Supplemental information

Hybridization underlies localized

trait evolution in cavefish

Rachel L. Moran, James B. Jaggard, Emma Y. Roback, Alexander Kenzior, Nicolas Rohner, Johanna E. Kowalko, Claudia Patricia Ornelas-García, Suzanne E. McGaugh, and Alex C. Keene

1 **Table S1. Population genetic summary statistics, Related to Figure 2 and STAR Methods.**

2 Population sample sizes, nucleotide diversity (Pi) within populations, absolute genetic divergence

3 (Dxy) between populations, and relative genetic divergence (Fst) between populations.

Diversity within populations			Divergence between populations			
Population	**n**	**Pi**	**Population 1**	**Population 2**	**Dxy**	**Fst**
Chica Pool 1	5	0.0021	Chica Pool1	Chica Pool2	0.0021	0.0000
Chica Pool 2	14*	0.0021	Chica Pool1	Río Choy	0.0033	0.1440
Río Choy	9	0.0028	Chica Pool1	Pachón	0.0030	0.3501
Pachón	10	0.0008	Chica Pool1	Rascón	0.0030	0.2592
Rascón	10	0.0013	Chica Pool1	Los Sabinos	0.0024	0.2029
Los Sabinos	3	0.0007	Chica Pool1	Tinaja	0.0024	0.2364
Tinaja	8	0.0008	Chica Pool2	Río Choy	0.0033	0.1651
			Chica Pool2	Pachón	0.0030	0.3693
			Chica Pool2	Rascón	0.0030	0.2923
			Chica Pool2	Los Sabinos	0.0024	0.1154
			Chica Pool2	Tinaja	0.0024	0.2477
			Río Choy	Pachón	0.0035	0.3654
			Río Choy	Rascón	0.0033	0.2507
			Río Choy	Sabinos	0.0034	0.1900
			Río Choy	Tinaja	0.0034	0.3555
			Pachón	Rascón	0.0026	0.4572
			Pachón	Los Sabinos	0.0020	0.2832
			Pachón	Tinaja	0.0020	0.4430
			Rascón	Los Sabinos	0.0023	0.2002
			Rascón	Tinaja	0.0023	0.4003
			Los Sabinos	Tinaja	0.0008	0.0164

4 *Note: Chica Pool 2 had a sample size of 14 for genomic analyses but a sample size of 15 for

5 morphological and sleep analyses (one individual died before a tissue sample was collected for

6 sequencing).

Table S2. Results of D statistic and f4 ratio tests for introgression, Related to Figure 2. *A. aeneus* served as the outgroup. BBAA = derived alleles shared by P1 and P2. ABBA = derived alleles shared by P2 and P3. BABA = derived alleles shared by P1 and P3. Significant p-values (< 0.05) indicate evidence of introgression between Choy, Chica, Rascón, and Tinaja.

P1	P2	P3	D statistic	Z score	p-value	f4 ratio	BBAA	ABBA	BABA
Río Choy	Chica	Rascón	0.27893	21.4698	< 0.0001	0.173087	8409.82	4100.59	2311.94
Río Choy	Tinaja	Chica	0.14629	7.98383	< 0.0001	0.262394	2195.94	9381.5	6986.95
Chica	Tinaja	Rascón	0.409914	33.6245	< 0.0001	0.230212	8116.3	3375	1412.52
Rascón	Tinaja	Río Choy	0.2558	23.5379	< 0.0001	0.11872	5652.15	3207.89	1901.03

Table S3. Summary of ancestry analysis in hybrid individuals from Chica Pool 1 and Pool 2, Related to Figure 2. Mean minor parent (i.e. surface) tract lengths and mean major parent (i.e. cave) global ancestry proportions were used to infer the timing since admixture (T_{admix}), summary statistics for minor parent ancestry tract lengths in base pair, and estimated mean ± SE number of generations since the onset of admixture (T_{admix}) in Chica cave Pool 1 (n = 5) and Pool 2 (n = 14).

