on expected heterozygosity was tested by a generalized linear model using the GLM function in R (Team R. Core 2018).

In order to examine the effect of riverscape on genetic differentiation, a resistance surface was firstly derived from four riverscape features using ArcGIS v10.2 (ESRI, 2013). We chose to adopt a resistance surface approach accounting for the combined effect of riverine distance, slope, altitude gradient, stream order changes and temperature increase as it was suggested to be a determinant factor driving *O. chrysogaster* genetic structure in a recently published simulation study (Escalante et al. 2018). A single point based environmental analysis is not able to quantify such physical corridors or boundaries to gene flow (Cushman and Landguth 2010; Landguth et al. 2012, 2016; Milanesi et al. 2017; Grummer et al. 2019). This surface was defined using the temperature of the warmest quarter, slope, stream order, and altitude raster files. Values were assigned to the pixels at each raster representing the extent to which movement is obstructed. The same criteria as those reported by Escalante et al. (2018) were considered, as the species and the study area fit with the current study. Then, resistance values from 0 to 10 were assigned to the raster pixels (30 m resolution) for each variable independently (see Online Resource

209 2). A riverscape resistance raster was generated by averaging the resistance values for the four environmental
210 variables at each pixel. For subsequent analyses in the gdistance package (van Etten, 2012), resistance values were
211 rescaled from 1 to 2, with 1 representing an absence of riverscape resistance and 2 maximum resistance. Further
212 information about the parameterisation is included in Online Resource 2.

213 The gdistance R package (van Etten 2012) was then used to calculate linear distance and riverscape
214 resistance matrices among sample sites within basins. This method simulates potential species movement in a
215 spatially structured landscape, linking different dispersal functions and connectivity thresholds using Djikstra's
216 shortest path algorithm (Dijkstra 1959). Two matrices were generated under the hypothesis of isolation by
217 Riverscape Resistance (RR) at intra-basin scale: RR for Río Fuerte populations (Matrix I) and RR for Río Sinaloa
218 populations (Matrix II). Additionally, two linear distance matrices were generated for the Río Fuerte populations
219 (Matrix III) and Río Sinaloa populations (Matrix IV) in order to control differences in distance in subsequent
220 analyses (for further details, see Online Resource 2). To define the influence of isolation by riverscape resistance
221 on genetic distances, Partial Mantel tests were applied using the PASSaGE package (Rosenberg and Anderson
222 2011). Partial Mantel tests were performed between the regression of $F_{ST}/(1-F_{ST})$ of Dataset D (Río Fuerte) and
223 Dataset E (Río Sinaloa) and corresponding riverscape resistance matrices at intra-basin scale (either Matrix I,
224 either Matrix II), using geographical linear distance as constant matrices (either Matrix III, either Matrix IV)
225 allowing the control for differences in distance (Online Resource 2). All tests were performed on under 10 000
226 permutations and assuming no correlation.
227
228 **2.5. Detection of SNPs under divergent selection**
229 Dataset B was analysed to detect *O. chrysogaster* SNPs potentially under selection using three different software
230 programs and two different approaches: a population (i.e. sampled sites) outlier detection approach (1) and
231 association tests between genotypes and continuous climatic variables (i.e. riverscape adaptive genomics) (2).

232 Firstly, the PCAdapt R package (Luu et al. 2016) was used to detect SNPs potentially under divergent
233 selection with approach 1. This method combines principal component analysis and Mahalanobis distances and
234 assumes that molecular markers excessively associated with population structure are candidates for local
235 adaptation. Based on the vector of z-scores, loci which do not follow the distribution of the main cluster of points
236 are considered outliers. The analysis was run with a threshold of 10% and K=6 based on fastStructure results
237 (observed spatial genetic structure).

For approach 2, two gene-environment association software programs were used to test the effect of temperature of the warmest quarter and precipitation of the driest month. These variables had previously been suggested as significant adaptation drivers in salmonids (Hecht et al. 2015; Hand et al. 2016). Using mixed models, both methods detect outlier loci through allele frequencies exhibiting strong statistical correlations with environmental variables. Initially, Bayenv2 was applied (Günther and Coop 2013), using an average of five independent runs (100 000 iterations). Also, the latent factor mixed models (LFMM) algorithm implemented in the R package LEA (Frichot et al. 2013; Frichot and François 2015) was run with five repetitions, 10 000 cycles, 5 000 burn-in, and K=6 (based on fastStructure outputs of the spatial genetic structure) as a random factor in the regression analysis. For both LFFM and Bayenv2, a threshold of 1% of total SNPs was defined to select the outlying SNPs with the highest posterior probabilities.

A gene ontology analysis was conducted on the nucleotide sequences (64 bp) containing all SNPs obtained from *O. chrysogaster*, using Dataset B. This analysis is based on a BLAST query (blastn) of the sequences against peptides from the rainbow trout genome database (Berthelot et al. 2014). Functional categorization by gene ontology terms (GO; http://www.geneontology.org) was carried out using Blast2GO software (version 4.1, http://www.blast2go.com/). Subsequently, protein annotations were filtered, retaining those from loci detected to be under divergent selection with an e-value cutoff of $\leq 10^{-6}$.

3. RESULTS

3.1. Genotyping by sequencing

The total number of raw sequences obtained was 722 836 026 with an average of 1 300 000 reads per individual. A genotyping rate of 82% was obtained for the total number of individuals sampled per population. After SNP calling, a total of 270 individuals (Table 1) and 9767 SNPs were retained for subsequent analysis.

3.2. Population genomics analyses

The highest H_O values were observed in *O. chrysogaster* and rainbow trout lab hybrids (0.22) whereas wild *O. chrysogaster* collected at CED, CER, and CER2 in Río Culiacán obtained the lowest scores (0.01). For H_E, the highest scores (0.16) were found in aquaculture trout collected at AQEB in Río Sinaloa, while the lowest values (0.01) were observed at CED, CER, and CER2 in Río Culiacán (Table 1). Focusing on wild *O. chrysogaster* only, the highest heterozygosity (both H_E and H_O) was found in the centre of the study area where the three basins meet

(Fig. 2). N_E were successfully estimated for 19 sample sites; the highest value (145.7) was found at SBA and the lowest (0.4) at SMA, both in Río Sinaloa (Table 1).

At the sampling sites, pairwise F_{ST} was highest (0.964) at the SPO and CED sample sites, in two different basins (Río Culiacán and Río Sinaloa), while the lowest (0.008) was between CER and CER2 (both in Río Culiacán). In terms of the basins, the highest F_{ST} averages (0.83) were observed between Río Fuerte and Río Culiacán (for further information, see Online Resource 3). The high F_{ST} values in some populations reflect high spatial genetic structure and are normally observed in trout inhabiting heterogeneous landscapes with rugged topography and complex hydrology (e.g. Abadía-Cardoso et al. 2015) or in small isolated populations (Perrier et al. 2017).

The Nei phenogram detected 27 well-supported clades (>97 % of bootstrapping value), all made up of geographically close sampling sites. Interestingly, the aquaculture trout (AQEB and AQSM) showed greater genetic similarity (given its lower branch length) with the lab hybrids (H, clearly a heterogeneous group, as it is known that hybrids have been artificially obtained). Furthermore, the trout collected in SSM falls within the aquaculture clade, suggesting that they are rainbow trout escaped from the farming facilities (see below, Fig. 3).

The unsupervised Bayesian clustering approach (fastStructure) identified six distinct wild genetic clusters defined by geography, and one aquaculture cluster: I. Eastern Fuerte; II. Central Fuerte; III. Southern Fuerte, Eastern Sinaloa, and Northern Culiacán; IV. Western Fuerte and Western Sinaloa; V. Central Sinaloa; VI. Southern Culiacán; and VII. Aquaculture (Fig. 3b and 4). Trout from Western Fuerte collected at FEM, FLC, and FLT showed admixture with trout from Central Sinaloa. Trout from Central Sinaloa collected at SBA showed genetic admixture with trout from Western Fuerte, while the trout collected at SHO and one individual from SLO showed admixture with aquaculture trout. Trout from Eastern Sinaloa collected at SCE, SCS, and SPE exhibited genetic admixture with Western Fuerte. Some trout from Southern Culiacán collected at CSJN exhibited genetic admixture with aquaculture trout, and one individual belonged entirely to the aquaculture cluster. Trout collected at SSM near the San Miguel aquaculture farm belonged to the aquaculture cluster and were identified as aquaculture trout, perhaps as a product of aquaculture escapes. Finally, for the lab hybrids, half of their genome belonged to native *O. chrysogaster* from the Central Fuerte cluster (parental population), and the other half to the aquaculture cluster. A comparison of different K values shows a similar genetic structure (across K=6, K=7, and K=8) with slight differences for K=6, where trout from FSJ (Central Fuerte in K=7 and 8) were clustered with trout from FCA, FON, CAB, SCE, SPE, and SCS (Online Resource 4). In general, the genetic admixture between native and farmed trout was very low. Moreover, for two genetic clusters (i.e. Southern Fuerte, Eastern Sinaloa, and Northern

297 Culiacán; and Western Fuerte and Western Sinaloa; Fig. 3b and 4c) the observed spatial genetic structure does not
298 follow a basin delineating, suggesting human translocations of native trout or potential connectivity among basins
299 during flood events, or both. Similar results were observed while analysing Dataset B, obtaining six native genetic
300 clusters with MGT individuals belonging to the same genetic clusters described above (Online Resource 5).
301 Interestingly, analysing the dataset without outliers (with only neutral SNPs) found the same genetic structure
302 (results not shown).
303
304 **3.3. Riverscape genetics analyses**
305 Both latitude and precipitation of the driest month showed significant correlations with H_E. These correlations
306 reflect the negative effect of latitude and precipitation of the driest month on genetic diversity (Table 2).
307 The partial Mantel test between pairwise $F_{ST}/(1-F_{ST})$ and riverscape resistance at Río Sinaloa was highly
308 statistically significant ($p = 0.0002$ and $r = 0.87$), suggesting that riverscape resistance strongly influences genetic
309 divergence within this basin. Remarkably, the effect of riverscape resistance was not observed at Río Fuerte,
310 probably due to the sampling strategy (Table 3). Is worth mentioning that at Río Sinaloa the sampling site
311 distribution was restricted to a small area (few streams) along the length of the river, which is not the case for Río
312 Fuerte.
313
314 **3.4. Detection of SNPs under divergent selection**
315 A total of 566 outlier loci were detected with PCAdapt, Bayenv2, and LFMM (Fig. 5). PCAdapt identified 278
316 outliers. On the other hand, Bayenv2 and LFMM identified 306 outliers correlated with environmental variables
317 (temperature of the warmest quarter and precipitation of the driest month). Few outliers (96) were detected twice
318 or more by the different approaches. No common outlier was shared among methods.
319 After applying quality filters in the gene ontology analysis, 21 SNP loci under divergent selection and
320 with protein annotations, were retained (Table 4). Most of these annotations were associated to biological functions
321 in the literature (e.g. growth, reproduction and thermal tolerance; Table 4).
322
323 **4. DISCUSSION**
324 Our main objective was to explore the effect of both riverscape and cultured rainbow trout farm escapes, on the
325 genetic diversity and local adaptation of an endemic salmonid. Low genetic admixture between aquaculture *O.*
326 *mykiss* and native trout was found for the populations sampled. This study also revealed high genetic differentiation

among geographically isolated locations, with populations in the south of the study area being the most isolated. A significant influence of latitude and precipitation of the driest month on genetic diversity was detected, in addition to evidence of isolation by riverscape resistance within a basin. Outlier detection and gene ontology analyses identified genes that could be implicated in adaptation to local climate heterogeneity. This integrative approach reveals that the riverscape influences different microevolutionary processes (i.e. gene flow and local adaptation) depending on the spatial scale: *i) at local scale:* genetic divergence might be shaped by hydrologic and topographic gradients and ruptures at intra-basin level; *ii) at regional scale:* temperature and precipitation influence genetic diversity within basins, but also among non-distant places at different basins; iii) at *broad scale:* temperature and precipitation gradients could be implicated in local adaptation. These findings, in addition to shedding light on riverscape genomics, are discussed in the context of the development of management strategies for endangered riverine species in order to preserve both genetic diversity and adaptive potential in the face of global change.

