

Critically endangered species facing range retreats are not necessarily genetically depleted: the case of the endemic freshwater fish *Anaocypris hispanica*

Sousa-Santos C¹, Robalo JI^{1,2}, Francisco SM¹, Carrapato C³, Cardoso AC³, Doadrio I⁴

¹MARE – Marine and Environmental Sciences Centre, Lisboa, Portugal
²ISPA – University Institute, Lisboa, Portugal
³ICNF – Parque Natural do Vale do Guadiana, Mértola, Portugal
⁴Museo Nacional de Ciencias Naturales - CSIC, Madrid, Spain



For more information: carla.santos@ispa.pt



Joana Robalo
Presenting author

Summary

Assessing genetic diversity levels and population structure is fundamental for conservation and management of highly threatened species. In this study we use mitochondrial and nuclear markers to address phylogeographic and demographic data on the critically endangered *Anaocypris hispanica* (Cyprinidae). The populations of this species are strongly differentiated (high and significant Φ_{ST} and F_{ST} values, corroborated by the results from AMOVA and SAMOVA) and genetically diversified. We suggest that the restricted gene flow between populations may have been potentiated by ecological, hydrological and anthropogenic causes. Bayesian skyline plots revealed a signal for expansion for all populations (t_{MRC} between 68kya and 1.33Mya) and a genetic diversity latitudinal gradient was detected between the populations from the Upper (more diversified) and the Lower (less diversified) Guadiana river basin. Distribution models inferred for this species highlight the significant contribution of temperature seasonality and isothermality for the occurrence of *A. hispanica* in Guadiana environments and emphasize the importance of stable climatic conditions for the preservation of this species. Given the strong population structure found, high percentage of private haplotypes and virtual absence of inter-basin gene flow we suggest that each *A. hispanica* population should be considered as an independent Operational Conservation Unit.

Introduction

- *Anaocypris hispanica*
- one of the most threatened Iberian cyprinids
- endemic to Guadiana and Guadalquivir Rivers (Fig. 1A), with a typical intermittent regime.
- suffered a dramatic decline in its abundance and distribution range
- at the present occasionally caught in some restricted stretches, usually in low numbers
- its major threats include construction of dams and weirs, receding water levels, habitat degradation, proliferation of exotic species, increasing pollution and eutrophication
- Previous genetic studies highlighted the presence of fragmented populations with restricted gene flow
- fundamental knowledge about genetic variation, population structuring and intrapopulation genetic variability was lacking.



Fig. 1 A) Distribution range of *A. hispanica*. B) Location of the *A. hispanica* samples used in this study and status of all of the known populations of the species: populations for which the field work conducted between 2001 and 2014 confirmed the presence of the species and those for which no individuals were collected since 2001 (Cardoso and Carrapato 2010, Doadrio et al. 2011). C) Currently available habitats for *A. hispanica* populations considering that this species is absent from deep water habitats, namely in main courses and dam reservoirs (Cardoso and Carrapato 2010, Doadrio et al. 2011). When compared to the previously known distribution range, the effective area occupied by the species suffered a significant reduction in the last 15 years, with the virtual extinction of the species from several sub-basins (Carreiras, Vascão, Hago, Alcarache, Martiño, Caia, Aljucén, Ortigas, Guadalquivir, Fresnedoso, Estena and Valdeanque).

Results and Discussion

mtDNA

Gene diversity, mean number of pairwise differences and nucleotide diversity values were high for most populations. Haplotype diversity was generally low, except for Zújar. Only 9% of *cytb* haplotypes were shared.

All populations showed private haplotypes (~5.79 haplotypes/population), ranging from two (in Caia, Gévora and Carreiras) to 20 in Zújar. Genetic diversity varies along a latitudinal gradient, with the eastern populations from medium and upper Guadiana (Ardila, Estena and Zújar) showing higher values than the westernmost populations from lower Guadiana (Foupana, Odeleite, Vascão and Chança) - Fig. 2. Strong population structuring supported by significant corrected average pairwise differences and Φ_{ST} values, high overall fixation index, AMOVA results and SAMOVA's 6 groups scheme: Estena; Chança; Guadalquivir; Zújar; Ardila+Sillo; Odeleite+Foupana; and Vascão.

A. hispanica populations predate the Last Glacial Maximum and BSP's show that they were all expanding around 20kya.

Estimated values of t_{MRC} for *A. hispanica* populations dated back to less than 1.3My ago, which is in agreement with their estimated Late Pleistocene and Holocene ages (0.04 to 1.01My) based on corrected pairwise differences - Fig. 3. The t_{MRC} is higher for Estena and Zújar, corroborating the more ancient age of these populations differences - Fig. 3.

Nuclear DNA

S7 and rhodopsin genes showed low resolution.

Beta-actin haplotypes are grouped according to geographical provenience (Fig. 4): one clade groups the Guadalquivir and Upper Guadiana populations of Estena, Guadarranque and Zújar; and the other clade groups the Lower Guadiana populations of Ardila, Chança, Odeleite and Vascão and also individuals from Zújar (N=4). Thus, Zújar is the only population comprising haplotypes assigned to both clades.

Westernmost populations showed lower diversity than the easternmost populations, resembling the latitudinal trend observed for the mtDNA.

