

1 Population genetics and species distribution modeling highlight conservation needs of the
2 endemic trout from the Northern Sierra Madre Occidental

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32 **Abstract**

33 Recent climatic conditions are affecting biodiversity, particularly those species highly isolated
34 and at their distribution limits such as trout (*Oncorhynchus* sp.) inhabiting the Northern Sierra Madre
35 Occidental (NSMO) in Mexico, which is a current conservation concern due to their fragmented
36 distribution and anthropogenic threads. Here, we compiled previously published data from 24
37 microsatellite loci from multiple localities of trout throughout the NSMO to construct geographic genetic
38 structuring hypotheses. Our results confirm two genetically distinct subgroups within the NSMO
39 corresponding to the northern populations from Río Yaqui-Río Bavispe and Río Casas Grandes, and the
40 southern population from Río Yaqui-Río Sirupa and Río Conchos. In addition, we found a strong genetic
41 differentiation between most of the locations sampled. We assembled species distribution models (SDM)
42 to estimate the current distribution of trout. The SDM show that suitable environmental conditions may
43 exist between northern and southern populations but are highly fragmented. In addition, association
44 analyzes between F_{ST} and environmental factors show that the minimum temperature of the coldest
45 month, the precipitation of wettest month, and the compound topographic index were responsible for the
46 genetic structure. These results along with the limited mobility of the species, support limited
47 connectivity as a possible explanation for current north and south arrangement of diversity. This study
48 provides deep insights into the ecology and divergence processes within Northern Sierra Madre
49 Occidental trout, and for the urgent development of effective conservation strategies for each of those
50 lineages.

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52 Key words: Mexican trout, *Oncorhynchus* sp., NSMO, population genetics, Species Distribution
53 Modeling.

54
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66 **Introduction**

67 Recent increases in the frequency and intensity of climatic events are threatening biodiversity
68 and, with it, the welfare of humanity (Díaz et al. 2006). There is a great deal of documentation about how
69 species change in abundance and distribution in response to climatic oscillations in different ecosystems
70 (Hewitt 2001; Penuelas and Filella 2001; Walther et al. 2002) and freshwater ecosystems seem to be the
71 most vulnerable (Dudgeon et al. 2006). Predictions indicate that cold-water species will be particularly
72 affected by environmental changes (Comte et al. 2012). Stream temperature increase are already reducing
73 suitable habitat of most cold-water fish species (Eby et al. 2014), resulting in habitat fragmentation and
74 population reductions (Isaak et al. 2012). These climatic changes have a negative effect on the genetic
75 diversity due to reduced gene flow, genetic drift, and limited further adaptive evolution (Schierenbeck
76 2017), increasing populations risk of local extinction (Fagan et al. 2002). In addition, anthropogenic
77 activities such as land use change, the introduction of non-natives species and human overpopulation,
78 further increase these risks (Baillie et al. 2004). There is a growing interest in understanding how genetic
79 diversity have been particularly affected in species distribution limits. Here, we present the case of trout
80 (*Oncorhynchus* sp.) inhabiting the remote Sierra Madre Occidental (SMO here after) in Mexico, the
81 southernmost native salmonid populations in the world (Hendrickson et al. 2002).

82 The SMO, situated on the eastern side of the Mexican Plateau, has a surface of more than 250,000
83 km² and an elevation ranging from 300 to 3340 m, which makes it the largest mountain range in Mexico
84 (González-Elizondo et al. 2013). The topographic complexity of the region created by high mountains,
85 deep canyons, and an intricate pluvial system results in a highly diverse ecosystem for land flora and
86 fauna (Felger and Wilson 1995), as well as for freshwater communities (Felger and Wilson 1995; Mayden
87 et al. 2010). Moreover, the area is characterized by a unique biota and abundance in endemic species, alas
88 little is known about them (Mittermeier et al. 2004; Mayden et al. 2010; González-Elizondo et al. 2012).
89 The SMO can be subdivided into three regions: northern, central, and southern. The Northern SMO
90 (NSMO here after) is characterized by an irregular topography of mountainous terrain with grasslands and
91 coniferous forests (Felger et al. 1997; González-Elizondo et al. 2012). Four major rivers run through the
92 NSMO: the endorheic Río Casas Grandes (also known as Río Guzmán); the Ríos Yaqui and Mayo that
93 drain into the Gulf of California; and the Río Conchos which drains into the Río Grande (also known as
94 Río Bravo) (Ruiz-Campos et al. 2003; Hendrickson et al. 2006).

95 The taxonomic relationships of SMO trout have been studied using multiple approaches (Nielsen
96 et al. 1998; Nielsen & Sage 2001; Ruiz-Campos et al. 2003; Camarena-Rosales et al. 2007). However, no
97 formal descriptions have been performed on the NSMO trout despite recent studies finding trout from
98 these four basins are separate lineages from the rest of the SMO trout with a subdivision between north
99 and south (Abadía-Cardoso et al. 2015), and should be considered as two Evolutionary Significant Units

100 (ESU): one formed by the populations of the Casas Grandes, Mayo, and Yaqui basins, and the second
101 formed by trout from the Río Conchos (García-De León et al. 2020). In March 2019, four groups of trout
102 from the NSMO were listed in the IUCN Red List of Threatened Species under different status categories
103 (IUCN 2020). Trout from the Río Conchos is separated into north and south, and listed as Critically
104 Endangered and Endangered respectively. Río Mayo trout is categorized as Endangered. Finally, only
105 trout from the northern tributary Río Bavispe of the Río Yaqui (named Bavispe trout) was listed as Near
106 Threatened, while trout from the southern region (Río Sirupa) was not included in the listing. Previous
107 studies have shown that NSMO trout is considerably distinct from any other species or group of trout both
108 morphologically (Ruiz-Campos et al. 2003) and genetically (Nielsen et al. 1998; Camarena-Rosales et al.
109 2007; Abadía-Cardoso et al. 2015; García-De León et al. 2020). Unfortunately, the NSMO trout –like the
110 rest of the SMO, is facing multiple anthropogenic impacts such as logging, overgrazing by livestock, and
111 conversion of land for cultivation directly threatening trout (Hendrickson et al. 2002). NSMO trout are a
112 current conservation concern due to their limited and fragmented distribution. Also, for decades, the non-
113 native, hatchery grown Rainbow trout (*Oncorhynchus mykiss*) has been introduced in many NSMO
114 watersheds for aquaculture, representing another threat –that of genetic introgression– for the native trout
115 (Escalante et al. 2014; Abadía-Cardoso et al. 2015).

116 Genetic resources should be a priority for management and governance to support continuation of
117 populations of NSMO trout. Thus, the focus of this study was to promote innovative tools to efficiently
118 understand the changes in genetic diversity related to environmental variables. We built on previous
119 genetic studies to better resolve geographic patterns of genetic diversity among NSMO trout. We then use
120 species distribution models (SDM) to predict the amount of habitat that is available to trout (Gotelli and
121 Stanton-Geddes 2015; Marcer et al. 2016; Carneiro-Muniz et al. 2019). Recognizing how environmental
122 factors relate to the distribution of genetic diversity across the species range is fundamental to understand
123 species responses to environmental conditions and global change, and potential declines and extinctions
124 (Mabel 2019). According to the above, we might expect that the population genetic differences of NSMO
125 trout respond to local environmental conditions and the structure of hydrological basins. The results of
126 this study will provide further insights into the NSMO trout ecology, and divergence processes within the
127 area.