4.1. Absence of high levels of admixture between *O. chrysogaster* and aquaculture *O. mykiss*

The low genetic admixture (introgression) between native and exotic trout found in this study can be explained by the lack of proximity to aquaculture sites, as intensive aquaculture activities were not detected in the study area (authors' field observations). Other studies at broader spatial scales found evidence of high genetic admixture, but mostly for undescribed Mexican trout forms in southernmost SMO areas, characterised by intensive aquaculture activities (Abadía-Cardoso et al. 2015; Escalante et al. 2014). In addition to reproductive behaviour, interspecific phenotypic variation and the overall geographic remoteness of aquaculture activities (Binder et al. 2015; Buchinger et al. 2017; Johnson et al. 2018); the low levels of exotic introgression found in this study might be explained by the local riverscape (i.e. altitude decrease; slope, stream order changes and temperature increase) acting as a boundary against exotic introgression. This physical barrier effect caused by the riverscape was suggested in recent simulation studies for this same species (Escalante et al. 2018), but also by empirical data for *O. mykiss*, *O. clarkii lewisi*, *O. clarkii bouvieri* and *Salmo trutta* (Weigel et al. 2003; Gunnell et al. 2008; Splendiani et al. 2013). The extensive introduction of exotic trout has been documented as one of the greatest threats to the entire endemic Pacific trout complex (Miller et al. 1989; Bahls 1992; Penaluna et al. 2016). Also, the harmful effects of exotic salmonid invasions have been recognised in native salmonid populations all over the world through parasites, native food web alterations, competition, and replacement of the native gene pool (Heggberget et al. 1993; Fausch 2007; Muhlfeld et al. 2009; Marie et al. 2012; Vera et al. 2017). In the SMO, particularly, due to economic interests,

357 it is expected that aquaculture activities will increase in coming years, while their impact on native trout
358 populations is still little known, making it challenging to implement management plans for conservation purposes
359 (Hendrickson et al. 2002).

360 However, the low level of admixture between native and exotic trout detected in this study highlights the
361 beneficial effect of environmental heterogeneity and low aquaculture activity, but would inevitably be reversed if
362 this activity increases. Therefore, the proper use of native trout for aquaculture activities in the area only using
363 closely-related populations and respecting the ecological niches, may offer an alternative which avoids the harmful
364 effects of exotic trout invasions. The low exotic admixture with aquaculture trout observed in *O. chrysogaster* in
365 previous works supports our results. Indeed, in the specific case of Arroyo Agua Blanca in Río Culiacán, previous
366 studies reported high levels of genetic admixture with *O. mykiss* in an analysis of wild samples collected in 1997
367 (Escalante et al. 2014), while samples collected in 2015 used in this study did not display genetic admixture. These
368 results are encouraging and revealed that the genetic pool of riverine species could still be preserved despite the
369 existence of aquaculture practices in their distribution area. Thus, conservation strategies may be considered to
370 avoid the probable harmful effects of exotic introgression in *O. chrysogaster* populations, such as banning or
371 strongly regulating rainbow trout aquaculture.

372

373 **4.2. Riverscape drivers of genetic diversity**

374 Low levels of heterozygosity were correlated with an increase in latitude, and precipitation of the driest month.
375 The genetic diversity values observed here are lower than those reported for trout from northern latitudes (e.g.
376 Carim et al. 2016; Linløkken et al. 2016; Wenne et al. 2016; Perreault-Payette et al. 2017; Winans et al. 2018;
377 Pearse and Campbell 2018), this is in agreement with former studies that already showed the lower genetic
378 diversity of *O. chrysogaster* in relation to trout form USA (Escalante et al. 2014; Abadía-Cardoso et al. 2015).
379 From an ecological point of view, the main sources of variation in genetic diversity are*: i) variation in effective*
380 *size*: populations with large effective sizes are expected to have higher heterozygosity than populations with
381 smaller effective sizes, as they have a larger number of breeders that keep allele frequencies stable (Höglund 2009);
382 *ii) space, mainly through variation in the environment and habitats:* isolated populations with low immigration
383 rates may exhibit inbreeding and reduced gene flow with closely related populations, as they are exposed to genetic
384 drift (Riginos and Liggins 2013); iii) *contemporary and historical events, and human disturbances*: the
385 fragmentation or connection of populations with consequent variations in genetic diversity due to
386 microevolutionary processes (e.g. gene flow and local adaptation) (Hewitt 2000; Banks et al. 2013).

The occurrence of extreme hydroclimatic events during embryo incubation may have catastrophic consequences on trout populations, causing strong variations in population sizes (Hand et al. 2016), as well as variations in the effect of genetic drift. In this study, a negative correlation was detected between precipitation of the driest month on H_E. This correlation may be explained by the fact that *O. chrysogaster* mates during the dry season (December – March; García-De León et al. 2016). Flash flood events during the reproductive period of *O. chrysogaster* may cause embryo mortality and, consequently, dramatic reductions in population size that translate into a decline in heterozygosity. A negative influence of precipitation on heterozygosity has also been observed in native steelhead trout in the USA, with similar life history traits and habitat conditions to *O. chrysogaster* (Narum et al. 2008).

A negative influence of latitude in H_E was also observed. The heterozygosity is actually lower on the periphery of the study area (i.e. east of Río Fuerte, southwest of Río Fuerte, northwest of Río Sinaloa and south of Río Culiacán) (Fig. 2). These peripheral zones have been defined in previous studies at the limits of the species' ecological niche (Ruiz-Luna et al. 2017; Escalante et al. 2018). This pattern of genetic diversity deficit on the periphery due to isolation has been broadly observed in a wide number of species (de Lafontaine et al. 2018). Taken together, the low genetic diversity, high F_{ST} values, geographical remoteness, high riverscape resistance (observed in riverscape resistance surfaces) and habitat suitability observed in previous studies using ecological niche models (Escalante et al. 2018) suggest that populations in the south of Río Culiacán (i.e. CED, CER, CER2 and CSJN) are the most isolated in this study.

The small N_E found in our study (≤146) fall far short of what is needed (≥ 500) to preserve the long-term viability of salmonids, which raises the question of the long-term survival of the populations studied (Koskinen et al. 2002; Rieman and Allendorf, 2001). Moreover, the low values of N_E (≤ 500) could be the reason for the lack of a positive correlation between the N_E and H_E reported in this study. Certainly, the effects of drift are shown in sites with high N_E values and low H_E values and vice versa, for example FLQ had N_E of 116.8 and H_E of 0.05, while FCA had N_E of 8.2 and H_E of 0.13 (Table 1). However, generally low N_E and H_E values are common in species that experience rapid expansions after glaciation periods because of habitat shifts and isolation (Hewitt 2000). This decline in genetic diversity may therefore be due to a combination of several factors: bottlenecks occurring after *O. chrysogaster* colonization of the SMO at the end of the Pleistocene (Behnke et al. 2002), and/or habitat fragmentation due to riverscape resistance impeding immigrant exchange among populations (Channell and Lomolino 2000; Behnke et al. 2002; Eckert et al. 2008; Ruzzante et al. 2016). Increasing the sample size in further studies would make it possible to test these hypotheses.

417 The highest parts of SMO inhabited by *O. chrysogaster* should be made into natural protected areas with
418 strictly regulated land use and land change activities. The aforementioned strategy can help to preserve dispersal
419 corridors among populations maintaining the gene flow but also habitat quality, which is necessary to ensure the
420 native genetic diversity (Olsen et al. 2017). Moreover, under any past or present scenarios explaining the low
421 levels of N_E, avoiding the loss of genetic diversity should be considered for the development of conservation
422 strategies. An alternative that could be explored in depth is the translocation of individuals between small and
423 isolated populations containing the same gene pool, to preserve the genes involved in local adaptation (Sato and
424 Harada, 2008). Translocation is a conservation strategy that can help to avoid genetic diversity reduction, and
425 could be achieved by ensuring founding populations including as many individuals as possible, and augmenting
426 the relocated populations after establishment with new trout from existing populations (Faulks et al. 2017).
427 However, this strategy requires extensive genetic monitoring of both existing and relocated populations in order
428 to avoid outbreeding problems.
429
430 **4.3. Riverscape drivers of genetic divergence**
431 The influence of riverscape structure on genetic divergence has already been tested in salmonids, with riverscape
432 defined as a fundamental driver of gene flow (Kanno et al. 2011; Torterotot et al. 2014; Landguth et al. 2016). In
433 this study, partial Mantel tests suggest an influence of riverscape resistance in genetic variation only in Río Sinaloa.
434 These outputs might be due to the sampling strategy, which was closer to a least cost path approach in Río Sinaloa
435 with the sampling sites homogeneously distributed along a few streams on a small spatial scale, compared with
436 Río Fuerte where the sampling sites are distributed over a larger spatial extent in several streams. Demo-genetic
437 simulations have already suggested that riverscape resistance has a strong influence on the genetic divergence of
438 *O. chrysogaster* at Río Fuerte (Escalante et al. 2018). Therefore, we expect that the effect of the riverscape at Río
439 Fuerte can be evidenced with a more continuous sampling strategy along the length of the streams (Cushman and
440 Landguth 2010). Moreover, the use of a point based approach to test the effect of local riverscape conditions on
441 genetic divergence might be considered to better understand the influence of environmental factors on the species'
442 genetic structure (Grummer et al. 2019).
443 The effect of riverscape resistance on gene flow may determine adaptation to local environments, due to
444 the fact that gene flow allows the entry of new genes, and then, through recombination during sexual reproduction,
445 a new combination of genes is produced and populations gain genetic advantages to cope with changing
446 environments (Kokko et al. 2017). For endangered species living in restricted heterogeneous habitats, it is vital to

447 understand the relationship between riverscape and gene flow in order to define management units based on
448 populations with historical gene flow, and which occupy similar ecological niches (Crandall et al. 2000; Olsen et
449 al. 2017; Schmidt et al. 2017). It is important to define the influence of landscape factors on gene flow processes
450 among populations at local scales (within basins), to then understand adaptive variation across wider scales (along
451 different basins). Thus, the development of riverscape resistance surfaces could help the conservation of native
452 trout, defining and preserving dispersal corridors to maintain gene flow among closely related populations with
453 low genetic diversity.
454

455 **4.4. Detection of SNPs under divergent selection**

456 The 306 SNPs correlated with hydroclimatic variables (i.e. precipitation of the driest month and temperature of
457 the warmest quarter), suggest that these environmental variables may act as selective factors in *O. chrysogaster*.
458 The influence of temperature and precipitation on adaptive genetic variation has also been suggested for steelhead
459 trout from the Inner Columbia River Basin and for cutthroat trout from the Great Basin Desert both in U.S.A.
460 (Hand et al. 2016; Amish et al. 2019). Temperature has been defined as a potential driver of adaptive processes in
461 salmonids mainly because ectothermic body temperature is closely associated with the environment (Hecht et al.
462 2015; Hand et al. 2016). Moreover, variations in temperature and precipitation affect phenomena such as changes
463 in dispersal and reproduction timing, age at maturity, growth, fecundity, and survival (Crozier & Hutchings, 2014;
464 Hecht et al., 2015). The very low overlap of outliers observed by the different approaches used in this study is
465 expected when using different methods, since their algorithms and prior assumptions differ considerably (Ahrens
466 et al. 2018; Dalongeville et al. 2018; Amish et al. 2019).

