# Supplementary Materials

**Genetic identification and reiterated captures suggest that the *Astyanax mexicanus* El Pachón cavefish population is closed and declining**

Laurent Legendre[1,#], Julie Rode[2,#], Isabelle Germon[1,#], Marie Pavie[3,#], Carla Quiviger[1], Maxime Policarpo[1,$], Julien Leclercq[3], Stéphane Père[3], Julien Fumey[4,5], Carole Hyacinthe[6], Patricia Ornelas-García[7], Luis Espinasa[8], Sylvie Rétaux[3,*], Didier Casane[1,9,*]

[1] Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, Gif-sur-Yvette 91190, France
[2] Objectif Sciences International NGO, OSI-Panthera Program, Geneva, Switzerland
[3] Institut de Neuroscience Paris-Saclay, Université Paris-Saclay and CNRS, Saclay 91400, France
[4] Institut Pasteur, Université Paris Cité, CNRS UMR 2000, Microbial Paleogenomics Unit, Paris 75015, France
[5] Institut Pasteur, Université Paris Cité, Bioinformatics and Biostatistics Hub, Paris F-75015, France
[6] Department of Genetics, Harvard Medical School, Blavatnik Institute, Boston MA 02115, USA
[7] Colección Nacional de Peces, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, México DF CP 04510, México
[8] School of Science, Marist College, Poughkeepsie, New York 12601, USA
[9] Université Paris Cité, UFR Sciences du Vivant, Paris 75013, France

[#]Authors contributed equally to this work
[$]Current affiliation: Zoological Institute, Department of Environmental Sciences, University of Basel, Basel, Switzerland
[*]Corresponding authors, E-mail: sylvie.retaux@cnrs.fr; didier.casane@universite-paris-saclay.fr