128

129 **Methods**

130

131 *Population genetics analysis*

132 Rivers of the NSMO in Mexico were surveyed for native trout between 2005 and 2007 (Figure 1;
133 Table 1). For details on collection, tissue preservation, and DNA extraction see Escalante et al. (2014),

134 Abadía-Cardoso et al. (2015), and García-De León et al. (2020). We combined genotypes previously
135 published in Abadía-Cardoso et al. (2015) and García-De León et al. (2020) consisting of 24
136 microsatellite loci (Online Resource 1). These previous studies evaluated the genetic associations of the
137 different groups of trout in Mexico in relation to other formally recognized trout species such as Rainbow
138 (*O. mykiss*), Cutthroat (*O. clarkii*), Apache (*O. apache*), and Gila (*O. gilae*) trout (Abadía-Cardoso et al.
139 2015), and recognized Evolutionary Significant Units for conservation (García-De León et al. 2020). Both
140 studies highlighted the importance and uniqueness of trout from the NSMO and recognized the need for a
141 more detailed evaluation of trout inhabiting the area. Here, we expect that, with the increase in the
142 number of loci, a better resolution of patterns of genetic diversity would be obtained. Therefore,
143 genotypes from a total of 232 individuals from 13 NSMO localities (one from Río Casas Grandes (n =
144 27); 10 from Río Yaquí (n = 175); one from Río Mayo (n = 15); and one from Río Conchos (n = 15)) were
145 analyzed (Table 2).

146 Stream sampling may accidentally capture family groups, and the inclusion of large numbers of full
147 sibling individuals may bias metrics of diversity and negatively impact the ability to infer population
148 structure. To avoid this issue, we used the program COLONY v2.0.6.5 (Wang 2004), which implements a
149 maximum likelihood method to infer kinship among individuals, using individual codominant multilocus
150 genotypes (Jones and Wang 2010). The program was run by locality, assigning males and females as
151 polygamous, without the presence of clones in the localities or inbreeding. Five long runs were performed
152 for each site with the full likelihood method with high precision and without updating allelic frequencies
153 because the sample sizes are less than 30, with 2000 random number of seeds. The analysis was
154 performed without defining sibship *a priori*. A genotyping error rate of 5% was considered and no
155 information about allelic frequencies was used. Individuals that presented a probability of sibling ratio
156 and half siblings greater than 0.7 were eliminated, taking this reference value as highly related.

157 Also, due to confirmed introductions of non-native hatchery Rainbow trout in some NSMO rivers
158 and streams, we explored the genetic introgression from Rainbow trout into native NSMO trout using the
159 admixture model in the program STRUCTURE 2.2 (Pritchard et al. 2000) with $k = 2$, and CLUMPP
160 (Jakobsson and Rosenberg 2007) and DISTRUCT (Rosenberg 2004) for visualization. We included
161 genotypes from four *O. mykiss* hatchery strains from California and two Rainbow trout hatcheries located
162 in Río Bavispe (data from Abadía-Cardoso et al. 2015). Due to data availability, we used a subset of 18
163 microsatellite loci from Abadía-Cardoso et al. (2015) to investigate this question.

164 We evaluated the genetic diversity within populations using different estimators. We estimated
165 expected (H_E) and observed (H_O) heterozygosities (Nei 1978), and tests on Hardy-Weinberg equilibrium
166 (HWE) using GENEPOP (Rousset 2008). Private alleles and allelic richness after rarefaction (El

167 Mousadik and Petit 1996) were calculated using the programming language R-based (4.0.3; R Core Team
168 2020) package *hierfstat* (Goudet 2005).

169 We applied complementary analytical methods that carry different assumptions (Bayesian,
170 multivariate, and dendrograms) to characterize population relationships and structure in NSMO trout at
171 the individual and population levels. The coefficient F_{ST} is widely used to assess genetic differences
172 between populations, and here we use it for the purpose of comparison. We estimated pairwise F_{ST} and
173 their significance after Bonferroni correction for multiple tests, evaluated with 10,000 permutations using
174 Arlequin 3.5 (Excoffier and Lischer 2010).

175 We explored genetic relationships at the individual level using the program STRUCTURE 2.2
176 (Pritchard et al. 2000) using all 24 microsatellite loci. This Bayesian admixture model-based clustering
177 analysis evaluates individual ancestry based on multilocus genotypes without using geographic
178 information –which reduces linkage disequilibrium and the level of Hardy-Weinberg disequilibrium
179 across separated loci. We evaluated k values from 2 to 5, with 10 iterations for each value and a burn - in
180 period of 50,000 steps and 150,000 Markov Chain Monte Carlo replicates. The software programs
181 CLUMPP (Jakobsson and Rosenberg 2007) and DISTRUCT (Rosenberg 2004) were used to visualize the
182 population clusters.

183 Principal components analysis (PCA) was performed on the genetic data using the R-based
184 package *adeigenet* 2.1 (Jombart 2008) to identify differences between individuals. This multivariate
185 approach of grouping is based on similarity coefficients or variance – covariance values of the component
186 traits of the entries.

187 Also, to illustrate the genetic relatedness among trout groups, a Neighbor joining unrooted
188 dendrogram of populations was generated using Cavalli-Sforza and Edwards (1967) chord distances in
189 the software package PHYLIP (Felsenstein 2005). Node support consistency was assessed using 1,000
190 bootstrap replicates and the resulting tree was visualized using Dendroscope 3 (Huson and Scornavacca
191 2012).

192 *Species distribution modeling and validation*

194 We assembled a georeferenced database of trout (*Oncorhynchus* sp.) inhabiting the SMO. The
195 location data of 13 records were from the rivers surveyed for native trout between 2005 and 2007 while
196 the other 86 records were obtained from the Hendrickson Lab Page
197 (<https://sites.cns.utexas.edu/hendricksonlab/map>). Preliminary data curation involved removing all
198 duplicate records of location. The remaining records were plotted on a digital map having a pixel of 1 km²
199 to detect geographically close records. Where two or more records occurred within a single pixel, only
200 one locality was retained in our final data set.

201 In this study, climatic and topographic variables were considered for developing the model. We
202 obtained current climatic data from the WorldClim–Global Climate Data v2 (Fick and Hijmans 2017).
203 Also, a digital elevation model (DEM) was downloaded from the USGS GTOPO HYDRO 1K project
204 (<https://earthexplorer.usgs.gov/>) and variables such as aspect, flow direction, flow accumulation, slope
205 and the compound topographic index (CTI) were derived from this DEM. All variables were fitted to a 1
206 km of pixel size and clipped to the study area whose lower boundaries were defined with elevations above
207 the 1,500 m.a.s.l. as recommended in Ruiz-Luna et al. (2017).

208 We extracted the values for each of the 25 variables (19 climatic + 6 topographic variables) for
209 each occurrence record using the package ‘raster’ v3.4-5 (Hijmans et al. 2020) and constructed a
210 correlation matrix in the R statistical software v4.0.3 (R Core Team 2020). To avoid collinearity and
211 provide non-redundant information, we removed highly correlated bioclimatic variables based on a
212 Pearson’s correlation coefficient greater than 0.8.

213 Once the variable set was reduced, we predicted the potential trout distribution by using six
214 commonly used single-model methods within the BIOMOD2 v3.4.6 package for R (Thuiller et al. 2009;
215 2020). These algorithms were generalized linear model (GLM), generalized additive model (GAM),
216 generalized boosting model (GBM), surface range envelop (SRE), classification tree analysis (CTA), and
217 random forest (RF). Considering that we did not have absence data, we created a 10,000 pseudo-absence
218 dataset randomly. Locality records were split into 75% for training and 25% to make model performance
219 evaluation. To reduce uncertainty, the procedure was repeated 10 times, which resulted in sixty models
220 for an ensemble forecast. We used the true skill statistics (TSS; Allouche et al. 2006) for a pre-evaluation
221 of the models. The TSS (sensitivity + specificity – 1) value has been suggested as an alternative to AUC
222 (area under the receiver operating curve) and Kappa statistics for measuring the accuracy of the SDM
223 projections (Allouche et al. 2006). In this study, we established a priori a TSS value > 0.7 as an
224 acceptable performance threshold (Swets 1988; Ribeiro et al. 2021). Thus, only those models with TSS >
225 0.7 were combined for the ‘ensemble’ map, that was then converted into binary presence-absence data by
226 setting the cut-off threshold determined by the minimum value of the confidence interval around the mean
227 probabilities.