467 Among the annotated gene functions detected by a gene ontology analysis in outlier loci, actin-binding
468 proteins might play an important role in water flow and temperature acclimation, as reported for other species such
469 as the European hake (Milano et al. 2014). Thus, adaptive genetic variation across the study area may increase
470 among populations exposed to different temperature and precipitation regimes. However, analyses of adaptive
471 variation, using the population genetics analyses of outlier loci, are needed to confirm this. Moreover, protein
472 binding is also associated with trout growth and flesh quality, which in turn may be associated with stream flow
473 and water temperature (Salem et al. 2010). Even though our results suggest potential adaptation of *O. chrysogaster*
474 to local conditions, these findings should be interpreted with caution. It is acknowledged that when sample size is
475 small and the averaged genetic differentiation is high, this may result in false positive gene-environment
476 associations (Hoban et al. 2016).

Our findings, together with previous works on related species, indicate that salmonids develop adaptations to cope with changing climatic conditions (Hecht et al. 2015; Bourret et al. 2013). However, faced with the threat of man-made global warming, habitat fragmentation and exotic introduction the species' risk of extinction is high, especially for small populations with putative fitness loss. Indeed, actions at local scale may not be enough to ensure the survival of the species (Rahel et al. 2008; Lawler et al. 2010). On this basis, federal agencies should consider climate change in all their management plans, integrating multidisciplinary approaches to understand the effect of climate fluctuations on native species at different scales. The landscape genetics approach considered in this study may be helpful in the development of these management strategies and illustrates how genomic data can inform monitoring programs and conservation actions (Flanagan et al. 2018; Hendricks et al. 2018). Finally, it is a matter of urgency that predictive models associating climate change with genomic diversity in order to maintain local adaptation skills are used to design the best conservation strategies for all endangered salmonid species in North America. This broad evolutionary perspective is crucial for the application of adaptive genomic variation to conservation efforts (Pearse 2016; Leitwein et al. 2017; Razgour et al. 2019). However, as in many other Latin American countries, until now Mexican federal agencies showed a lack of interest to invest financial resources and integrate genomic tools into conservation strategies (Torres-Florez et al. 2017), making more challenging the preservation of native salmonids in relation to USA and Canada. Therefore, Government authorities should understand the importance of the incorporation of evolutionary research and management actions, linking theory and practice in order to ensure the native biodiversity in the face of global change. Undoubtedly, improving this requires improving connectivity between scientists and policy makers (Torres-Florez et al. 2017).

4.5. Conclusions

The integrative study presented here describes the effect of both landscape and anthropogenic factors on microevolutionary processes providing information about possible effects of hydroclimatic variables on local adaptation. This type of approach has not been widely considered in conservation genomics studies, especially in Latin America. Our findings suggest that both low genetic introgression, and that riverscape factors have an effect on genetic diversity, connectivity and adaptive genetic variation. The approach presented here may be useful for developing conservation strategies for endangered riverine fish species that consider both the ecological and evolutionary aspects.

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FIGURES CAPTIONS

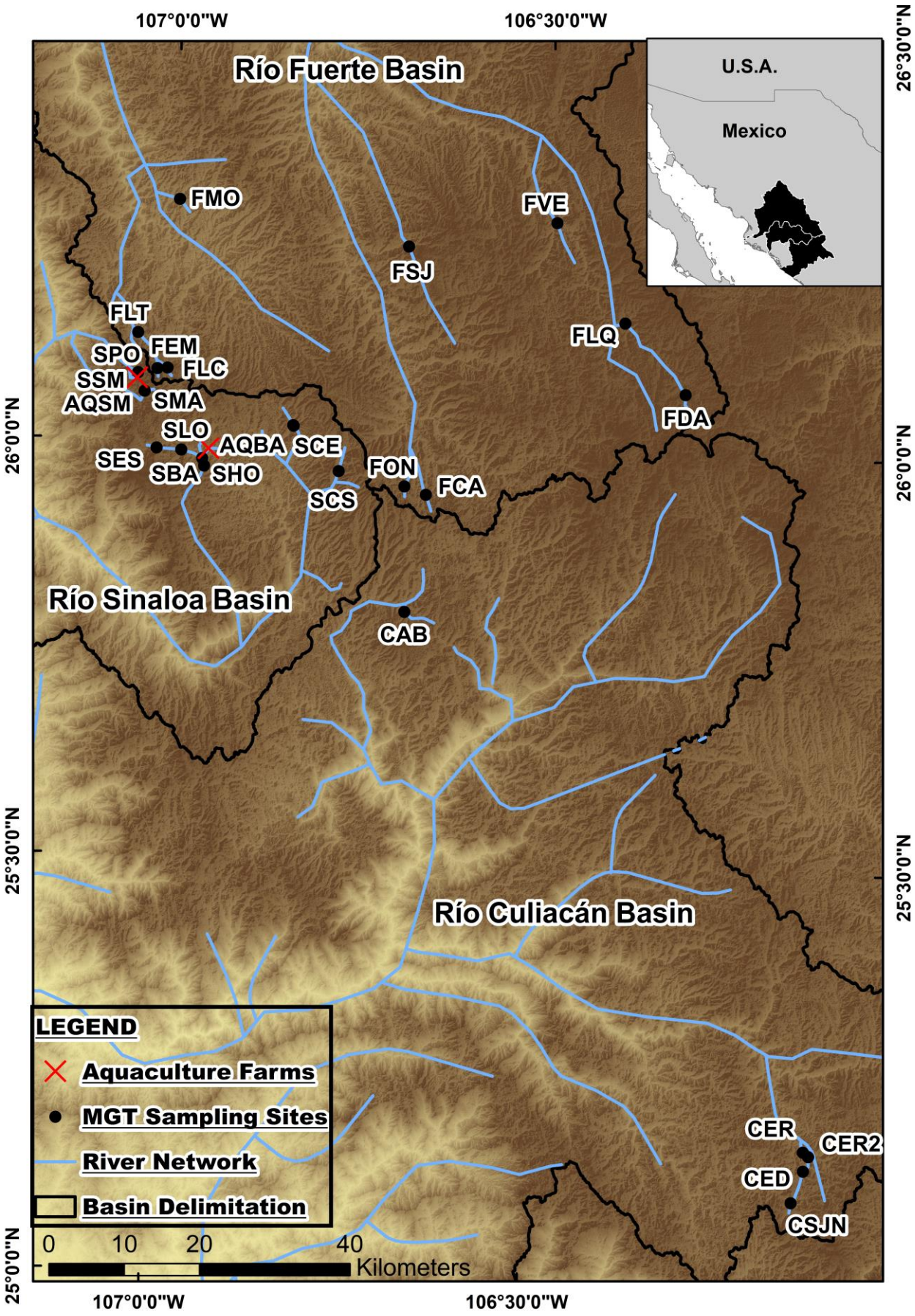
Fig. 1 Study area. Mexican golden trout (MGT) and cultured rainbow trout (Aquaculture farms) sample sites in the Río Fuerte, Río Sinaloa and Río Culiacán basins. See table 1 for the explanation of the sample site codes

Fig. 2 Geographical distribution of genetic diversity values for native *O. chrysogaster*. a) Expected heterozygosity; b) Observed heterozygosity. See table 1 for the explanation of the sample site codes

Fig. 3 Genetic structure of *O. chrysogaster* and aquaculture rainbow trout defined by Nei distances (Tamura and Nei, 1993) in adegenet and a Bayesian assignment test in fastStructure. (a) Nei phenogram, the bootstrapping values for each clade are represented by numbers, see table 1 for the explanation of the sampling site codes. (b) Bayesian assignment test barplot, each color represents a different genetic cluster and the genome of each individual is represented by a horizontal line: Eastern Fuerte (EF); Central Fuerte (CF); Southern Fuerte, Eastern Sinaloa and Northern Culiacán (SFESNC); Western Fuerte and Western Sinaloa (WFWS); Central Sinaloa (CS); Southern Culiacán (SC); Aquaculture (AQ).

Fig. 4 Spatial distribution of native *O. chrysogaster* and aquaculture rainbow trout genetic clusters defined in FastStructure. a) Spatial genetic structure across all the study area. b) Trout from Eastern Fuerte; Central Fuerte; Southern Fuerte, Central Sinaloa and Northern Culiacán genetic clusters; and FMO sample site from Western Fuerte and Western Sinaloa genetic cluster. c) Trout from Western Fuerte and Western Sinaloa; Central Sinaloa; and Aquaculture genetic clusters. d) Trout from Southern Culiacán genetic cluster. See table 1 for the explanation of the sample site codes.

Fig. 5 Venn diagram of outliers detected for *O. chrysogaster* among PCAdapt, LFMM using temperature of the warmest quarter (LFMM TWQ), LFMM using precipitation of the driest month (LFMM PDM), Bayenv2 using temperature of the warmest quarter (BAYENV TWQ), and Bayenv2 using precipitation of the driest month (BAYENV TWQ). The total amount of outliers (SNPs) detected by each method are represented in bold. Values outside of the curve intersections represent the number of outliers detected by only one method and the values inside the intersections represent the number of outliers detected by two methods or more.