Pool	ID	MeanSurface TractLengthBP	MeanSurfaceTract LengthMorgans	MeanCaveGlobal Ancestry	T_{admix}
1	Chica5_1	47096.4	0.000546	0.770	2377.275
1	Chica5_2	49508.8	0.000574	0.759	2294.262
1	Chica5_3	49475.3	0.000574	0.750	2324.321
1	Chica5_4	51214.4	0.000594	0.744	2261.418
1	Chica5_5	49574.2	0.000575	0.751	2315.212
2	Chica1_1	51893.7	0.000602	0.739	2246.508
2	Chica1_10	48084.2	0.000558	0.755	2374.725
2	Chica1_11	48674.3	0.000565	0.762	2323.384
2	Chica1_12	47755.7	0.000554	0.755	2389.959
2	Chica1_13	51812.5	0.000601	0.768	2166.720
2	Chica1_14	52055.8	0.000604	0.755	2193.243
2	Chica1_2	50207.8	0.000582	0.761	2257.289
2	Chica1_3	48756.4	0.000566	0.754	2343.562
2	Chica1_4	49882.2	0.000579	0.755	2289.383
2	Chica1_5	48534.3	0.000563	0.767	2315.360
2	Chica1_6	49014.7	0.000569	0.773	2276.677
2	Chica1_7	48674.4	0.000565	0.755	2345.607
2	Chica1_8	49867.9	0.000578	0.741	2333.061
2	Chica1_9	51125.9	0.000593	0.745	2262.986
Pool	Min	Max	Median	Mean	SE
1	1,350	2,069,769	29,372	48,042	263
2	1,031	2,077,917	29,561	48,435	162
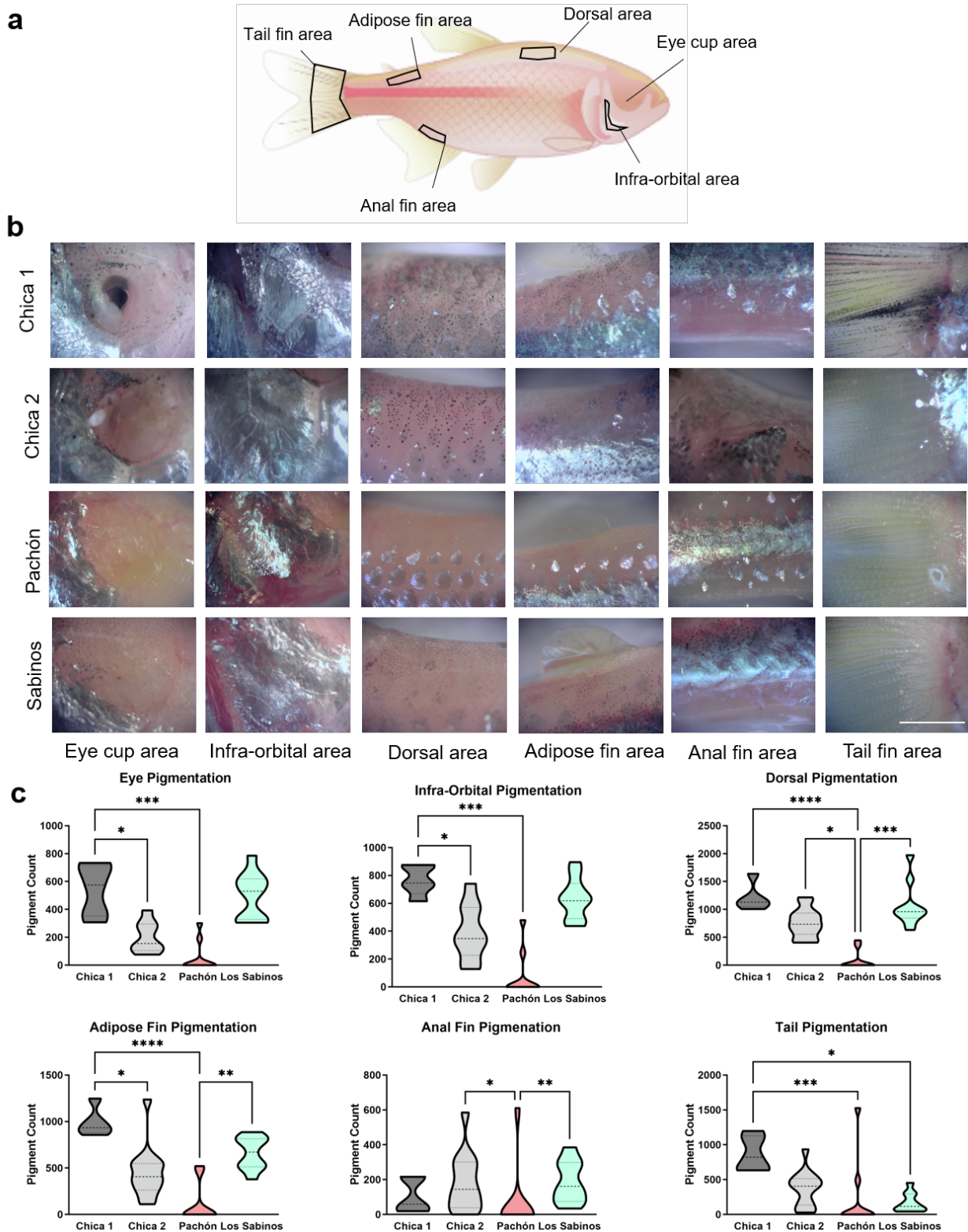
Figure S1. Eye and pigment morphology variations among cave populations, Related to Figure 1. (A) Diagram of areas used for pigmentation quantifications. (B) Brightfield images of