228 Finally, to associate the genetic diversity of trout (*Oncorhynchus sp.*) with topographic and
229 environmental factors, we implemented a Generalized Dissimilarity Model (GDM; Ferrier et al. 2007) to
230 transform our predictors and visualize genetic patterns. We extracted the values of all variables from each
231 location and the F_{ST} table was used as the site-by-site genetic distance (dissimilarity) matrix within the R
232 package GDM v1.4.2 (Fitzpatrick and Keller 2015; Fitzpatrick et al. 2020). To fit the GDM model, the
233 standard three I-spline (partial regression fits) basis functions were calculated (Ferrier et al. 2007), while
234 the variable importance was quantified as the percent change of deviance explained permutating 50 times

235 the GDMs fitting. After that, we transform the geographic and environmental predictors using the fitted
236 functions into a multi-dimensional grid with predicted ecological distances. Finally, to reduce
237 redundancy, our results were submitted to a Principal Component Analysis (PCA) and the first three
238 principal components were mapped using a scaled (i.e., 0-255) RGB color space. These options
239 correspond to default values implemented in the R package GDM (Fitzpatrick et al. 2020).

240

241

242 **Results**

243

244 *Population genetics analysis*

245 Previously published genotypes from a total of 24 microsatellite loci from 232 individuals from
246 13 NSMO trout populations were examined (Abadía-Cardoso et al. 2015 and García-De León et al. 2020;
247 Online Resources 1). These data were first filtered for the presence of family structure. We detected full -
248 siblings with probabilities > 0.7 in trout from Río Casas Grandes, Río Bavispe (Las Guacamayas, Los
249 Cuarteles, and El Salto creeks), Río Sirupa, and Río Mayo (Online Resource 2). A total of 19 individuals
250 were removed from the database for a final number of 213 trout (Río Casas Grandes ($n = 24$), Río Yaqui
251 ($n = 160$), Río Mayo ($n = 14$), and Río Conchos ($n = 15$)) that were retained for downstream analyses
252 (Table 2). Also, we found minimal evidence of hybridization/genetic introgression from hatchery *O.*
253 *mykiss* into native NSMO trout as showed in the STRUCTURE analysis (Online Resource 3).

254 Estimated expected heterozygosity ranged from 0.05 (Río Conchos) to 0.52 (Río YS -
255 Banderella), with a mean value of 0.35 over the 13 population samples. Observed heterozygosities did not
256 depart from Hardy-Weinberg expectations at most locations except for the three localities with the lowest
257 diversity: Río Casas Grandes, Río Mayo and Río Conchos (Table 2). These deviations were driven by a
258 few markers in each locality and may be due to small population sizes and inbreeding. Allelic rarefied
259 richness ranged between 1.13 - 2.54, with Río Conchos showing the lowest allelic richness and Río YS -
260 Banderella the highest (Table 2). Private alleles were observed in all localities except for Río YB -
261 Guacamayas with the greatest number observed in arroyos from Río Yaqui-Sirupa (Table 2).

262 Pairwise F_{ST} values across all sampling locations were very high and significantly different from
263 zero ($P < 0.001$), except for ríos YB - Pedernal and YB - Largo (Table 3). Also, the STRUCTURE
264 analysis shows an overall pattern of population clustering according to geographic location. At $k = 2$,
265 trout from Río Yaqui separates into two clusters that concur with a geographic north (Río Bavispe) to
266 south (Río Sirupa) separation. There is an association between trout from Río Casas Grandes with that
267 from Río Bavispe, while the same association pattern is observed between Río Sirupa, Río Mayo, and Río
268 Conchos trout to the south (Figure 2). At higher k values, watersheds split (i.e., Río Casas Grandes),

269 however, there is not a clear geographic pattern of differentiation in the southern localities, where Río YS
270 - Salto separates at $k = 4$, while there is recent ancestry shared between the other Río Yaqui - Sirupa
271 tributaries, Río Mayo and Río Conchos that is not clearly resolved at $k = 5$.

272 The first three principal components (PCs) of the individual - based PCA plotted in Figure 3
273 account for 43.5% of the underlying variation. Locations Río Bavispe and Río Casas Grandes cluster
274 together in the left area of the plot and the Río Sirupa, Río Mayo and Río Conchos group roughly in the
275 right side. This result is consistent with that obtained with the STRUCTURE analysis.

276 The genetic dendrogram topography corroborates the patterns obtained with both STRUCTURE
277 and PCA. Population samples from both Río Bavispe and Río Sirupa form different clusters on the tree.
278 Also, Río Conchos is interspersed with Río Sirupa cluster. However, branch support was not strong for
279 most of the groups, and Río YB - Cuarteles and Río Casas Grandes do not seem to be resolved (Figure 4).

280

281 *Species distribution model (SDM)*

282 After data curation, a total of 60 occurrence records are reported, 13 of them corresponding to the
283 rivers surveyed for native trout, and the remaining 47 records from the Hendrickson Lab Page. After
284 removing variables that were highly correlated, the number of topographic variables was reduced from six
285 to three while the bioclimatic variables were reduced from 19 to six. The importance of each layer to the
286 formal model varied depending on the algorithm used. Each variable provided non-redundant information
287 that contributed to determining the optimal habitat for trout species (see Online Resource 4). Overall, the
288 variables with the greatest contribution were Precipitation of Driest Quarter (BIO17) followed by the
289 Minimum Temperature of Coldest Month (BIO06). The topographic variables CTI and slope were those
290 that contributed least to the models. Trout distribution seems to be favored when the precipitation of the
291 driest quarter is greater than 40 mm and the minimum temperature of the coldest month is below -2°C .
292 Where positive values in BIO06 are present, the potential distribution is low or null. The predictive
293 accuracies of the individual models are summarized in the Online Resource 5. Bold values in the Online
294 Resource 5 are those models that were retained and combined for the final 'ensemble' model, while the
295 shaded was considered the best model. TSS values for retained models (those with $\text{TSS} > 0.7$) ranged
296 from 0.705 (GLM and GBM) to 0.815 (GBM), while the mean TSS was 0.7451 (± 0.04). All models of
297 the SRE algorithms shows a poor performance with TSS ranged from 0.084 to 0.434 (mean = 0.263).

298 After clipping the watershed boundaries with elevations above 1,500 m, the study area was about
299 54,590 km^2 . The predicted distribution area using the new set of variables was about 14,447 km^2 . A wide
300 potential distribution is observed as an almost continuous patch distributed from north to south along the
301 headwaters of the basins except in the southeast part where the Río Conchos is located, and the potential
302 distribution is practically null. The predicted area is mainly characterized by evergreen forest (12,730

303 km²) followed by agriculture (1,106 km²) and grasslands (460 km²) in elevation ranges from 1,500 to
304 3,030 m.a.s.l., terrestrial diurnal temperatures from 9.5 to 16.2°C and annual accumulated precipitation of
305 327 – 1,109 mm. It is worth mentioning that, although two patches with the highest potential distribution
306 can be seen, one to the central - north part of the study area and the other less evident to the southeast
307 part, the locations within these areas of high habitat suitability are not always connected. These two main
308 regions with the highest probability of occurrence correspond to a north-south partition described above
309 (Figure 5).

310 The total deviance explained by the GDM including all the nine environmental and topographic
311 attributes plus geographic distances between sites was 85.6%. In terms of variable importance (obtained
312 by summing its I-spline coefficients), we found Minimum Temperature of Coldest Month (I-spline value
313 = 14.902), Precipitation of Wettest Month (2.423), and the Compound Topographic Index (2.118) that
314 represent the 65.7 %, 10.7 %, and 9.3%, respectively. However, two predictors (Slope and Max
315 Temperature of Warmest Month) were not important in determining patterns of genetic differentiation in
316 *Oncorhynchus* sp. (Online Resource 6). The results of the association of the genetic structure and
317 environmental factors indicate that genetic dissimilarity F_{ST} values start to appear significant at a distance
318 of around 0.5 degrees (~ 55km), elevations above 1900 m, and when the precipitation of the wettest
319 month is above 180 mm. This dissimilarity is greater when the minimum temperature of the coldest
320 month is below -4.0°C. The map of expected patterns in genetic turnover spatially projected by the GDM
321 is shown in Figure 6. The rapid turnover is predicted to the central and western part of the study area
322 (yellow and green colors), and comparative little (pink) elsewhere, consistent with the output produced by
323 the SDM.