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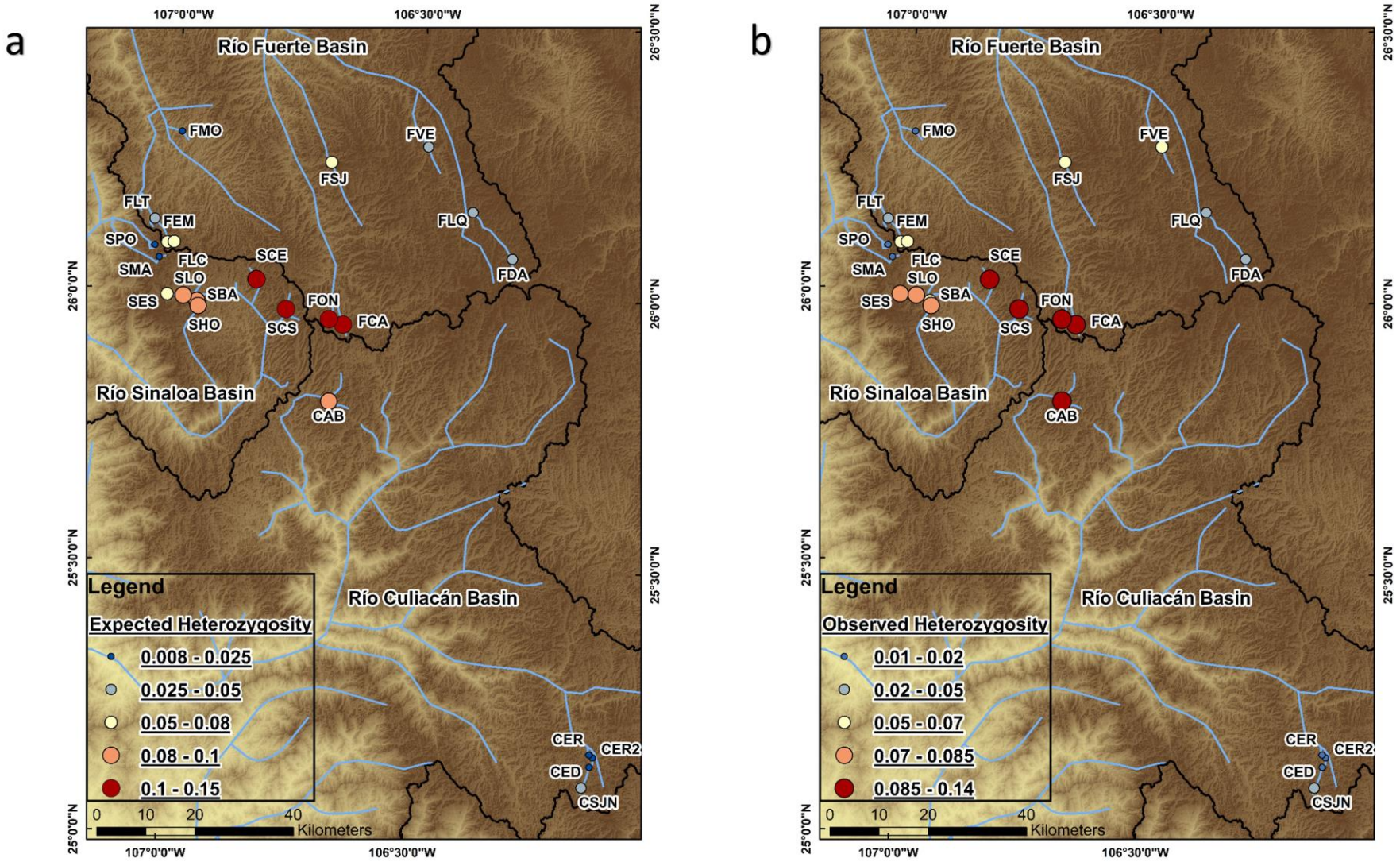
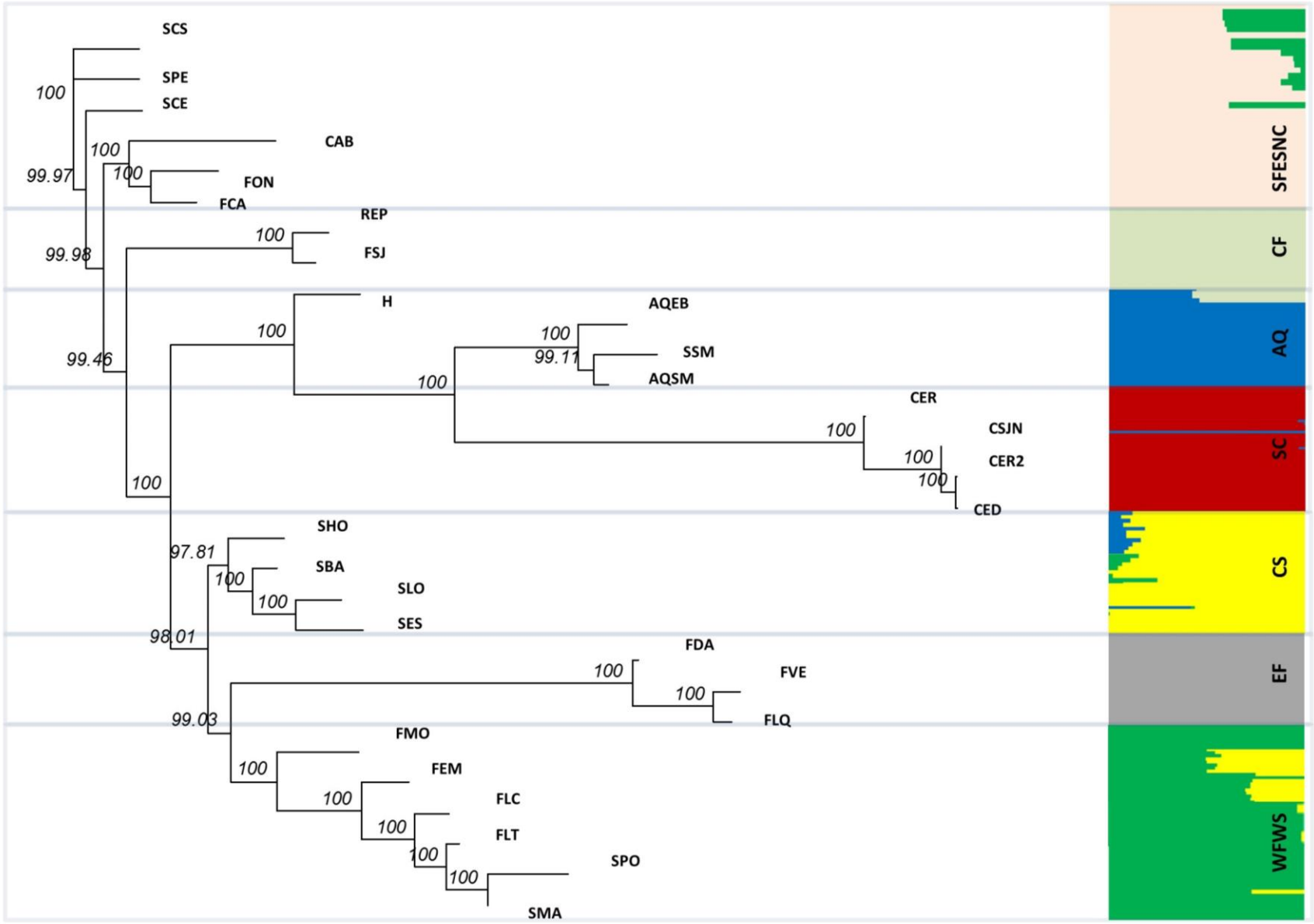

a
107°0'0"W
106°30'0"W
26°30'0"N
26°0'0"N
25°30'0"N
25°0'0"N
Río Fuerte Basin
Río Sinaloa Basin
Río Culiacán Basin
FMO
FVE
FSJ
FLT
FEM
FLQ
SPO
FLC
SCE
SMA
SLO
FDA
SES
SBA
FON
SHO
SCS
FCA
CAB
CER
CER2
CED
CSJN
Legend
Expected Heterozygosity
0.008 - 0.025
0.025 - 0.05
0.05 - 0.08
0.08 - 0.1
0.1 - 0.15
0
10
20
40
Kilometers
b
Legend
Observed Heterozygosity
0.01 - 0.02
0.02 - 0.05
0.05 - 0.07
0.07 - 0.085
0.085 - 0.14


SCS
SPE
SCE
CAB
FON
FCA
REP
FSJ
H
AQEB
SSM
AQSM
CER
CSJN
CER2
CED
SHO
SBA
SLO
SES
FDA
FVE
FLQ
FMO
FEM
FLC
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SPO
SMA
100
99.97
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100
97.81
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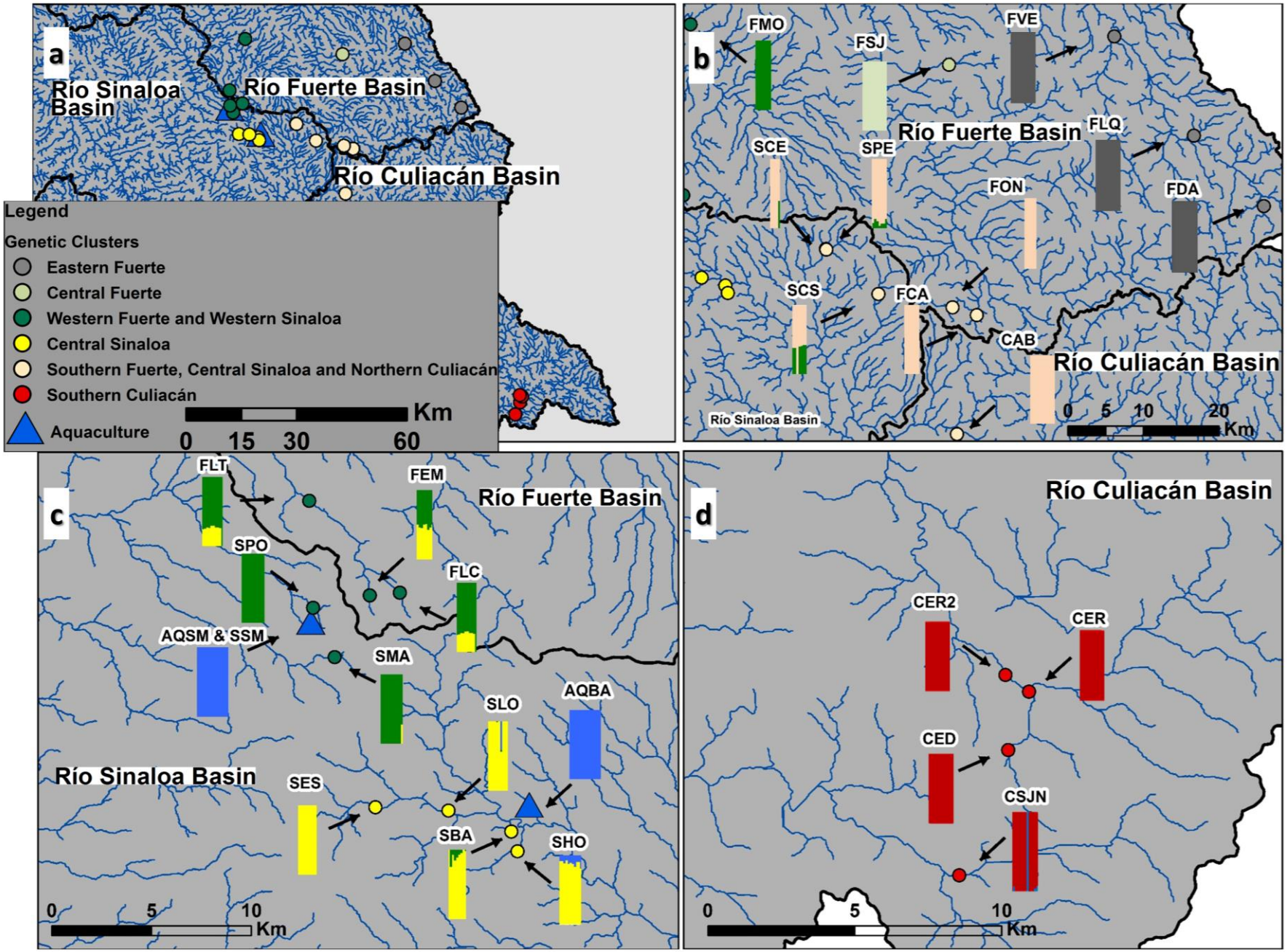
SFESNC
CF
AQ
SC
CS
EF
WFWS


a
Río Sinaloa Basin
Río Fuerte Basin
Río Culiacán Basin
Legend
Genetic Clusters
Eastern Fuerte
Central Fuerte
Western Fuerte and Western Sinaloa
Central Sinaloa
Southern Fuerte, Central Sinaloa and Northern Culiacán
Southern Culiacán
Aquaculture
0 15 30 60 Km
b
FMO
FSJ
FVE
Río Fuerte Basin
FLQ
SCE
SPE
FON
FDA
SCS
FCA
CAB
Río Culiacán Basin
Río Sinaloa Basin
0 5 10 20 Km
c
FLT
FEM
Río Fuerte Basin
SPO
FLC
AQSM & SSM
SMA
AQBA
SLO
Río Sinaloa Basin
SES
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SHO
0 5 10 Km
d
Río Culiacán Basin
CER2
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CED
CSJN
0 5 10 Km