**Supplementary Figure S1.** Analysis of allele frequency differences between sampling sessions using Fisher's exact tests. A) without correction for multiple tests, B) with Bonferroni correction, C) with Benjamini-Hochberg procedure to decrease the false discovery rate.
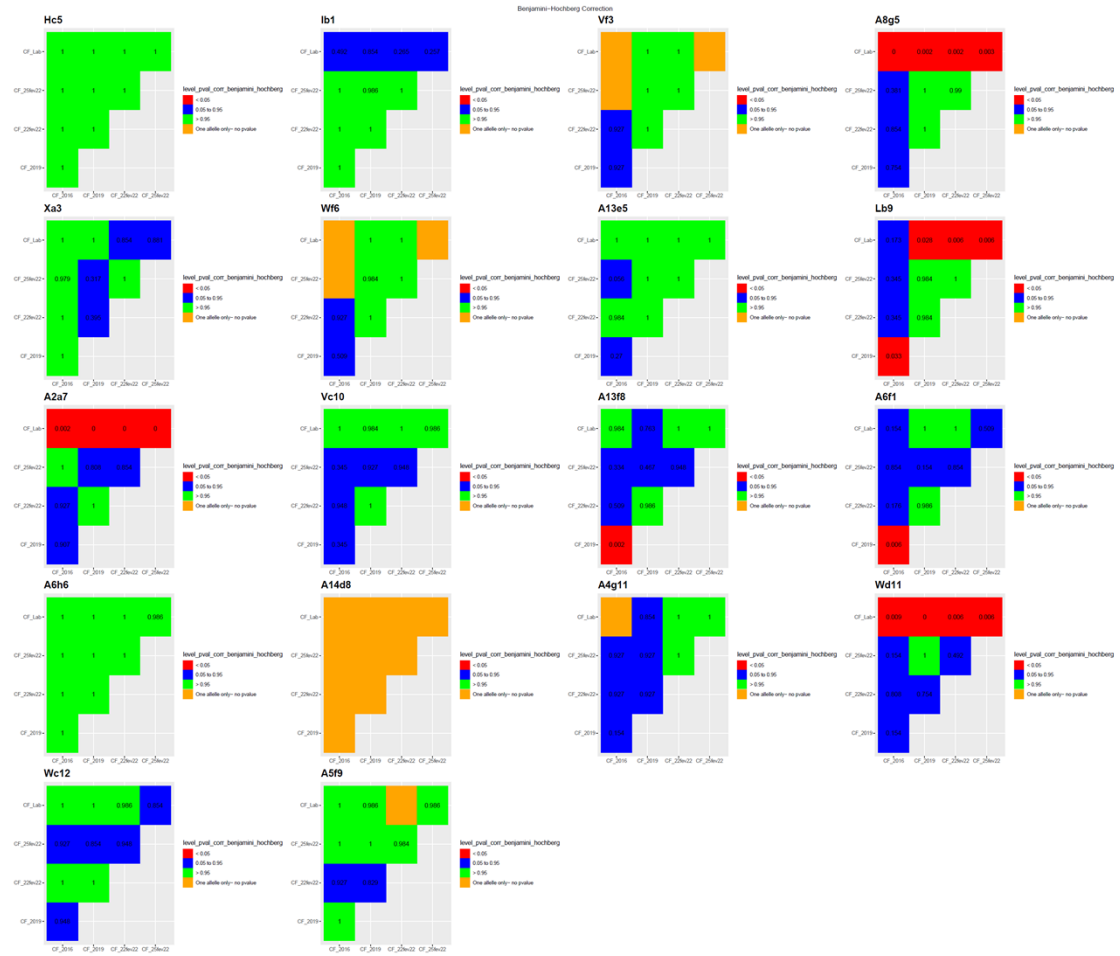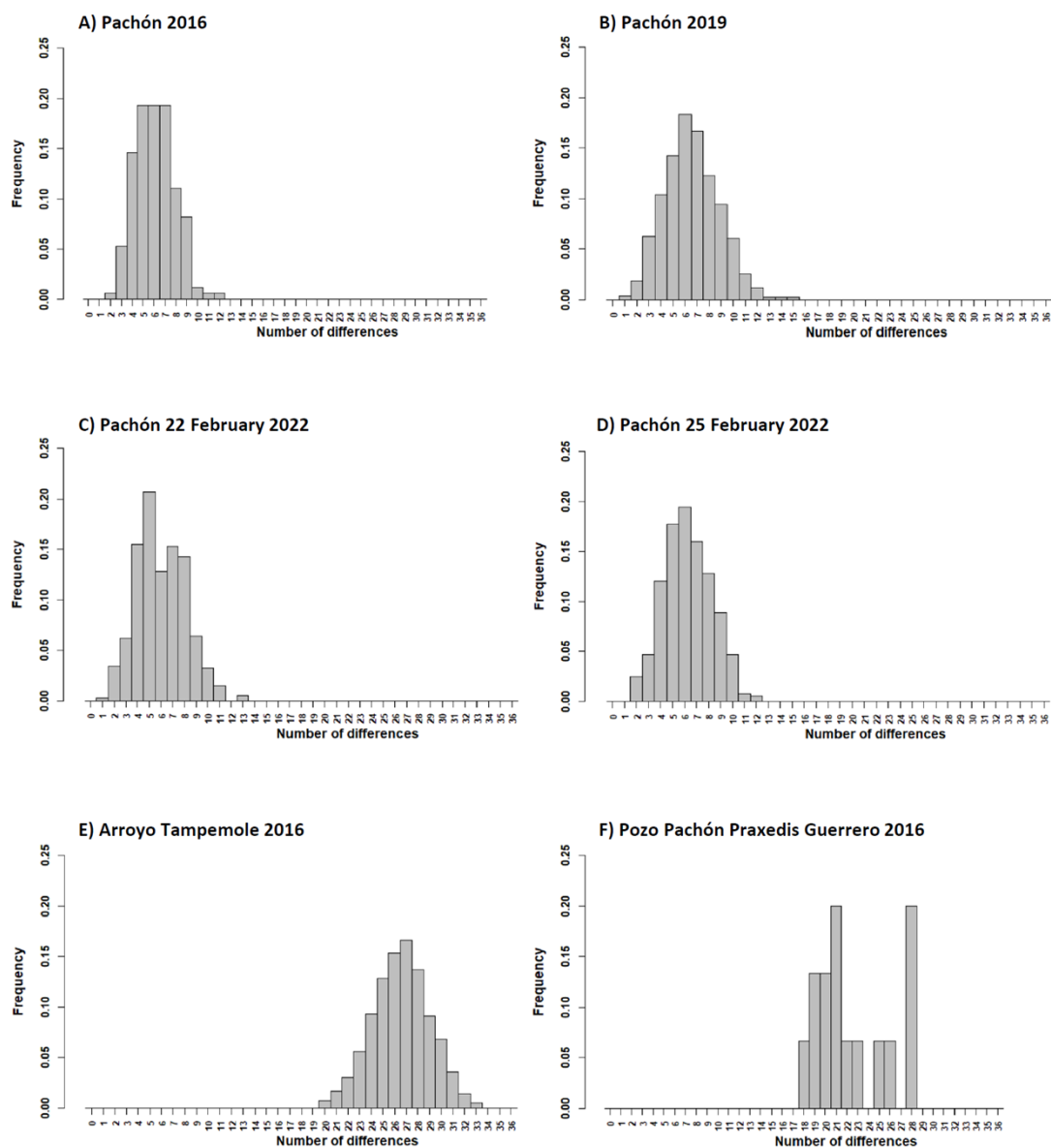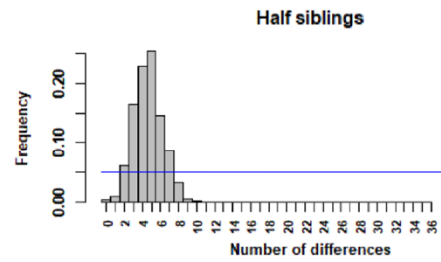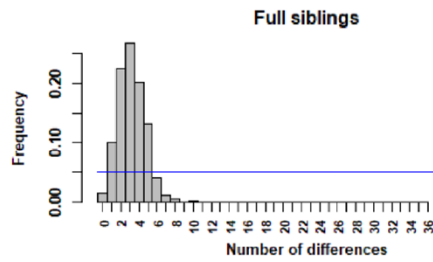
A)