324

325 **Discussion**

326 Our results confirm the overall low genetic diversity observed in NSMO trout (Table 2). We
327 recorded low levels of observed heterozygosity (mean H_o = 0.32) and allelic richness (mean A_r = 1.96)
328 compared to other trout species (*O. mykiss*: H_o = 0.7 and A_r = 6.12; Garza et al. 2014). Within NSMO,
329 trout from Río Yaqui showed higher observed heterozygosity (mean H_o = 0.37) and allelic richness
330 (mean A_r = 2.9) than other NSMO trout (Río Casas Grandes: H_o = 0.16 and A_r = 1.4; Río Conchos: H_o =
331 0.04 and A_r = 1.13). Nevertheless, a high number of private alleles was found across localities (Table 2).
332 These results could be the consequence of genetic drift, indication of long isolation periods and related to
333 small population sizes. Unfortunately, there are no studies on the abundance and biological data of the
334 populations in the NSMO trout, so it is urgent to monitor the species to know the dynamics of natural
335 populations.

336 Strong significant genetic differentiation was evident among all NSMO trout populations (mean
337 $F_{ST} = 0.33$) but one pair of sampled localities: YB - Arco - Arroyo Pederal and Arroyo Largo, which are
338 the closest localities geographically from the Río Yaqui basin (Table 3). F_{ST} values in this study are
339 among the highest reported in fish populations and similar to those reported in other isolated and
340 threatened trout species (e.g., brown trout, *Salmo trutta* (Apostolidis et al. 2008); marvel trout, *S.*
341 *marmoratus* (Fumagalli et al. 2002). This high genetic differentiation suggests interrupted gene flow
342 between tributaries as well as a long-term isolation and genetic drift owing to low effective population
343 sizes; the low heterozygosity and allelic richness values support this hypothesis.

344 Despite the strong genetic differentiation among localities, the data show two main clusters
345 consistent to a geographic (north and south of basin) division and it is consistent with previous reports
346 (Abadía-Cardoso et al. 2015; García-De León et al. 2020). Yaqui trout from the northern tributary Río
347 Bavispe shares ancestry with Río Casas Grandes, while the southern tributary Río Sirupa clusters with
348 Mayo and Conchos rivers (Figure 2). This result is invariable throughout the three different approaches
349 (STRUCTURE, PCA and Neighbor-joining dendrogram) used here. Particularly, the dendrogram
350 topology shows very long branches for the southern cluster localities supporting trout populations
351 (evolutionary lineages) that presumably have evolved in geographic isolation. This has been previously
352 observed. For example, *Oncorhynchus gilae*, a species with a presumed similar evolutionary origin than
353 the NSMO trout (Behnke 1992), patterns of differentiation and structure are most likely a consequence of
354 isolation and unique evolutionary diversity among lineages, possibly correlated to local adaptation
355 (Camak et al. 2021). Another example in the same SMO is *O. chrysogaster*, where river resistance is one
356 of the causes of isolation and genetic structure (Escalante et al. 2020).

357 One population of trout was particularly distinct relative to all other localities. Trout from Río YS
358 - Salto presented the highest F_{ST} (0.522 – 0.761) estimates among all Río Yaqui localities (Table 3). Also,
359 both the PC and STRUCTURE analyses shows a separate cluster for this locality. Geographically, Arroyo
360 El Salto is the most distant from the rest of the Río Sirupa tributaries (Figure 1). The robust differentiation
361 of trout of this tributary has not been previously identified and could be the result from an even longer
362 period of geographic isolation resulting in a very distinctive trout that could be consider a unique
363 Evolutionary Significant Unit or even a subspecies. Morphological and more genetic studies are necessary
364 to determine the taxonomic identity of this group of trout. These results and the vast geographic
365 complexity of the SMO suggest that undiscovered lineages may still be found.

366 Another interesting feature observed in the analyses is the genetic similarity between Río YB -
367 Cuarteles and Río Casas Grandes despite being geographically distant from one another. One explanation
368 for the observed pattern could be the inter - basin translocations by humans, which has been previously
369 assumed for Yaqui trout in Arroyos La Presita, Las Nutrias and El Largo (Ballesteros-Córdova et al.

370 2019), and observed in the area for other fish groups (i.e., stonerollers (*Campostoma* spp.; Schönhuth et
371 al. 2011)).

372 The strong genetic differentiation between tributaries in the Yaqui basin is concordant with the
373 topographic complexity of the study area and with the potential optimal distribution estimated by the
374 ensemble model. Bearing in mind that several authors (Allouche et al. 2006; Hodd et al. 2014) provide
375 interpretive guidelines for classifying model predictive accuracy (e.g., 0.01–0.20, Fail or null; 0.21–0.40,
376 Poor; 0.41–0.60, Fair; 0.61–0.80, Good; and 0.81–1.00, Excellent or high), and Ribeiro et al. (2021)
377 suggest that values above 0.5 are acceptable and values above 0.7 are considered good, 19 of our models
378 have TSS < 0.5 (32%), and the remaining 41 models (68%) have TSS > 0.5. Therefore, we presume our
379 final model is reliable. Excluding the SRE models, our results suggested at least one of the remaining
380 algorithms have a good to excellent ability for recognizing areas with elevated potential for the
381 occurrence of trout. Even when distribution models for freshwater fish species regularly include variables
382 of temperature, precipitation, drainage area, elevation, stream slope, discharge, wetted width, bankfull
383 width, depth, land use, and geology (McNyset 2005; Filipe et al. 2013; Mostafavi et al. 2014; Ruiz-Luna
384 et al. 2017), most of these variables were not evaluated in this study. The habitat suitability map for
385 NSMO trout was primarily affected by variables related to precipitation and temperature. Both variables
386 have been previously noted as influencing adaptive genetic variation in other trout species (Hand et al.
387 2016; Amish et al. 2019; Escalante et al. 2020) and affecting life history traits (i.e., dispersal, age at
388 maturity, fecundity, and survival) in salmonids (Crozier and Hutchings 2014; Hecht et al. 2015; Hand et
389 al. 2016). Temperature is particularly important for salmonids because they are ectothermic fish that
390 require cold and clear waters (Penaluna et al. 2016). Temperature and precipitation are tightly related
391 environmental conditions. Significant changes in precipitation regimes have been observed as
392 temperatures increase which, in turn, cause negative effects in salmonid species population growth and
393 survival (Haak et al. 2010; Ward et al. 2015). Our results are also concordant with those published for the
394 Mexican golden trout (*Oncorhynchus chrysogaster*), a species inhabit near the study area (Ruiz-Luna et
395 al. 2017; Escalante et al. 2018; 2020).

396 We did not detect strong signals of hybridization between native NSMO trout and introduced
397 Rainbow trout (Online Resource 3). The results of the association of F_{ST} and environmental variables
398 indicated that significant genetic differences are observed at distances of 55 km, perhaps these distances
399 are so small that they could prevent hybridization (Online Resource 6). Furthermore, no trout farms were
400 reported in the study area. However, previous studies have shown genetic introgression between other
401 native Mexican trout groups and non-native Rainbow trout (Escalante et al. 2014; Abadía-Cardoso et al.
402 2015). Isolation between native and non-native trout is strongly related to riverscape characteristics
403 (Escalante et al. 2020), nevertheless, the risk of introgression is still high as more aquaculture facilities

404 are established in the area. Moreover, studies in other trout species have predicted an increase in
405 hybridization as river temperatures increase because of global warming (Muhlfeld et al. 2014). On the
406 other hand, the ability to detect false negatives of introgression could be influenced by several aspects
407 such as sample size (too few individuals may fail to capture hybrid individuals), absence of fixed
408 differences in genetic markers selected (Allendorf et al. 2001), and stocking history where the
409 introgression can be impacted by number of stocking events, number of fish stocked, and distance to
410 stocking site (Escalante et al 2020). As mentioned above, the microsatellites used in this study have been
411 proven to detect hybridization when it exists (Escalante et al. 2014; Abadía-Cardoso et al. 2015), so it is
412 likely that non-detection of hybridization is real phenomenon.