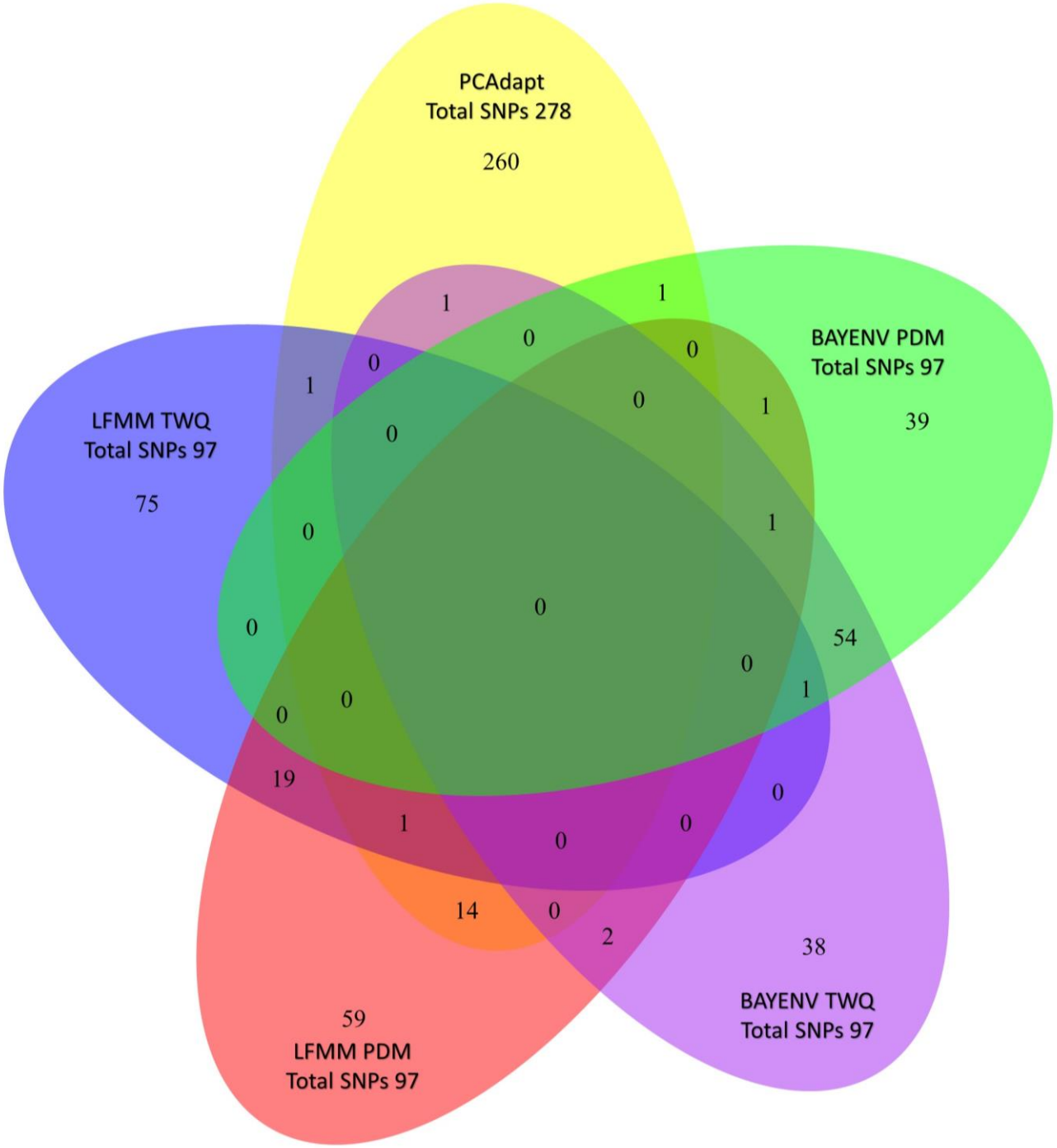

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TABLE 1 Genetic diversity values for Mexican golden trout, aquaculture rainbow trout and lab hybrids.

River Basin	CODE	Latitude	Longitude	Location	Description	N[a]	H_O[b]	H_E[c]	N_E[d]	95% CI N_E[e]
Río Fuerte	FDA	26.07	-106.31	Arroyo del Agua	Wild trout	10	0.03	0.04	44.2	38.3–51.9
	FEM	26.09	-107.02	Arroyo El Manzano	Wild trout	8	0.06	0.06	35.3	32.4-38.7
	FLC	26.09	-107.00	Arroyo Las Cuevas	Wild trout	10	0.06	0.07	33.7	32.2–35.3
	FLQ	26.16	-106.40	Arroyo La Quebrada	Wild trout	12	0.04	0.05	116.8	110.3-139.6
	FLT	26.13	-107.04	Arroyo Las Truchas	Wild trout	8	0.04	0.04	-	Infinite
	FMO	26.29	-106.99	Arroyo Momorita	Wild trout	5	0.02	0.02	-	Infinite
	FSJ	26.24	-106.69	Arroyo San José	Wild trout	12	0.07	0.07	14.7	14.4-15
	FCA	25.94	-106.65	Arroyo Calera	Wild trout	9	0.14	0.13	8.2	8.1-8.2
	FON	25.95	-106.68	Arroyo La Onza	Wild trout	6	0.14	0.12	-	Infinite
	FVE	26.28	-106.49	Río Verde	Wild trout	12	0.05	0.05	11	10.8-11.2
	Totals and means	-	-	-	-	**92**	**0.065**	**0.065**	**37.7**	
Río Sinaloa	SBA	25.98	-106.95	Arroyo Baluarte	Wild trout	9	0.07	0.09	145.7	114.8-198.7
	SES	25.99	-107.01	Arroyo El Soldado	Wild trout	9	0.08	0.08	5.9	5.8-6
	SHO	25.97	-106.95	Arroyo Hondo	Wild trout	11	0.09	0.11	2.4	2.3-2.4
	SLO	25.99	-106.98	Arroyo La Osera	Wild trout	10	0.08	0.09	1.5	1.5-1.5
	SMA	26.06	-107.03	Arroyo Macheras	Wild trout	10	0.02	0.02	0.4	0.4-0.4
	SPO	26.08	-107.04	Arroyo Potrero	Wild trout	11	0.02	0.02	-	Infinite
	SSM	26.07	-107.04	Arroyo San Miguel	Trout in aquaculture proximities	4	0.17	0.15	-	Infinite
	SCS	25.97	-106.77	Arroyo Cerro Solo	Wild trout	9	0.11	0.15	1.4	1.4-1.4
	SCE	26.02	-106.83	Arroyo Cebollín	Wild trout	7	0.09	0.12	1.8	1.8-1.8
	SPE	26.02	-106.83	Arroyo Pericos	Wild trout	7	0.12	0.12	7.5	7.3-7.6

	Totals and means	-	-	-	-	**87**	**0.085**	**0.095**	**20.83**	
Río Culiacán	CED	25.14	-106.13	Arroyo El Desecho	Wild trout	12	0.009	0.01	-	Infinite
	CER	25.16	-106.12	Arroyo El Río 1	Wild trout	12	0.008	0.01	-	Infinite
	CER2	25.17	-106.13	Arroyo El Río 2	Wild trout	12	0.009	0.01	-	Infinite
	CSJN	25.10	-106.14	Arroyo San Juan del Negro	Wild trout	12	0.05	0.04	8	7.9-8.2
	CAB	25.80	-106.68	Arroyo Agua Blanca	Wild trout	10	0.11	0.1	42.7	41.4-44
	Totals and means	-	-	-	-	**58**	**0.038**	**0.034**	**25.35**	
Lab Trout	H			INAPESCA Cultive Center	Mexican golden trout and aquaculture rainbow trout lab hybrids	4	0.22	0.15	-	Infinite
	REP			INAPESCA Cultive Center	Domestic Mexican golden trout	7	0.08	0.08	-	Infinite
	Totals and means	-	-	-	-	**11**	**0.15**	**0.12**		
Aquaculture Farms	AQEB	25.99	-106.95	El Barro Aquaculture Farm	Aquaculture rainbow trout	14	0.15	0.16	19.1	18.9-19.2
	AQSM	26.07	-107.04	San Miguel Aquaculture Farm	Aquaculture rainbow trout	10	0.13	0.15	22.1	21.7-22.5
	Totals and means	-	-	-	-	**24**	**0.14**	**0.16**	**20.6**	

a-Number of genotyped samples (N), *b*-expected heterozygosity (H_E), *c*-observed heterozygosity (H_O), *d*-effective population size (N_E) and *e*-95% confidence intervals for effective population size (95% CI N_E).

TABLE 2 Generalized linear model explaining the effect of riverscape variables on expected heterozygosity (H_E) for Mexican golden trout populations (Dataset C).

Variable	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept	7.527140	1.624313	4.634	0.000237
Latitude	-0.281141	0.061273	-4.588	0.000261
Precipitation of the driest month	-0.008457	0.003796	-2.228	0.039706

TABLE 3 Partial Mantel test performed under 9,000 permutations between (F_{ST}/(1-F_{ST}) and riverscape resistance matrices.

Dataset	R	p-value
Dataset D (Río Fuerte populations)	-0.05	0.73
Dataset E (Río Sinaloa populations)	0.872	0.0002

Table 4 Blast hits from sequences containing an SNP found to be putatively under selection by PCAdapt, BAYENV and LFMM. SNPs are identified by locus ID. Sequence names are identified as in Berthelot *et al.* (2014). Statistical significance of the hits is represented by the e-Value. Functional characterizations by gene ontology terms obtained in Blast2GO are identified by GO IDs.