B)

C)



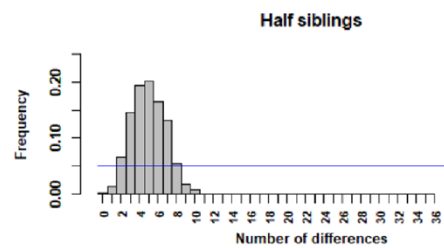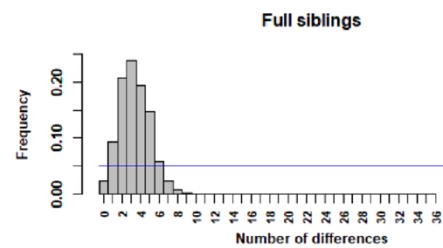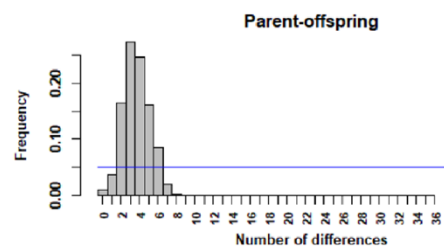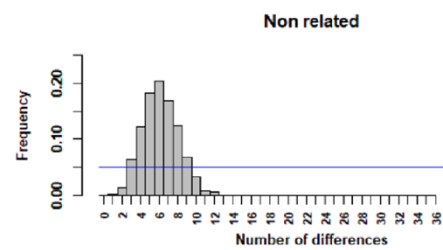Benjamini-Hochberg Correction
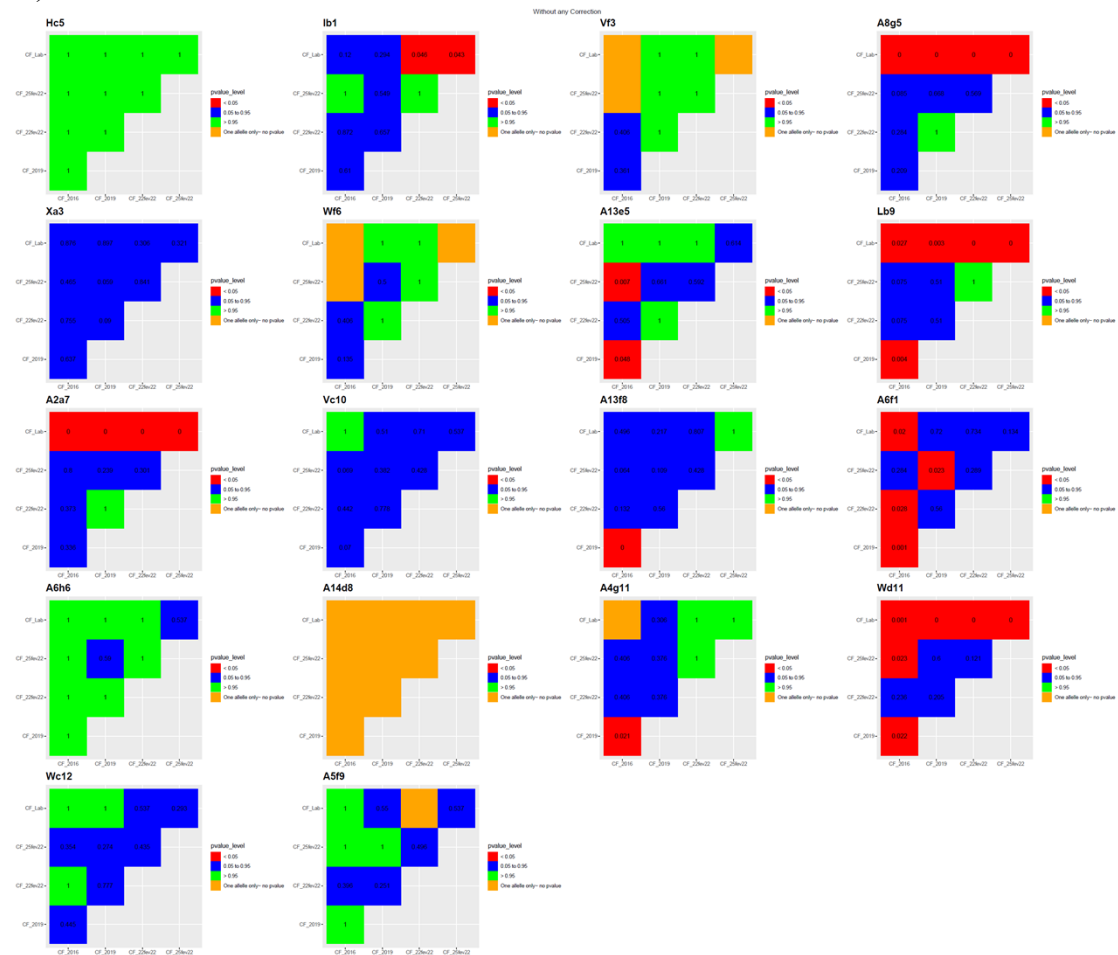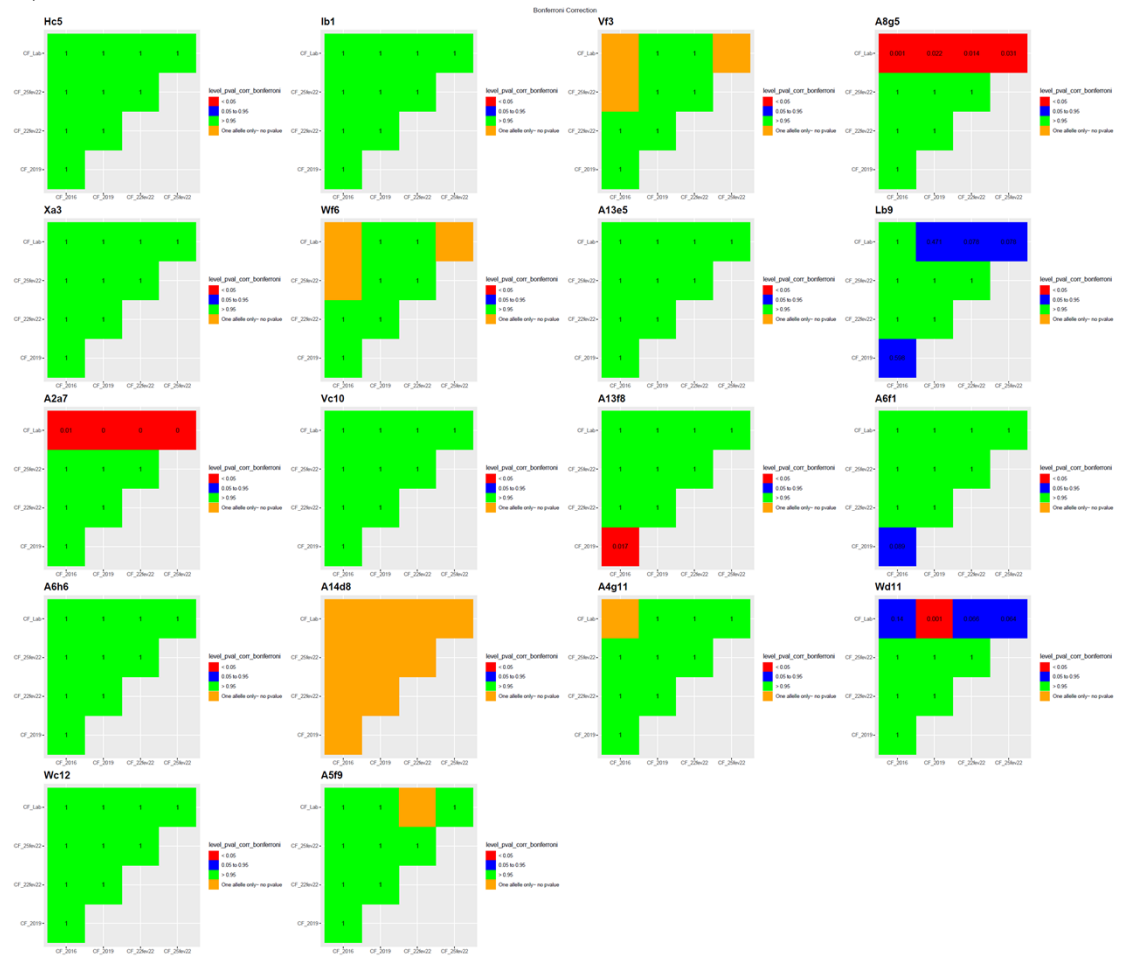
**Supplementary Figure S2.** Distribution of pairwise genetic distances (number of allelic differences). A) El Pachón 2016; B) El Pachón 2019; C) El Pachón 22 February 2022; D) El Pachón 25 February 2022; E) Arroyo Tampemole 2016; F) Pozo Pachón Praxedis Guerrero 2016; G) Expected distribution of pairwise genetic distances according to genealogical relationship in El Pachón 2016; H) Expected distribution of pairwise genetic distances according to genealogical relationship in El Pachón 2019; I) Expected distribution of pairwise genetic distances according to genealogical relationship in El Pachón 22 February 2022; J) Expected distribution of pairwise genetic distances according to genealogical relationship in El Pachón 25 February 2022; K) Expected distribution of pairwise genetic distances according to genealogical relationship in Arroyo Tampemole 2016; L) Expected distribution of pairwise genetic distances according to genealogical relationship in Pozo Pachón Praxedis Guerrero 2016.
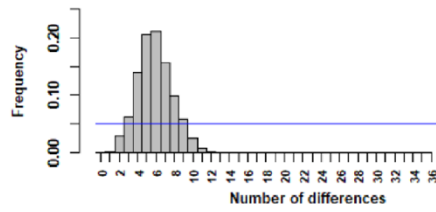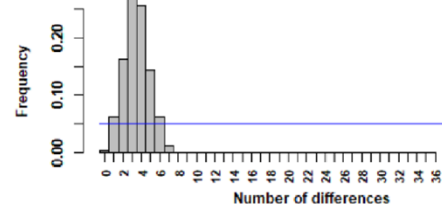
**G) Pachón 2016**