413 It has been recognized that freshwater ecosystems are among the most endangered ecosystems in
414 the world with habitat fragmentation as one of the central threats to freshwater megafauna (Vörösmarty et
415 al. 2010; Reid et al. 2019; Rodríguez-Rey et al. 2019). The SDM indicates two strongly fragmented
416 potential suitable areas for NSMO trout, corresponding with the higher elevations near the headwaters of
417 the basins of the study area (above 1,500 m.a.s.l). These results are confirmed by the full GDM model. In
418 this study, both approaches identified the Minimum Temperature of Coldest Month (BIO06) between the
419 most important variables explaining trout distribution patterns. GDM additionally finds the Compound
420 Topographic Index (a dimensionless value that models water flow accumulation as a function of upstream
421 contributing area and slope) between the variables with the highest percentage of variance explained. The
422 CTI curve increases abruptly until a value of 500, suggesting that low values in CTI (usually related to
423 small streams and creeks) have effects on the genetic structure of trout. These two variables had already
424 been identified for their contribution to explaining the potential distribution of the Mexican golden trout
425 (*O. chrysogaster*; Ruiz-Luna et al. 2017). It is worth highlight that, although 13 records seem too few
426 records to examine multiple environmental correlations, the method used in the present study has been
427 previously reported for small sample sizes and with good predictions (Xu et al. 2017; Kaliontzopoulou et
428 al. 2018; Ingvarsson and Bernhardsson 2019).

429 The observed scenario reveals that these unique trout have limited opportunity for expansion,
430 therefore they are being put in serious challenge and warming stream temperatures will likely drive
431 declines of these cold-water specialists on the southern edge of their distribution (Escalante et al. 2020).
432 Particularly, Río Casas Grandes and Río Conchos trout seem to be outside the potentially suitable habitat,
433 which could indicate these trout are already experiencing very extreme environmental conditions such as
434 heat and drought that could be outside of the trout tolerance limits reducing the population size, as
435 evidenced by its lower values of intra-population genetic diversity (Table 2). The binational group
436 Truchas Mexicanas has previously recognized evident signs of population reduction of trout from Río
437 Conchos and the urgency of establishing recovery measures (Truchas Mexicanas 2006).

438 Our results indicate that it might be necessary to reconsider the categorizations of NSMO trout in
439 the IUCN Red List based on all evidence available. We believe that immediate conservation actions are
440 needed to reassure a successful conservation strategy for NSMO trout, such as ‘genetic rescue’. However,
441 all efforts to conserve this diverse group would be to no purpose if these trout are not formally described,
442 given that in Mexico the only taxonomic units under legal conservation status are named species.
443 Contrarily, significant evolutionary units and genetic populations are outside this protection. Then, a
444 long-term protection project should be undertaken by local communities so that effective conservation
445 methods ensure the survival of trout in the NSMO. Freshwater salmonids live in restricted areas at
446 considerable altitudes and many of the populations are small and highly sensitive to genetic drift and / or
447 local adaptation, due to this they are more exposed to local extinctions because of global warming and
448 various anthropogenic activities. The trout of NSMO represent a particular case little known in North
449 America.

450 In conclusion, delimitation of these groups of trout in the NSMO was supported by both
451 molecular population genetics and quantitative ecological space. This study provides deep insights into
452 the ecology and divergence processes within trout in the NSMO, and for the urgent development of
453 effective conservation strategies.

454

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729

730 **Figure captions**

731

732 **Fig. 1** Map of the Northern Sierra Madre Occidental, Mexico, including four basins: Río Casas Grandes,
733 Río Yaqui, Río Mayo, and Río Conchos. Sampling locations indicated in circles and numbers correspond
734 to localities in Table 1

735

736 **Fig. 2** STRUCTURE analysis of 213 trout from 13 localities from the Northern Sierra Madre Occidental,
737 Mexico (separated by black vertical lines). The analysis was used to group all individuals into different
738 numbers of genetic clusters (K), from 2 to 5. Individuals are represented by single vertical lines and the
739 colors in each line indicates the estimated fraction of ancestry from the inferred clusters. Individuals that
740 consistently share colors across K values are considered genetically similar. A total of 10 runs per K was
741 performed and the right column indicates the number of times that pattern was observed for each K value

742

743 **Fig. 3** Principal components analysis (PCA) of allele frequencies from 213 trout from 13 localities from
744 the Northern Sierra Madre Occidental, Mexico. a) First vs second principal components (PC1 vs PC2). b)
745 First vs third principal components (PC1 vs PC3). Coloration pattern indicates divergence between
746 individuals using the first three principal components

747

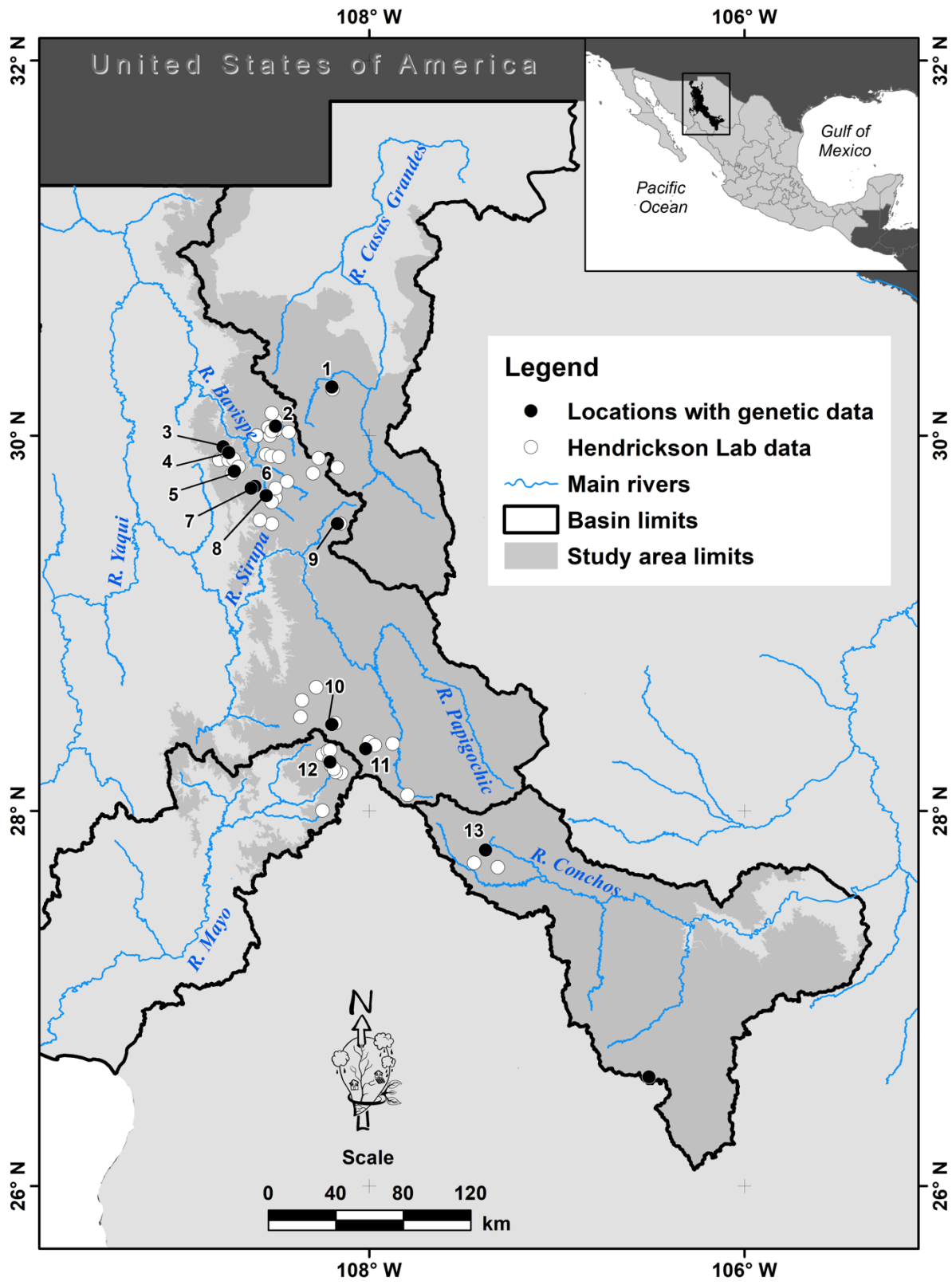
748 **Fig. 4** Unrooted neighbor-joining chord distance dendrogram of 13 trout populations from the Northern
749 Sierra Madre Occidental, Mexico. Branch labels are percentages from 1,000 bootstrap replicates. Internal
750 branches length is in proportion with the number of trees in which the branch was found