Locus ID	Sequence Name	e-Value	GO ID's	Known functions	Associated functions
CLocus_63824	GSONMP00076296001	1.93E-08	F:GO:0003676	F:nucleic acid binding	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)
CLocus_17128	GSONMP00061816001	0.0000145	F:GO:0008270; P:GO:0030163	F:zinc ion binding; P:protein catabolic process	Adaptation to elevated concentrations of waterborne (Hogstrand et al. 1994)
CLocus_24893	GSONMP00073281001	0.000209	F:GO:0003676	F:nucleic acid binding	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)
CLocus_34692	GSONMP00068395001	0.00000574	F:GO:0005524; F:GO:0004672; P:GO:0006468	F:ATP binding; F:protein kinase activity; P:protein phosphorylation	Water flow and temperature acclimation (Milano et al. 2014)
CLocus_39212	GSONMP00076439001	0.00000039	P:GO:0097264; C:GO:0005887; F:GO:0005515; P:GO:0007165; C:GO:0016021; F:GO:0005102	C:integral component of plasma membrane; P:self proteolysis; P:signal transduction; F:receptor binding	Temperature adaptation (Crockett 1998)
CLocus_40408	GSONMP00028710001	0.000684	P:GO:0055085; C:GO:0016021	P:transmembrane transport; C:integral component of membrane	Temperature adaptation (Crockett 1998)
CLocus_40780	GSONMP00020503001	0.000225	F:GO:0005515	F:protein binding	Water flow and temperature acclimation (Milano et al. 2014)
CLocus_41412	GSONMP00076296001	0.000311	F:GO:0003676	F:nucleic acid binding	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)
CLocus_45731	GSONMP00008827001	0.000766	F:GO:0008641	F:small protein activating enzyme activity	
CLocus_48136	GSONMP00021876001	0.000665	C:GO:0005615; P:GO:0010506	C:extracellular space; P:regulation of autophagy	
CLocus_5089	GSONMP00082580001	0.000619	F:GO:0005515	F:protein binding	Water flow and temperature acclimation (Milano et al. 2014)
CLocus_54656	GSONMP00051628001	0.00000314	F:GO:0005515	F:protein binding	Water flow and temperature acclimation (Milano et al. 2014)
CLocus_55309	GSONMP00044696001	0.000544	F:GO:0003677; C:GO:0005634; F:GO:0008270;	C:nucleus; F:DNA binding; F:zinc ion binding; F:steroid hormone receptor activity;	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)

			F:GO:0003707; P:GO:0006355; P:GO:0043401	P:regulation of transcription, DNA-templated; P:steroid hormone mediated signaling pathway	
CLocus_5644	GSONMP00023521001	0.000247	F:GO:0004725; P:GO:0006470	P:tyrosine metabolic process; F:protein tyrosine phosphatase activity; P:protein dephosphorylation	Water flow and temperature acclimation (Milano et al. 2014)
CLocus_58838	GSONMP00030033001	0.00000662	P:GO:0008360; F:GO:0003779; P:GO:0007010; P:GO:0016043; F:GO:0017048; F:GO:0005488; P:GO:0030036	P:regulation of cell shape; F:actin binding; F:Rho GTPase binding; P:actin cytoskeleton organization	Water flow and temperature acclimation (Milano et al. 2014)
CLocus_59529	GSONMP00030319001	0.0000646	P:GO:0016337; F:GO:0005515; P:GO:0007155; F:GO:0005488; P:GO:0032467; P:GO:0043547	P:single organismal cell-cell adhesion; F:protein binding; P:positive regulation of cytokinesis; P:positive regulation of GTPase activity	Water flow and temperature acclimation (Milano et al. 2014)
CLocus_63824	GSONMP00059101001	6.73E-07	F:GO:0003676; F:GO:0005524; C:GO:0005634; F:GO:0003677; P:GO:0015074; P:GO:0006355; P:GO:0006313	C:nucleus; F:DNA binding; F:ATP binding; P:DNA integration; P:regulation of transcription, DNA-templated; P:transposition, DNA-mediated	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)
CLocus_6876	GSONMP00019151001	0.00052	F:GO:0003676	F:nucleic acid binding	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)
CLocus_69641	GSONMP00059101001	0.0000108	F:GO:0003676; F:GO:0005524; C:GO:0005634; F:GO:0003677; P:GO:0015074; P:GO:0006355; P:GO:0006313	C:nucleus; F:DNA binding; F:ATP binding; P:DNA integration; P:regulation of transcription, DNA-templated; P:transposition, DNA-mediated	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)
CLocus_71994	GSONMP00069497001	0.0000141	F:GO:0005515; F:GO:0005509	F:protein binding; F:calcium ion binding	Water flow and temperature acclimation (Milano et al. 2014)

CLocus_77569	GSONMP00045823001	0.0000424	F:GO:0003676	F:nucleic acid binding	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)

SUPPORTING INFORMATION

Additional Supporting Information can be found in the online version of this article:

Online Resource 1. Six datasets on four different spatial scales.

Online Resource 2. Riverscape resistance surfaces and matrices.

Online Resource 3. F_{ST} coefficients among sample sites of Mexican golden trout, aquaculture rainbow trout and lab hybrids.

Online Resource 4. Assignment probabilities obtained in fastStructure for different K values.

Online Resource 5. Ancestry coefficients obtained in faststructure for Dataset B with native trout without significant introgression.

Genotyping-by-sequencing reveals the effects of riverscape, climate and interspecific introgression on the genetic diversity and local adaptation of the endangered Mexican golden trout (*Oncorhynchus chrysogaster*).

Conservation Genetics

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Online Resource 1. Five datasets on four different spatial scales.

Five datasets at four different spatial scales were constituted after quality filters (Table S1.1). Dataset A: to perform population genetics analyses, all samples after bioinformatics filtering were kept (native, aquaculture and hybrids) including 272 genotyped individuals (Table S1.2). Dataset B: To conduct landscape genomics analyses (gene-environment associations) individuals with aquaculture ancestry coefficients ≥ 20% (based on fastStructure outputs) were removed, the trout collected at Arroyo San Miguel (SSM) was discarded since it is close to aquaculture facilities. Aquaculture rainbow trout was also discarded. Similarly, one individual from Arroyo La Osera (SLO) and one individual from Arroyo San Juan del Negro (CSJN) who presented significant aquaculture ancestry coefficients in fastStructure outputs were not considered either (Table S1.3). Dataset C: In order to conduct landscape genetics analyses with spatially continuous populations, trout from the southernmost part of Río Culiacán was discarded from Dataset B to constitute this new dataset (Table S1.4). Additionally, riverscape genomics analyses were performed at basin scale using two datasets. Dataset D: including trout from 7 sampling sites at Río Fuerte Basin, three sampling sites (FDA, FLQ and FFVE) at the easternmost part of Río Fuerte were discarded due to their large riverine isolation (long riverine distance) in relation with the other sampling sites (Table S1.5). Dataset E: including non-significantly introgressed native trout from the 9

sampling sites at Río Sinaloa Basin (Table S1.6). Native trout from Río Culiacán Basin were not analyzed independently at basin scale due to the small amount of sample sites.

Table S1.1 Characteristics of the six genetic datasets. (MGT. Mexican golden trout)

Dataset	**MGT without significant introgression**	**Wild MGT with significant aquaculture ancestry coefficients**	**Aquaculture rainbow trout**	**Lab hybrids from MGT and aquaculture trout.**	**Cultured MGT**
Dataset A (all study area)	X	X	X	X	X
Dataset B (all study area)	X				
Dataset C (center of the study area)	X				
Dataset D (Río Fuerte Basin)	X				
Dataset E (Río Sinaloa Basin)	X				

33 **Table S1.2**. Dataset A.

Río Fuerte					
CODE	**Latitude**	**Longitude**	**Locality**	**Description**	**N**
FDA	26.07	-106.31	Arroyo del Agua	Wild trout	10
FEM	26.09	-107.02	Arroyo El Manzano	Wild trout	8
FLC	26.09	-107.00	Arroyo Las Cuevas	Wild trout	10
FLQ	26.16	-106.40	Arroyo La Quebrada	Wild trout	12
FLT	26.13	-107.04	Arroyo Las Truchas	Wild trout	8
FMO	26.29	-106.99	Arroyo Momorita	Wild trout	5
FSJ	26.24	-106.69	Arroyo San José	Wild trout	12
FCA	25.94	-106.65	Arroyo Calera	Wild trout	9
FON	25.95	-106.68	Arroyo La Onza	Wild trout	6
FVE	26.28	-106.49	Río Verde	Wild trout	12
Total	-	-	-	-	**92**
Río Sinaloa					
CODE	**Latitude**	**Longitude**	**Locality**	**Description**	**N**
SBA	25.98	-106.95	Arroyo Baluarte	Wild trout	9
SES	25.99	-107.01	Arroyo El Soldado	Wild trout	9
SHO	25.97	-106.95	Arroyo Hondo	Wild trout	11
SLO	25.99	-106.98	Arroyo La Osera	Wild trout	10
SMA	26.06	-107.03	Arroyo Macheras	Wild trout	10
SPO	26.08	-107.04	Arroyo Potrero	Wild trout	11
SSM	26.07	-107.04	Arroyo San Miguel	Cultured trout	4
SCS	25.97	-106.77	Arroyo Cerro Solo	Wild trout	9
SCE	26.02	-106.83	Arroyo Cebollín	Wild trout	7
SPE	26.02	-106.83	Arroyo Pericos	Wild trout	7
Total	-	-	-	-	**87**
Río Culiacán					
CODE	**Latitude**	**Longitude**	**Locality**	**Description**	**N**
CED	25.14	-106.13	Arroyo El Desecho	Wild trout	12
CER	25.16	-106.12	Arroyo El Río 1	Wild trout	12
CER2	25.17	-106.13	Arroyo El Río 2	Wild trout	12
CSJN	25.10	-106.14	Arroyo San Juan del Negro	Wild trout	12
CAB	25.80	-106.68	Arroyo Agua Blanca	Wild trout	10
Total	-	-	-	-	**58**
Lab Trout					
CODE	**Latitude**	**Longitude**	**Locality**	**Description**	**N**

H			INAPESCA Aquaculture Center	Hybrids from MGT and rainbow trout	4
REP			INAPESCA Aquaculture Center	Cultured MGT	7
Total	-	-	-	-	**11**

Aquaculture farms

CODE	**Latitude**	**Longitude**	**Locality (farm)**	**Description**	**N**
AQEB	25.99	-106.95	El Barro Aquaculture	rainbow trout	14
AQSM	26.07	-107.04	San Miguel Aquaculture	rainbow trout	10
Total	-	-	-	-	**24**
Dataset total					**272**

56 **Table S1.3**. Dataset B.