**Non related**

**Parent-offspring**

**Full siblings**

**Half siblings**

**H) Pachón 2019**



**Non related**

**Parent-offspring**
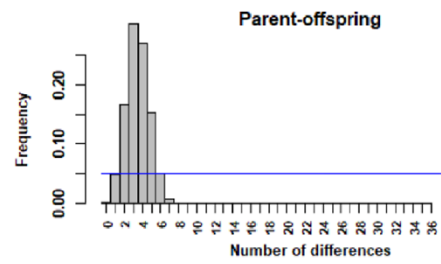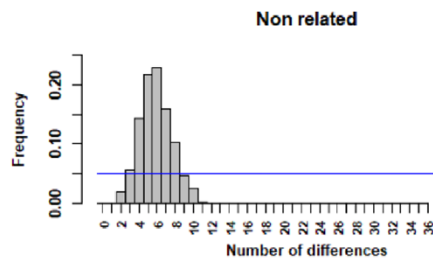
**Full siblings**

**Half siblings**

## I) Pachón 22 February 2022

**Non related**



**Parent-offspring**
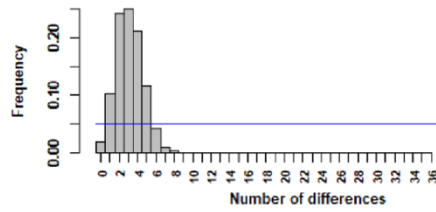


**Full siblings**
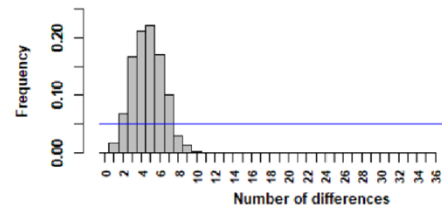


**Half siblings**



## J) Pachón 25 February 2022

**Non related**
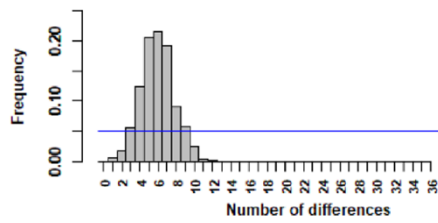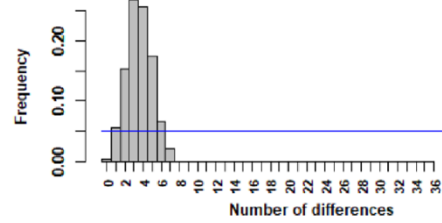


**Parent-offspring**
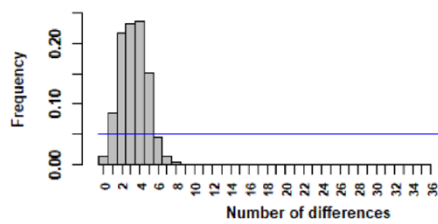


**Full siblings**
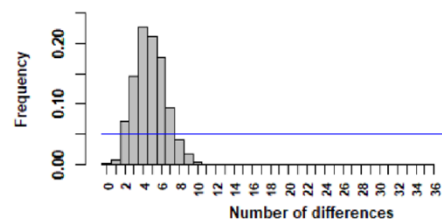


**Half siblings**

## K) Arroyo Tampemole 2016
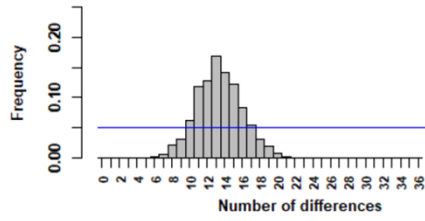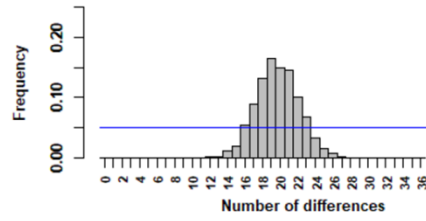
**Non related**
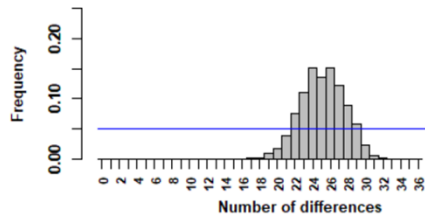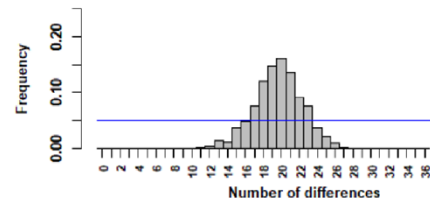


**Parent-offspring**
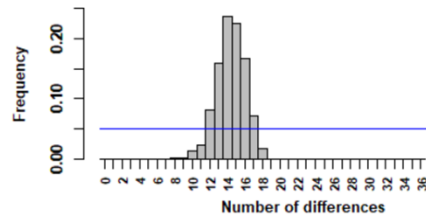


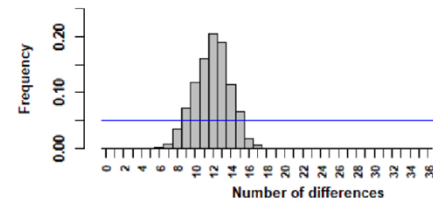**Full siblings**