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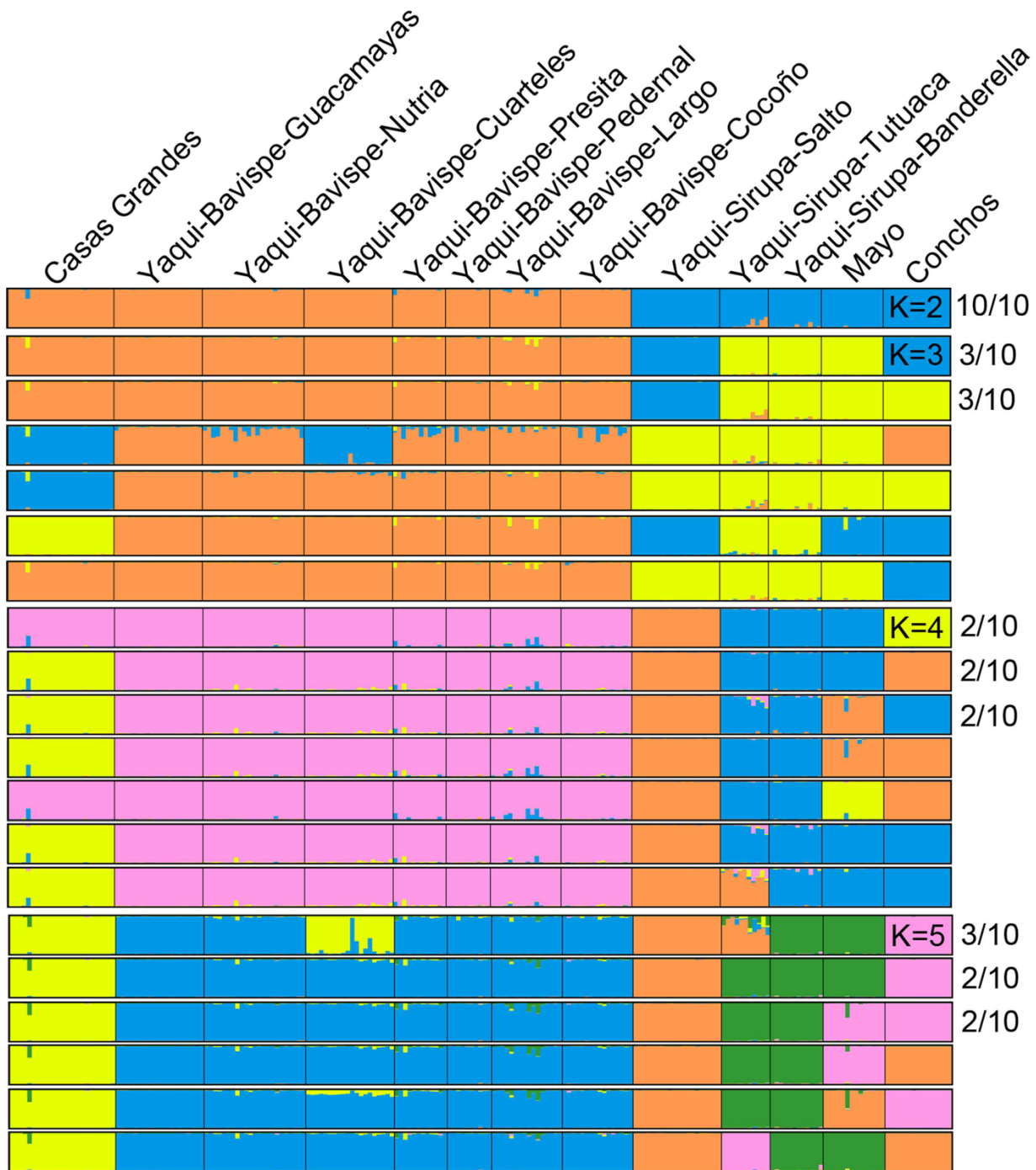
752 **Fig. 5** Ensemble distribution map of the genetic groups showing suitable areas under current climatic
753 conditions based on nine noncorrelated variables. Warmer colors show areas with higher probabilities of
754 occurrence

755

756 **Fig. 6** RGB color composites derived from a Principal Component Analysis of GDM-transformed
757 environmental predictors. Similarity of colors represent similarity in group relationships