Río Fuerte					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
FDA	26.07	-106.31	Arroyo del Agua	Wild trout	10
FEM	26.09	-107.02	Arroyo El Manzano	Wild trout	8
FLC	26.09	-107.00	Arroyo Las Cuevas	Wild trout	10
FLQ	26.16	-106.40	Arroyo La Quebrada	Wild trout	12
FLT	26.13	-107.04	Arroyo Las Truchas	Wild trout	8
FMO	26.29	-106.99	Arroyo Momorita	Wild trout	5
FSJ	26.24	-106.69	Arroyo San José	Wild trout	12
FCA	25.94	-106.65	Arroyo Calera	Wild trout	9
FON	25.95	-106.68	Arroyo La Onza	Wild trout	6
FVE	26.28	-106.49	Río Verde	Wild trout	12
Total	-	-	-	-	**92**
Río Sinaloa					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
SBA	25.98	-106.95	Arroyo Baluarte	Wild trout	9
SES	25.99	-107.01	Arroyo El Soldado	Wild trout	9
SHO	25.97	-106.95	Arroyo Hondo	Wild trout	11
SLO	25.99	-106.98	Arroyo La Osera	Wild trout	9
SMA	26.06	-107.03	Arroyo Macheras	Wild trout	10
SPO	26.08	-107.04	Arroyo Potrero	Wild trout	11
SCS	25.97	-106.77	Arroyo Cerro Solo	Wild trout	9
SCE	26.02	-106.83	Arroyo Cebollín	Wild trout	7
SPE	26.02	-106.83	Arroyo Pericos	Wild trout	7
Total	-	-	-	-	**82**
Río Culiacán					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
CED	25.14	-106.13	Arroyo El Desecho	Wild trout	12
CER	25.16	-106.12	Arroyo El Río 1	Wild trout	12
CER2	25.17	-106.13	Arroyo El Río 2	Wild trout	12
CSJN	25.10	-106.14	Arroyo San Juan del Negro	Wild trout	11
CAB	25.80	-106.68	Arroyo Agua Blanca	Wild trout	10
Totals	-	-	-	-	**57**
Lab Trout					
CODE	**Latitude**	**Longitude**	**Locality**	**Description**	**N**
REP			INAPESCA Aquaculture Center	Cultured MGT	7
Total	-	-	-	-	**7**

Dataset total					**238**

86 **Table S1.4**. Dataset C.

Río Fuerte					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
FDA	26.07	-106.31	Arroyo del Agua	Wild trout	10
FEM	26.09	-107.02	Arroyo El Manzano	Wild trout	8
FLC	26.09	-107.00	Arroyo Las Cuevas	Wild trout	10
FLQ	26.16	-106.40	Arroyo La Quebrada	Wild trout	12
FLT	26.13	-107.04	Arroyo Las Truchas	Wild trout	8
FMO	26.29	-106.99	Arroyo Momorita	Wild trout	5
FSJ	26.24	-106.69	Arroyo San José	Wild trout	12
FCA	25.94	-106.65	Arroyo Calera	Wild trout	9
FON	25.95	-106.68	Arroyo La Onza	Wild trout	6
FVE	26.28	-106.49	Río Verde	Wild trout	12
Total	-	-	-	-	**92**
Río Sinaloa					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
SBA	25.98	-106.95	Arroyo Baluarte	Wild trout	9
SES	25.99	-107.01	Arroyo El Soldado	Wild trout	9
SHO	25.97	-106.95	Arroyo Hondo	Wild trout	11
SLO	25.99	-106.98	Arroyo La Osera	Wild trout	9
SMA	26.06	-107.03	Arroyo Macheras	Wild trout	10
SPO	26.08	-107.04	Arroyo Potrero	Wild trout	11
SCS	25.97	-106.77	Arroyo Cerro Solo	Wild trout	9
SCE	26.02	-106.83	Arroyo Cebollín	Wild trout	7
SPE	26.02	-106.83	Arroyo Pericos	Wild trout	7
Total	-	-	-	-	**82**
Río Culiacán					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
CAB	25.80	-106.68	Arroyo Agua Blanca	Wild trout	10
Total	-	-	-	-	**10**
Dataset total					**184**

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94 **Table S1.5**. Dataset D.

Río Fuerte					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
FLC	26.09	-107.00	Arroyo Las Cuevas	Wild trout	10
FLT	26.13	-107.04	Arroyo Las Truchas	Wild trout	8
FMO	26.29	-106.99	Arroyo Momorita	Wild trout	5
FSJ	26.24	-106.69	Arroyo San José	Wild trout	12
FCA	25.94	-106.65	Arroyo Calera	Wild trout	9
FON	25.95	-106.68	Arroyo La Onza	Wild trout	6
FVE	26.28	-106.49	Río Verde	Wild trout	12
Dataset total	-	-	-	-	**62**

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116 **Table S1.6**. Dataset E.

Río Sinaloa					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
SBA	25.98	-106.95	Arroyo Baluarte	Wild trout	9
SES	25.99	-107.01	Arroyo El Soldado	Wild trout	9
SLO	25.99	-106.98	Arroyo La Osera	Wild trout	11
SHO	25.97	-106.95	Arroyo Hondo	Wild trout	9
SMA	26.06	-107.03	Arroyo Macheras	Wild trout	10
SPO	26.08	-107.04	Arroyo Potrero	Wild trout	11
SCS	25.97	-106.77	Arroyo Cerro Solo	Wild trout	9
SCE	26.02	-106.83	Arroyo Cebollín	Wild trout	7
SPE	26.02	-106.83	Arroyo Pericos	Wild trout	7
Dataset total	-	-	-	-	**82**

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Genotyping-by-sequencing reveals the effects of riverscape, climate and interspecific introgression on the genetic diversity and local adaptation of the endangered Mexican golden trout (*Oncorhynchus chrysogaster*).

Conservation Genetics

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Online Resource 2. Riverscape resistance surfaces and matrices.

To analyze connectivity among Mexican golden trout populations, a riverscape resistance surface was created. Four environmental variables were selected (altitude, slope, temperature of the warmest quarter and stream order). Using a raster format (30 m pixel size) values from 0 to 10 were assigned to the pixels by variable based on the criteria reported by Escalante et al. (2018). Then, a resistance surface was produced averaging the values for the four variables (Table 1).

For subsequent analyses with the gdistance package (van Etten 2012), the output resistance values were rescaled between 1 and 2, the minimum or absence of riverscape resistance is represented by 1, while 2 is the maximum resistance. All those analyses were conducted in ArcGIS v 10.2 (ESRI 2013).

Table S2.1 Resistance values for riverscape variables: altitude, slope, temperature of the warmest quarter (TWQ) and stream order (SO).

Altitude (m)	Slope (° Slope)	TWQ (°C)	SO	Resistance value
3009 - 1950	0 - 6.74	≤ 14	1 - 3	0
1949 - 1900	6.75 - 13.48	15		1
1899 - 1850	13.49 - 20.22	16		2
1849 - 1800	20. 23 - 26.96	17	4	3
1799 - 1750	26.97 - 33.70	18		4
1749 - 1700	33.71 - 40.44	19		5
1699 - 1650	40.45 - 47.18	20	5	6
1649 - 1600	47.19 - 53.92	21		7
1599 - 1550	53.93 - 60.66	22	6	8
1549 - 1500	60.67 - 67.40	23		9
≤ 1499	67.41 - 74.14	≥ 24	7	10

To calculate isolation by riverscape resistance and linear distance, four matrices among sample sites within basins were generated in gdistance R package (van Etten, 2012): Isolation by riverscape resistance among Dataset D for Río Fuerte populations (Matrix I), Isolation by riverscape resistance among Dataset E for Río Sinaloa populations (Matrix II), Isolation by distance among Dataset D for Río Fuerte populations (Matrix III), and Isolation by distance among Dataset E for Río Sinaloa populations (Matrix IV). Matrix I and Matrix II were calculated using the riverscape resistance surface, while Matrix III and Matrix IV were calculated taking into account only the geographical coordinates of the sampling sites (Table S2.2).

41 **Table S2.2.** Isolation by distance and riverscape resistance matrices.

Matrix	Dataset	Type of isolation	Spatial scale
Matrix I	Dataset D	Isolation by riverscape resistance	Río Fuerte
Matrix II	Dataset E	Isolation by riverscape resistance	Río Sinaloa
Matrix III	Dataset D	Isolation by distance	Río Fuerte
Matrix IV	Dataset E	Isolation by distance	Río Sinaloa

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43 **LITERATURE**

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48 1500–1514. https://doi.org/10.1111/jbi.13246

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50 *Available a t CRAN. R-Project. Org/Package= Gdistance*.

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Online Resource 3. F_{ST} coefficients among sample sites of Mexican golden trout.

Table S3.1. F_{ST} coefficients among sample sites of Mexican golden trout, aquaculture rainbow trout and lab hybrids. Each sample site is represented by a code, including the initial of the river basin (F. Río Fuerte; S. Río Sinaloa; C. Río Culiacán) and an acronym of the locality Spanish name: Arroyo del Agua (FDA), Arroyo El Manzano (FEM), Arroyo Las Cuevas in Río Fuerte (FLC), Arroyo La Quebrada (FLQ), Arroyo Las Truchas (FLT), Arroyo Momorita (FMO), Arroyo San José (FSJ), Arroyo Caleras (FCA), Arroyo La Onza (FON), Arroyo Río Verde (FVE), Arroyo Baluarte (SBA), Arroyo El Salto (SES), Arroyo Hondo (SHO), Arroyo La Osera (SLO), Arroyo Macheras (SMA), Arroyo El Potrero (SPO), Arroyo San Miguel (SSM), Arroyo Cerro Solo (SCS), Arroyo Cebollín (SCE), Arroyo Pericos (SPE), Arroyo El Desecho (CED), Arroyo El Río (CER), Arroyo El Río 2 (CER2), Arroyo San Juan del Negro (CSJN) and Arroyo Agua Blanca (CAB). Additionally, trout from aquaculture facilities are included. El Barro Aquaculture Farm (AQBA), San Miguel Aquaculture Farm (AQSM), Mexican golden trout generated in laboratory (REP), and hybrids from Mexican golden trout and rainbow trout, produced in laboratory (H). The F_{ST} coefficients among simple sites at the same basin are shown in colors: Río Fuerte in yellow, Río Sinaloa in green, Río Culiacán in red, Lab trout in orange and aquaculture trout in blue.