40 fish bodies showing pigmentation across multiple cave populations. (C) Pigment count

41 quantifications by area. Eye cup: Kruskal-Wallis test, $p < 0.001$, KW statistic = 24.28. Chica Pool

42 1 vs. Chica Pool 2, $p < 0.05$; Chica Pool 1 vs. Pachón, $p < 0.001$. Infra-Orbnital: Kruskal-Wallis

43 test, $p < 0.001$, KW statistic = 22.70. Chica Pool 1 vs. Chica Pool 2, $p < 0.05$; Chica Pool 1 vs.

44 Pachón, $p < 0.001$. Dorsal area: Kruskal-Wallis test, $p < 0.001$, KW statistic = 25.66. Chica Pool

45 1 vs. Pachón, $p < 0.001$; Chica Pool 2 vs. Pachón, $p < 0.05$; Pachón vs. Los Sabinos, $p < 0.001$.

46 Adipose area: Kruskal-Wallis test, $p < 0.001$, KW statistic = 23.65. Chica Pool 1 vs. Chica Pool 2,

47 $p < 0.05$; Chica Pool 1 vs. Pachón, $p < 0.001$, Pachón vs. Los Sabinos, $p < 0.01$. Anal fin: Kruskal-

48 Wallis test, $p < 0.01$, KW statistic = 12.44. Chica Pool 2 vs. Pachón, $p < 0.05$, Pachón vs. Los

49 Sabinos, $p < 0.01$. Tail area: Kruskal-Wallis test, $p < 0.001$, KW statistic = 16.87. Chica Pool 1 vs.

50 Pachón, $p < 0.001$, Chica Pool 1 vs. Los Sabinos, $p < 0.05$.

51

52

53

54

55

56

57

58

59

60

61

62
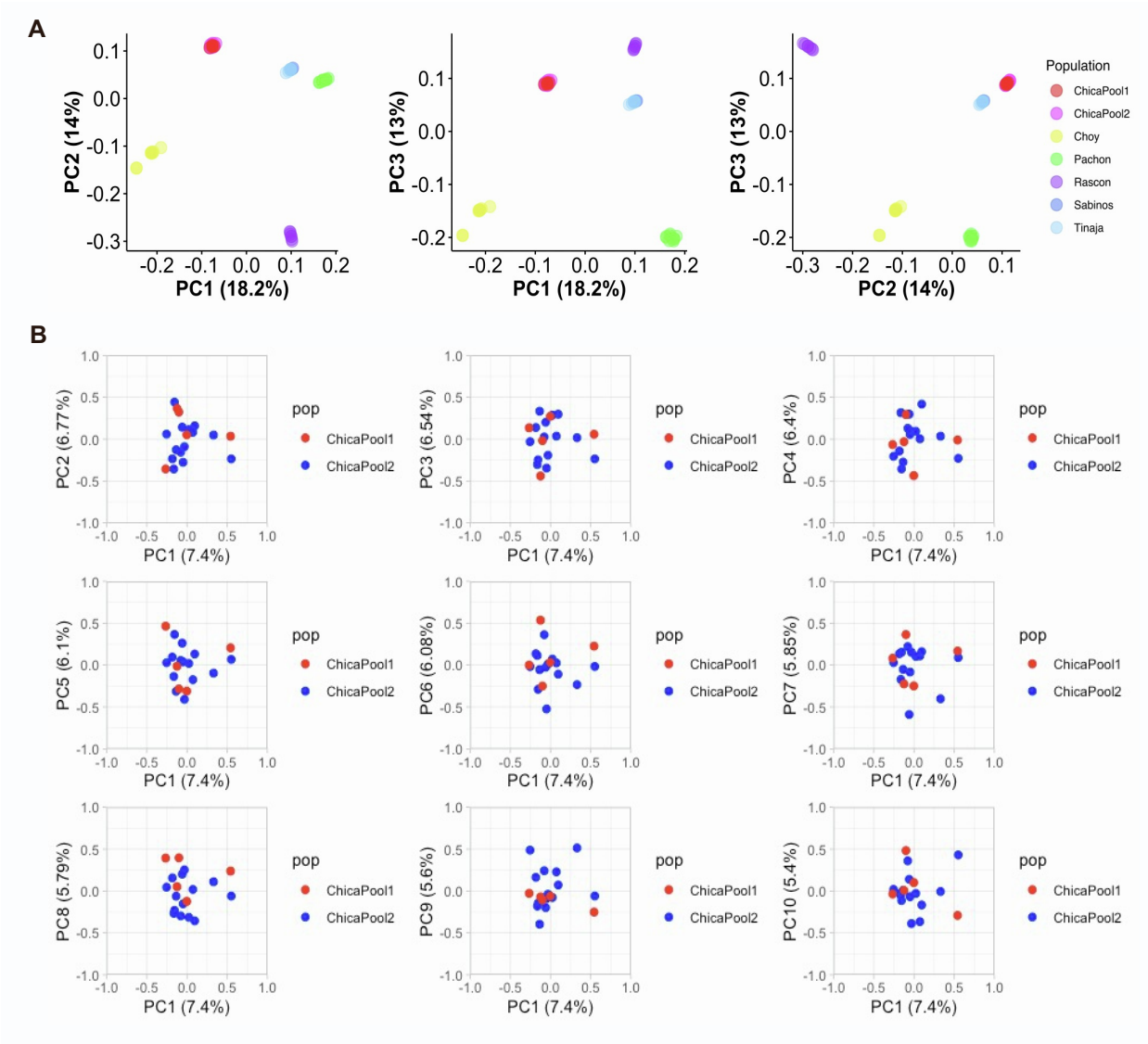
Figure S2. Visualization of PCA analyses, Related to Figure 1. (A) Biplots of scores for the first three PCs from the PCA on SNPs from cave (Chica, Pachón, Tinaja, Los Sabinos) and surface (Río Choy and Rascón) populations. Note that individuals from Chica Pool 1 and Pool 2 overlap and individuals from Tinaja and Los Sabinos overlap. (B) Biplots of PCs 1-10 for PCA including only Chica Pools 1 and 2. Note overlap of individuals from both pools.