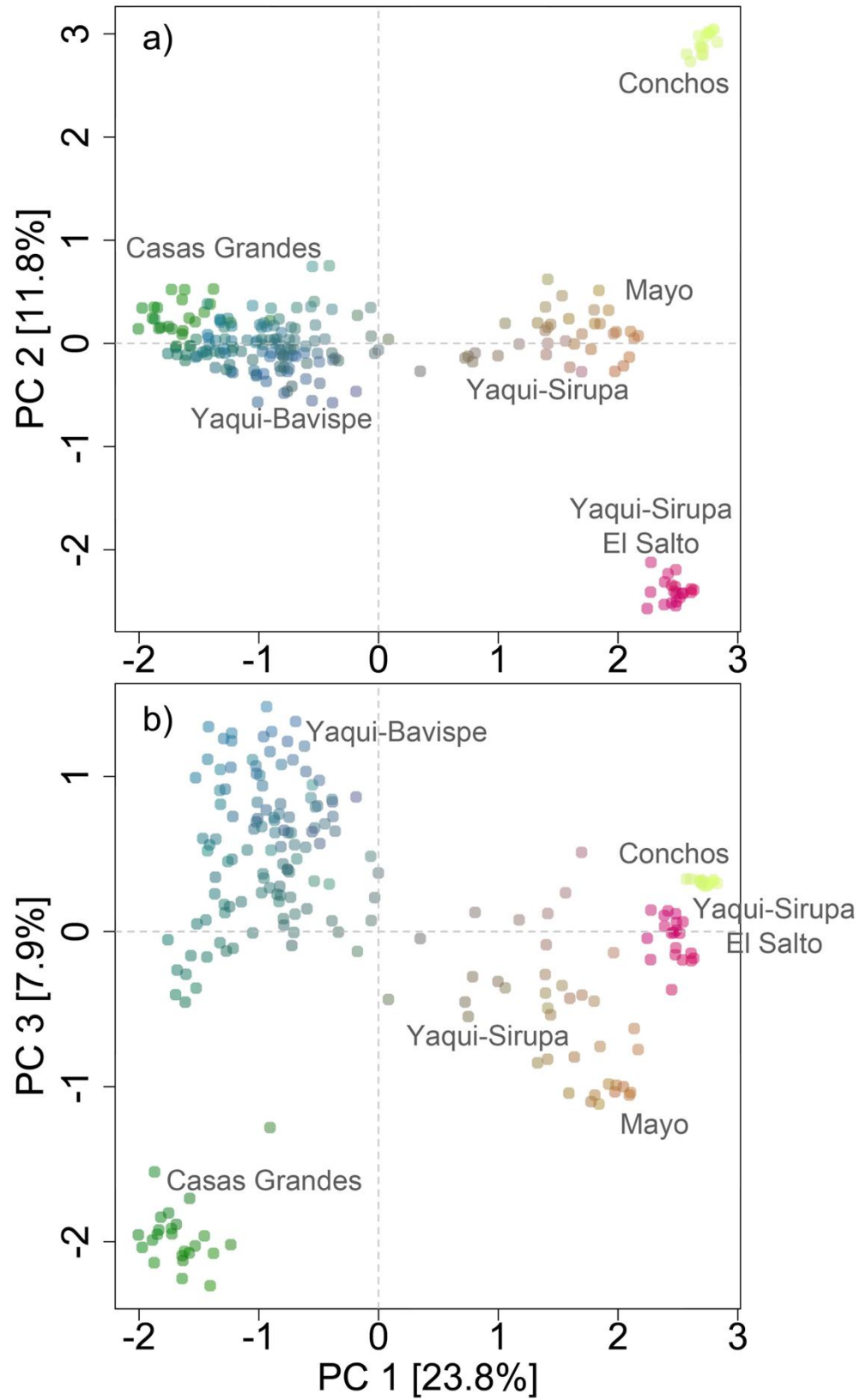


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760 Figure 1



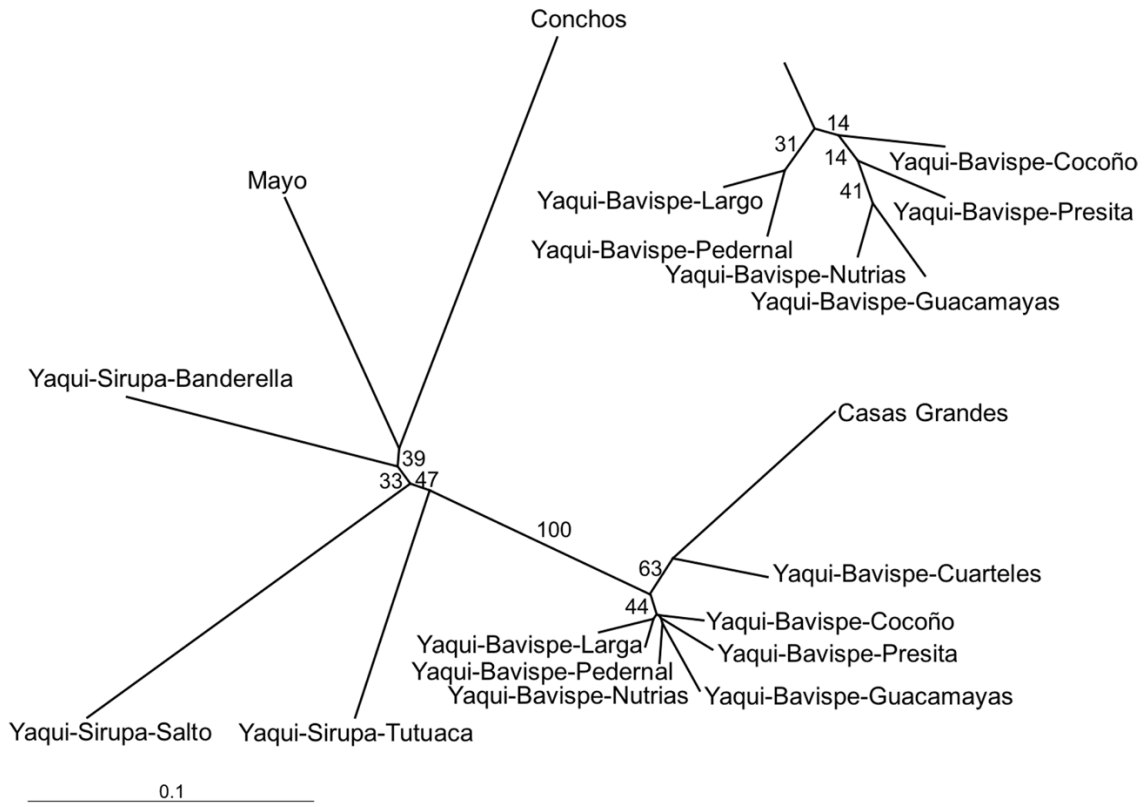
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Figure 2



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769 **Figure 3**



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771 **Figure 4**

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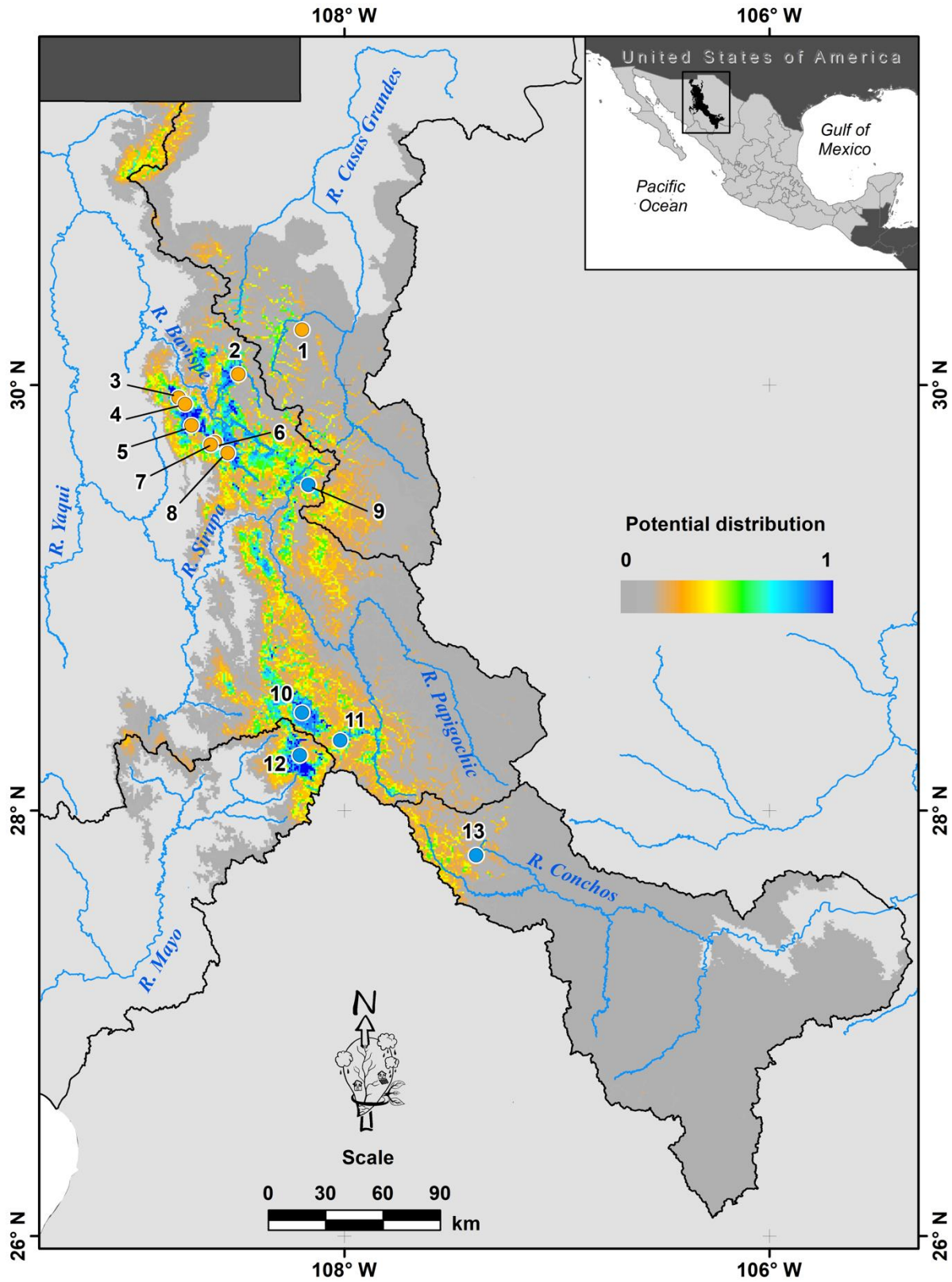
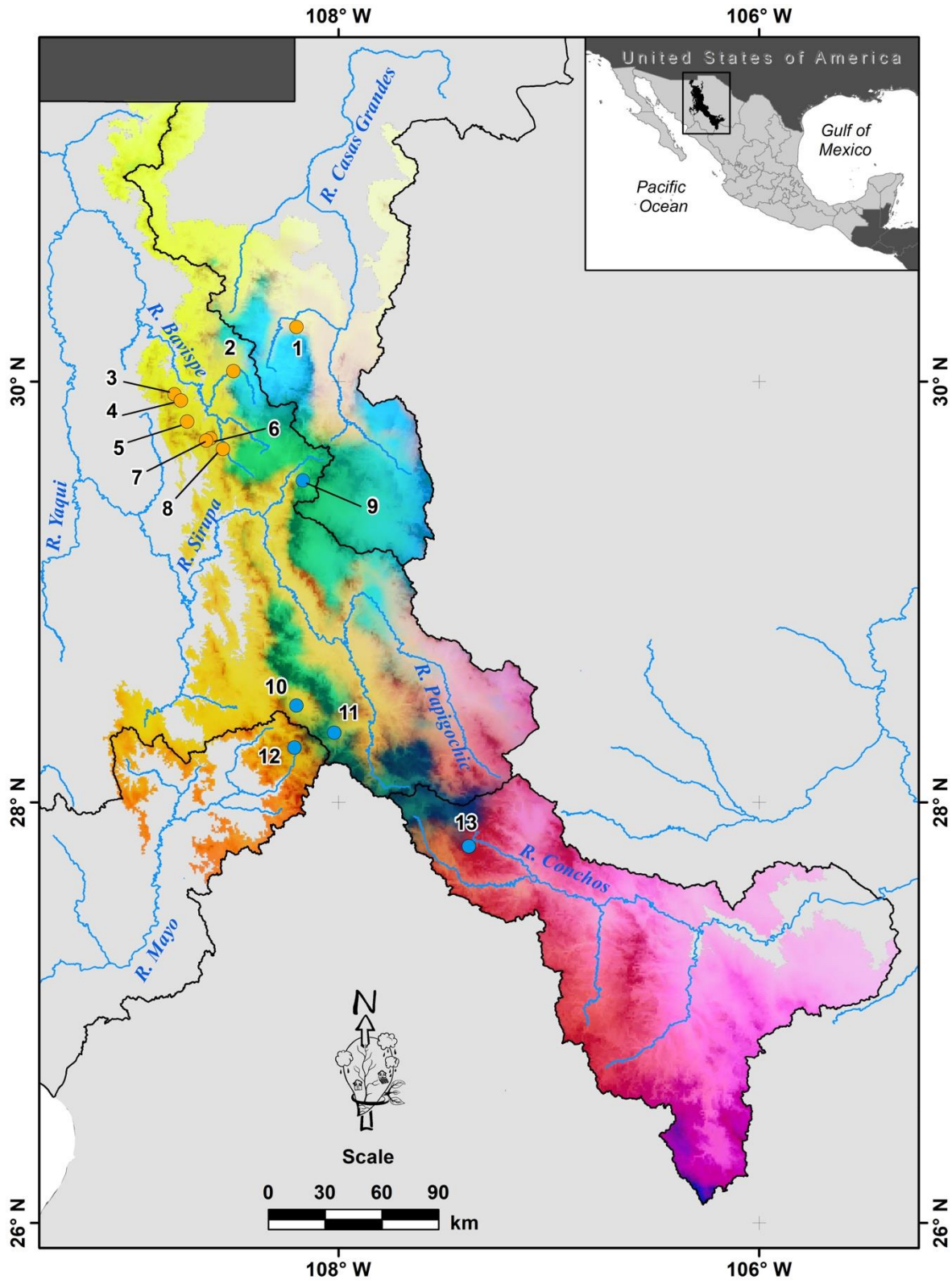