Fst	FDA	FEM	FLC	FLQ	FLT	FMO	FSJ	FCA	FON	FVE	SBA	SES	SHO	SLO	SMA	SPO	SSM	SCS	SCE	SPE	CED	CER	CER2	CSJN	CAB	H	REP	AQEB	AQSM
FDA	0.00	0.78	0.76	0.07	0.83	0.84	0.78	0.66	0.74	0.10	0.71	0.75	0.68	0.71	0.86	0.89	0.76	0.62	0.69	0.70	0.95	0.94	0.95	0.90	0.74	0.73	0.78	0.67	0.71
FEM		0.00	0.24	0.78	0.41	0.61	0.67	0.51	0.56	0.77	0.27	0.43	0.38	0.33	0.51	0.61	0.67	0.39	0.52	0.51	0.91	0.91	0.91	0.85	0.60	0.59	0.65	0.60	0.64
FLC			0.00	0.76	0.21	0.47	0.67	0.54	0.58	0.75	0.35	0.51	0.46	0.43	0.27	0.39	0.68	0.39	0.53	0.52	0.89	0.88	0.89	0.84	0.61	0.60	0.65	0.62	0.65
FLQ				0.00	0.81	0.81	0.78	0.67	0.74	0.07	0.71	0.76	0.69	0.72	0.84	0.87	0.77	0.63	0.70	0.70	0.93	0.93	0.93	0.89	0.74	0.73	0.78	0.69	0.73
FLT					0.00	0.52	0.73	0.59	0.66	0.80	0.48	0.63	0.53	0.56	0.13	0.25	0.72	0.43	0.59	0.58	0.94	0.94	0.94	0.88	0.67	0.66	0.72	0.63	0.67
FMO						0.00	0.74	0.59	0.67	0.80	0.55	0.66	0.55	0.60	0.64	0.75	0.72	0.39	0.57	0.55	0.96	0.96	0.96	0.89	0.67	0.66	0.74	0.61	0.64
FSJ							0.00	0.40	0.46	0.77	0.57	0.61	0.56	0.58	0.77	0.81	0.67	0.42	0.42	0.44	0.88	0.88	0.88	0.83	0.52	0.40	0.06	0.60	0.64
FCA								0.00	0.10	0.67	0.41	0.45	0.40	0.42	0.64	0.69	0.51	0.22	0.18	0.21	0.78	0.78	0.78	0.73	0.28	0.35	0.37	0.50	0.51
FON									0.00	0.73	0.43	0.50	0.41	0.44	0.73	0.79	0.50	0.20	0.17	0.21	0.87	0.87	0.87	0.79	0.31	0.35	0.42	0.48	0.50
FVE										0.00	0.70	0.75	0.68	0.71	0.83	0.86	0.76	0.63	0.69	0.69	0.92	0.92	0.92	0.88	0.73	0.72	0.77	0.68	0.72
SBA											0.00	0.13	0.18	0.06	0.56	0.64	0.56	0.32	0.40	0.41	0.86	0.86	0.86	0.80	0.51	0.47	0.54	0.51	0.54
SES												0.00	0.24	0.12	0.69	0.75	0.62	0.40	0.46	0.47	0.88	0.87	0.88	0.82	0.55	0.52	0.59	0.56	0.59
SHO													0.00	0.20	0.60	0.66	0.45	0.35	0.39	0.39	0.80	0.79	0.80	0.73	0.49	0.39	0.53	0.41	0.43
SLO														0.00	0.62	0.69	0.58	0.36	0.41	0.42	0.85	0.85	0.85	0.79	0.52	0.48	0.55	0.53	0.56
SMA															0.00	0.10	0.77	0.49	0.66	0.64	0.95	0.95	0.95	0.90	0.71	0.73	0.78	0.66	0.71
SPO																0.00	0.82	0.56	0.72	0.71	0.96	0.96	0.96	0.92	0.76	0.79	0.82	0.70	0.75
SSM																	0.00	0.47	0.51	0.51	0.80	0.78	0.79	0.68	0.59	0.28	0.63	0.04	0.05
SCS																		0.00	0.11	0.12	0.78	0.76	0.78	0.72	0.33	0.32	0.38	0.47	0.48
SCE																			0.00	0.13	0.84	0.83	0.84	0.77	0.31	0.34	0.38	0.49	0.51
SPE																				0.00	0.83	0.82	0.83	0.76	0.33	0.35	0.40	0.49	0.51
CED																					0.00	0.00	0.01	0.11	0.84	0.83	0.89	0.64	0.69
CER																					0.00	0.00	0.00	0.11	0.83	0.82	0.89	0.63	0.68
CER2																							0.00	0.11	0.83	0.83	0.89	0.64	0.69
CSJN																								0.00	0.78	0.73	0.83	0.57	0.61
CAB																									0.00	0.45	0.49	0.54	0.57
H																										0.00	0.35	0.29	0.29
REP																											0.00	0.58	0.61

AQEB																											0.00	0.04
AQSM																												0.00

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28 **Table S3.2.** Avergae F_{ST} coefficients within and among basins, lab trout and aquaculture farms.

F_{ST}	Río Fuerte	Río Sinaloa	Río Culiacán	Lab Trout	Aquaculture
Río Fuerte	0.60	0.56	0.83	0.59	0.62
Río Sinaloa		0.45	0.77	0.51	0.50
Río Culiacán			0.36	0.77	0.63
Lab Trout				0.35	0.44
Aquaculture					0.04

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Genotyping-by-sequencing reveals the effects of riverscape, climate and interspecific introgression on the genetic diversity and local adaptation of the endangered Mexican golden trout (*Oncorhynchus chrysogaster*).

Conservation Genetics

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Online Resource 4. Ancestry coefficients obtained in faststructure for different K values (6, 7 and 8).

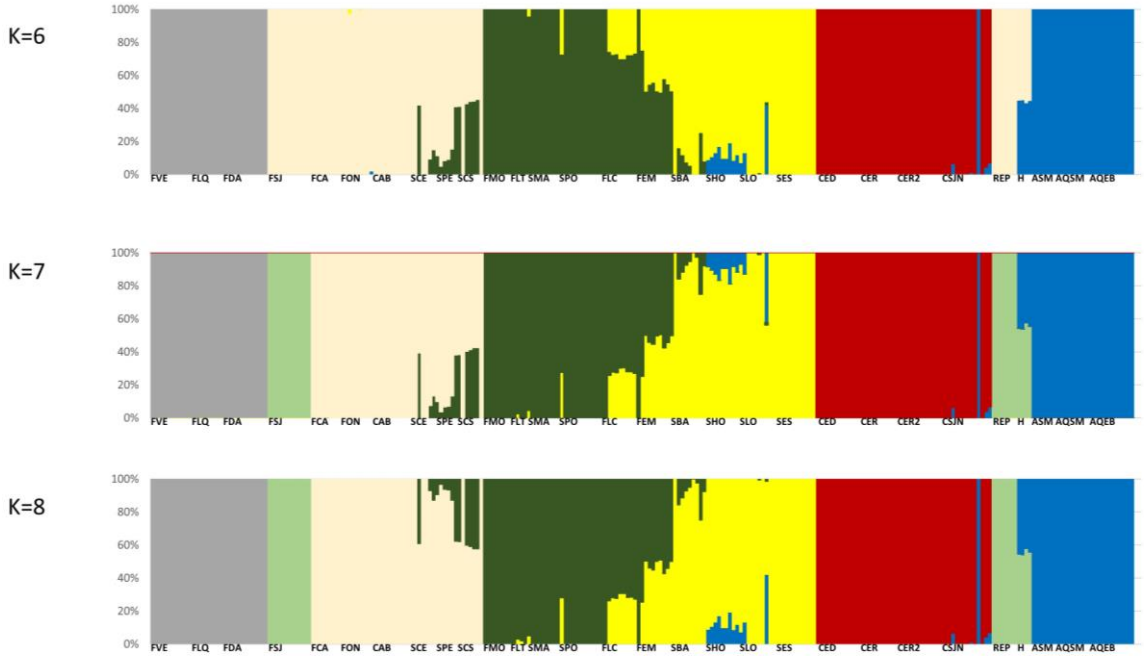

Figure S4.1. Ancestry coefficients obtained in faststructure for different K values (6, 7 and 8). Each color represents a different genetic cluster, the genome of each individual is represented by a vertical line and each sample site is represented by codes, including the initial of the river basin (F. Río Fuerte; S. Río Sinaloa; C. Río Culiacán) and an acronym of the locality Spanish name: Arroyo del Agua (FDA), Arroyo El Manzano (FEM), Arroyo Las Cuevas (FLC), Arroyo La Quebrada (FLQ), Arroyo Las Truchas (FLT), Arroyo Momorita (FMO), Arroyo San José (FSJ), Arroyo Caleras (FCA), Arroyo La Onza (FON), Arroyo Río Verde (FVE), Arroyo Baluarte (SBA), Arroyo El Salto (SES), Arroyo Hondo (SHO), Arroyo La Osera (SLO), Arroyo Macheras (SMA), Arroyo El Potrero (SPO), Arroyo San Miguel (SSM), Arroyo Cerro Solo (SCS), Arroyo Cebollín (SCE), Arroyo Pericos (SPE), Arroyo El Desecho (CED), Arroyo El Río (CER), Arroyo El Río 2 (CER2), Arroyo San Juan del Negro (CSJN) and Arroyo Agua Blanca (CAB). Additionally, trout from aquaculture facilities are included. El Barro Aquaculture Farm (AQBA), San Miguel Aquaculture Farm (AQSM), Mexican golden trout generated in laboratory (REP), and hybrids from Mexican golden trout and rainbow trout, produced in laboratory (H).

Genotyping-by-sequencing reveals the effects of riverscape, climate and interspecific introgression on the genetic diversity and local adaptation of the endangered Mexican golden trout (*Oncorhynchus chrysogaster*).

Conservation Genetics

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Online Resource 5. Ancestry coefficients obtained in faststructure for Dataset B with native trout without significant introgression.

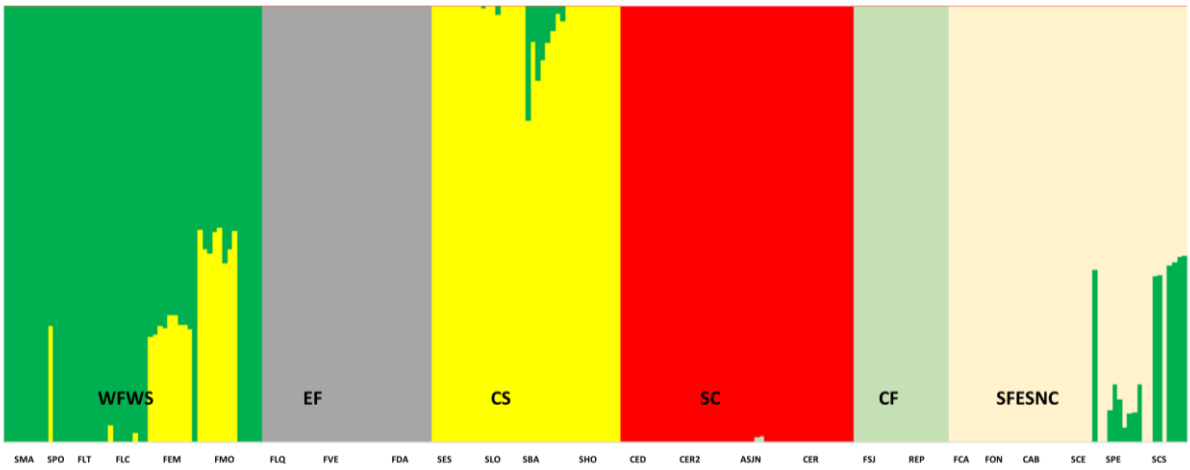

Figure S5.1. Ancestry coefficients obtained in faststructure for Dataset B with native trout without significant introgression. Each color represents a different genetic cluster: Eastern Fuerte (EF); Central Fuerte (CF); Southern Fuerte, Eastern Sinaloa and Northern Culiacán (SFESNC); Western Fuerte and Western Sinaloa (WFWS); Central Sinaloa (CS); Southern Culiacán (SC). The genome of each individual is represented by a vertical line and each sample site is represented by codes, including the initial of the river basin (F. Río Fuerte; S. Río Sinaloa; C. Río Culiacán) and an acronym of the locality Spanish name: Arroyo del Agua (FDA), Arroyo El Manzano (FEM), Arroyo Las Cuevas (FLC), Arroyo La Quebrada (FLQ), Arroyo Las Truchas (FLT), Arroyo Momorita (FMO), Arroyo San José (FSJ), Arroyo Caleras (FCA), Arroyo La Onza (FON), Arroyo Río Verde (FVE), Arroyo Baluarte (SBA), Arroyo El Salto (SES), Arroyo Hondo (SHO), Arroyo La Osera (SLO), Arroyo Macheras (SMA), Arroyo El Potrero (SPO), Arroyo Cerro Solo (SCS), Arroyo Cebollín (SCE), Arroyo Pericos (SPE), Arroyo El Desecho (CED), Arroyo El Río (CER), Arroyo El Río 2 (CER2), Arroyo San Juan del Negro (CSJN) and Arroyo Agua Blanca (CAB). Additionally, Mexican golden trout generated in laboratory (REP) are included.