70
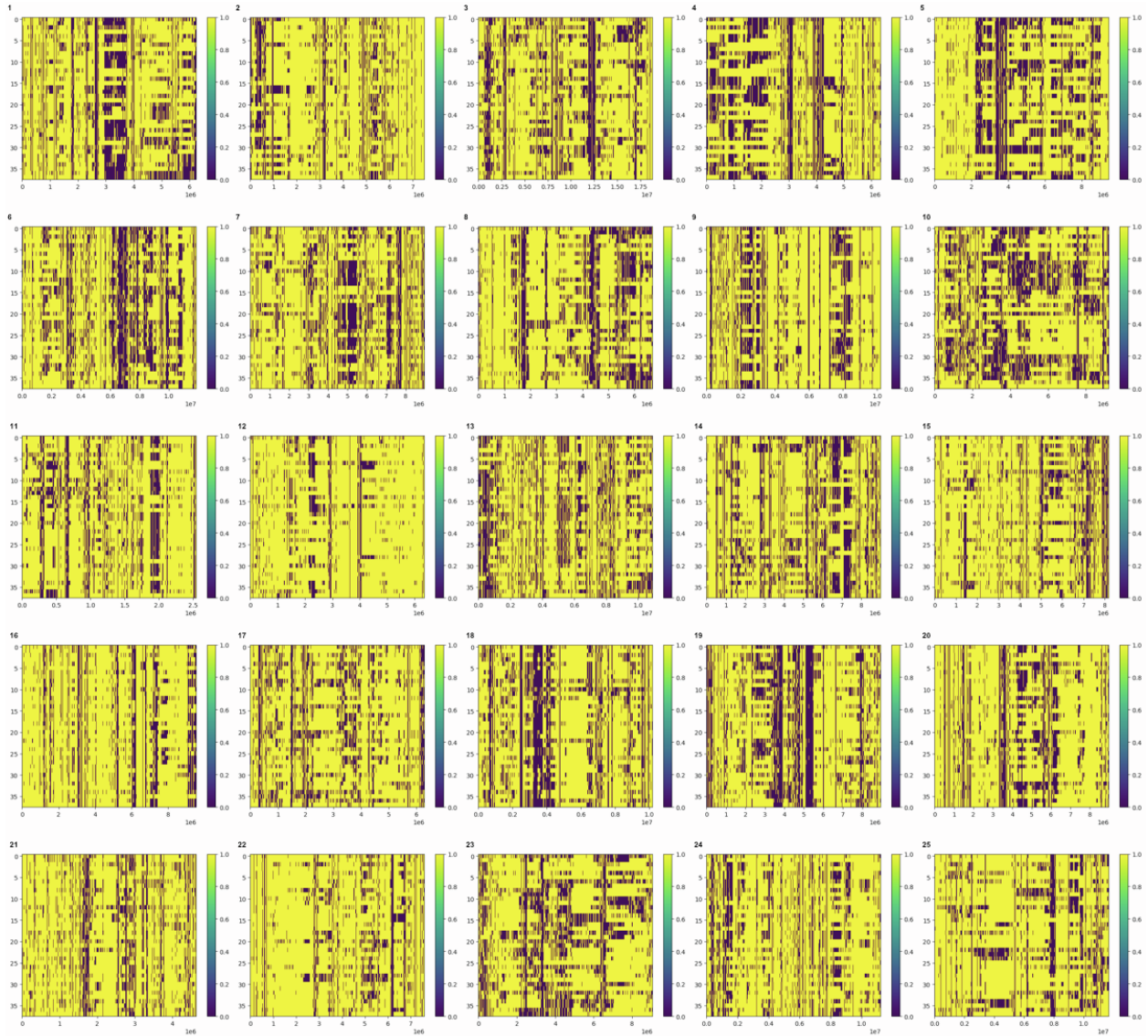
Figure S3. Visualization of local ancestry mapping in fish from Chica Pool 1 and Pool 2, Related to Figure 2. Local ancestry tracts in Chica samples inferred using a Hidden Markov Model approach along each of the 25 chromosomes. Yellow represents cave ancestry and purple represents surface ancestry. The y axis shows haplotypes 1 - 38, with haplotypes 0 - 27 corresponding to Chica Pool 2 (n = 14 diploid individuals), and haplotypes 28 - 38 corresponding to Chica Pool 1 (n = 5 diploid individuals). The x axis shows bp position along each chromosome.