Figure 5



776

777

Figure 6

778 **Table 1** Sampling location of trout from the Northern Sierra Madre Occidental in México used in this
 779 study organized from north to south.

Pop no.	Basin	Subsain	Tributary	Creek	Location ID
1	Río Casas Grandes	Río Piedras Verdes	Arroyo Escalariado		Casas Grandes
2	Río Yaqui	Río Bavispe	Arroyo Las Guacamayas		YB - Guacamayas
3			Arroyo La Nutria		YB - La Nutria
4			Arroyo Los Cuarteles ¹		YB - Cuarteles
5			Arroyo La Presita		YB - Presita
6			Arroyo El Arco	Arroyo Pedernal	YB - Arco - Pedernal
7			Arroyo El Arco	Arroyo Largo	YB - Arco - Largo
8			Río Negro	Arroyo El Coccoño	YB - Negro - Coccoño
9		Río Sirupa	Río Papagochic	Arroyo El Salto	YS - Papagochic - Salto
10			Río Papagochic	Río Tutuaca	YS - Papagochic - Tutuaca
11			Arroyo Banderella ²		YS - Banderella
12	Río Mayo	Río Candameña			Mayo
13	Río Conchos		Arroyo Ureyna		Conchos

¹Also known as Arroyo El Cuartel

²Also known as Arroyo El Tecacote

780
781

782 **Table 2** Sampling location of trout used in this study from north to south and genetic diversity
783 estimations by population. N: number of samples; no sibs: total number of samples after removing
784 siblings detected with Colony; Lat: Latitude; Long: Longitude are given in decimal degrees; Ho:
785 Observed heterozygosity; He: Expected heterozygosity; Hardy-Weinberg probability test (*significant);
786 No Alleles: mean number of alleles per locus; pA: number of private alleles and Ar: allelic richness was
787 calculated after rarefaction.

Pop no.	Location ID	N	no sibs	He	Ho	HWE p-val	No Alleles	pA	Ar
1	Casas Grandes	27	24	0.17	0.16	<0.022*	1.96	6	1.41
2	YB - Guacamayas	23	20	0.35	0.30	0.33	2.48	0	1.94
3	YB - La Nutria	23	23	0.42	0.39	0.28	2.92	1	2.12
4	YB - Cuarteles	26	20	0.22	0.20	0.70	1.79	2	1.49
5	YB - Presita	12	12	0.46	0.39	0.49	3.16	5	2.30
6	YB - Arco - Pedernal	10	10	0.48	0.46	0.73	3.00	2	2.36
7	YB - Arco - Largo	16	16	0.48	0.45	0.20	3.76	15	2.43
8	YB - Negro - Coccoño	16	16	0.41	0.40	0.23	3.12	2	2.13
9	YS - Papagochic - Salto	21	20	0.18	0.19	0.80	2.12	18	1.52
10	YS - Papagochic - Tutuaca	13	11	0.45	0.41	0.49	2.88	18	2.21
11	YS - Banderella	15	12	0.52	0.52	0.65	3.72	22	2.54
12	Mayo	15	14	0.32	0.28	0.002*	2.64	17	1.90
13	Conchos	15	15	0.05	0.04	0.04*	1.24	6	1.13
Total		232	213						
Mean				0.35	0.32		2.68		1.96

788

789 **Table 3** Pairwise F_{ST} (below diagonal) and significance of p - values > 0.005 indicated by * (above
 790 diagonal) for all sampling location pairs of NSMO trout
 791

No.	Location (Creek or River)	1	2	3	4	5	6	7	8	9	10	11	12
1	Casas Grandes												
2	YB - Guacamayas	0.463		*	*	*	*	*	*	*	*	*	*
3	YB - La Nutria	0.367	0.114		*	*	*	*	*	*	*	*	*
4	YB - Cuarteles	0.484	0.357	0.250		*	*	*	*	*	*	*	*
5	YB - Presita	0.388	0.101	0.074	0.285		*	*	*	*	*	*	*
6	YB - Arco - Pedernal	0.393	0.110	0.050	0.229	0.039		-	*	*	*	*	*
7	YB - Arco - Largo	0.377	0.113	0.077	0.242	0.056	0.000		*	*	*	*	*
8	YB - Negro - Coccoño	0.416	0.119	0.041	0.242	0.073	0.021	0.056		*	*	*	*
9	YS - Papagochi - Salto	0.761	0.627	0.556	0.716	0.582	0.546	0.523	0.577		*	*	*
10	YS - Papagochi - Tutuaca	0.588	0.404	0.323	0.506	0.309	0.258	0.276	0.320	0.530		*	*
11	YS - Bandarella	0.579	0.424	0.359	0.546	0.316	0.294	0.292	0.368	0.528	0.267		*
12	Mayo	0.662	0.525	0.455	0.637	0.443	0.405	0.407	0.451	0.589	0.368	0.345	
13	Conchos	0.841	0.718	0.649	0.820	0.684	0.667	0.636	0.671	0.822	0.664	0.610	0.701

792