**Half siblings**



## L) Pozo Pachón Praxedis Guerrero 2016
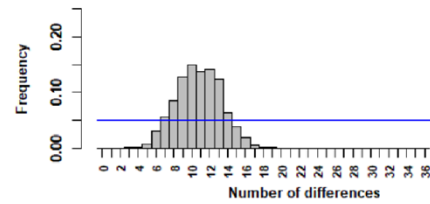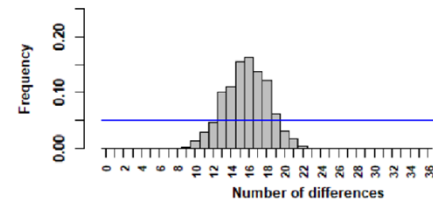
**Non related**



**Parent-offspring**



**Full siblings**



**Half siblings**

**Supplementary Table S1**: Sequences of the primers used to amplify the 18 microsatellite loci.

**Supplementary Table S**2: Fin clips and swabs genotypes obtained in the present study. Genotypes highlighted
in red are incomplete and were not used in demographic analyses.

**Supplementary Table S**3: Allele frequencies in A) Arroyo Tampemole 2016 and B) Pozo Pachón Praxedis Guerrero 2016.

**Supplementary Table S**4: Allele lengths and frequencies in El Pachón cave in 2008 as reported in Bradic *et al.* 2012.

**Supplementary Table S**5: Matrix of genetic distances between El Pachón genotypes obtained in 2016, 2019 and 2022.

**Supplementary Table S**6: Reliability of inferences of different kinships using ML Relate and according to allelic frequencies estimated during different sampling sessions.

**Supplementary Table S1–S6** are listed as separate Excel files due to their large size.