77
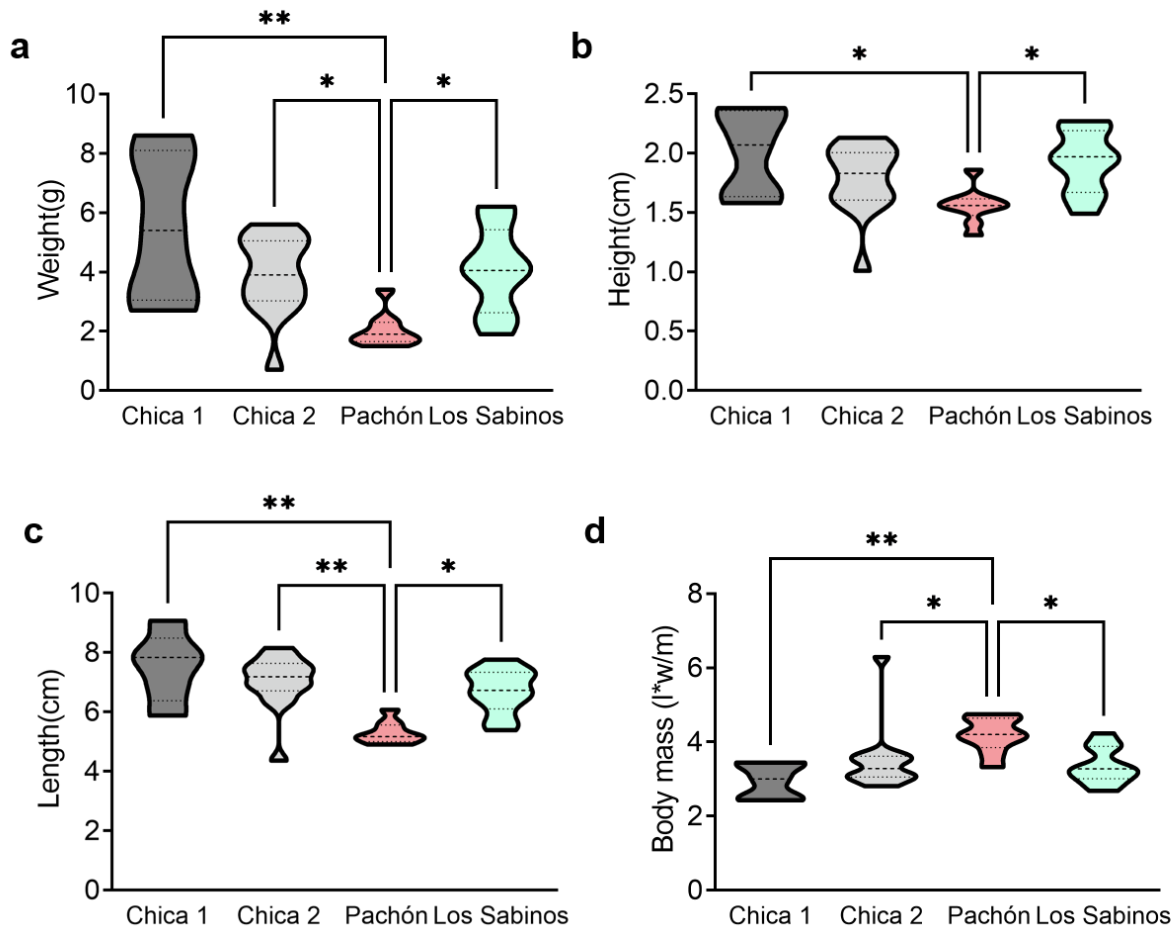
Figure S4. Physical morphology in cave populations of *A. mexicanus*, Related to Figure

1. (A) Pachón cavefish weigh significantly less than Chica Pool 1 and 2 and Los Sabinos

Kruskal-Wallis test, $p < 0.01$, KW statistic = 15.19. Chica Pool 1 vs. Pachón, $p < 0.01$; Chica

Pool 2 vs. Pachon, $p < 0.05$, Pachón vs. Los Sabinos, $p < 0.05$. (B) Body height from dorsal fin

to stomach is smaller in Pachón cavefish compared to Chica Pool 1 and Pool 2 as well as Los

Sabinos. Kruskal-Wallis test, $p < 0.01$, KW statistic = 11.90. Chica Pool 1 vs. Pachón, $p < 0.05$;

Pachon vs. Los Sabinos, $p < 0.05$. (C) Body length measured from mouth to tail is significantly

smaller in Pachón cavefish compared to all other cave populations. Kruskal-Wallis test, $p <$

0.001, KW statistic = 17.84. Chica Pool 1 vs. Pachón, $p < 0.01$; Chica Pool 2 vs. Pachón, $p <$

0.01, Pachón vs. Los Sabinos, $p < 0.05$. (D) Body mass is significantly larger in Pachón

90   cavefish compared to other populations Kruskal-Wallis test, $p < 0.01$, KW statistic = 14.41.

91   Chica Pool 1 vs. Pachón, $p < 0.01$; Chica Pool 2 vs. Pachón, $p < 0.05$, Pachón vs. Los Sabinos,

92    $p < 0.05$.