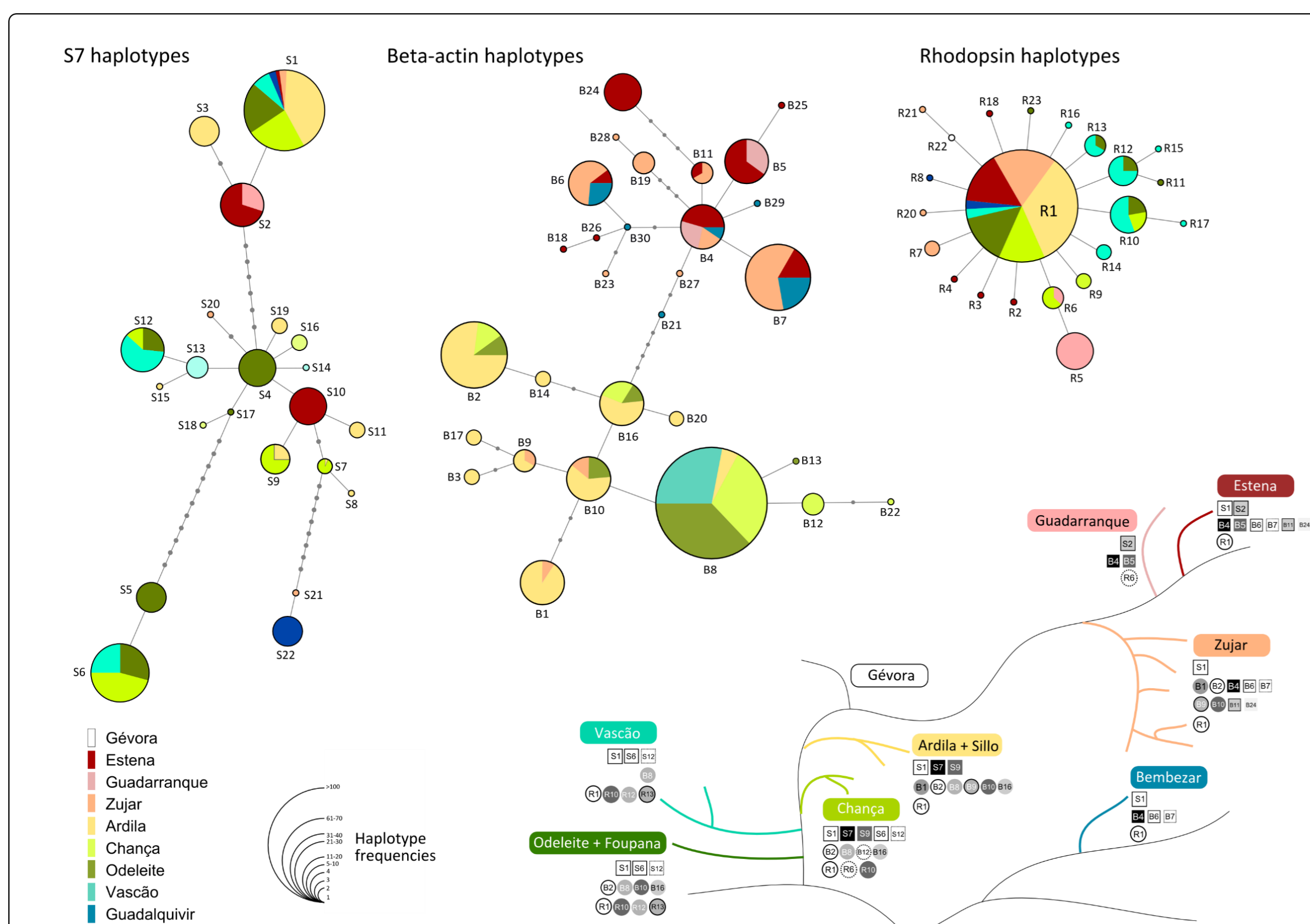


Fig. 4 – Top: Network diagrams of S7 (N=22), beta-actin (N=21) and rhodopsin (N=21) haplotypes. Bottom: Schematic representation of the sampled sub-basins from rivers Guadiana and Guadalquivir. For each population, shared haplotypes for the three molecular markers are indicated so that connections between populations may be easily visualized along with the geographical location of the sampled sub-basins.

Strong population structuring: significant F_{ST} values for all pairs except Chança-Odeleite+Foupana and Zújar-Guadalquivir; high overall fixation index and variation among populations accounted for 69.95% of the total variation.

SAMOVA yielded a maximized F_{CT} for a 2-groups scheme: Estena+Zújar+Guadalquivir ("Easternmost group") and Ardila+Sillo+Chança+Odeleite+Foupana+Vascão ("Westernmost group"), corroborating the existence of two distinct clades (Fig.4).

Other results also support two clearly differentiated groups: inexistence of migration, significant Φ_{ST} value, high overall fixation index, and most of the variation explained by variation among groups. The "Easternmost group" has higher observed mismatch mean and variance values and a wider mismatch distribution, reflecting an older genealogy.

Methods

Sampling by electrofishing throughout the distribution area.

N=205 samples from 8 distinct sub-basins: Bembezar (n=13), Ardila (n=42), Chança (n=27), Estena (n=26), Guadarranque (n=8), Odeleite (n=45), Vascão (n=24) and Zújar (n=34) (Fig. 1B).

47 additional *A. hispanica cytb* sequences were retrieved from Genbank.

One mitochondrial (cytochrome *b* - *cytb*) and three nuclear genes (beta-actin, rhodopsin, and S7 - first intron of the S7 ribosomal protein) were amplified.

DNAcollapser, ARLEQUIN, BEAST, SAMOVA, Network and TCS.

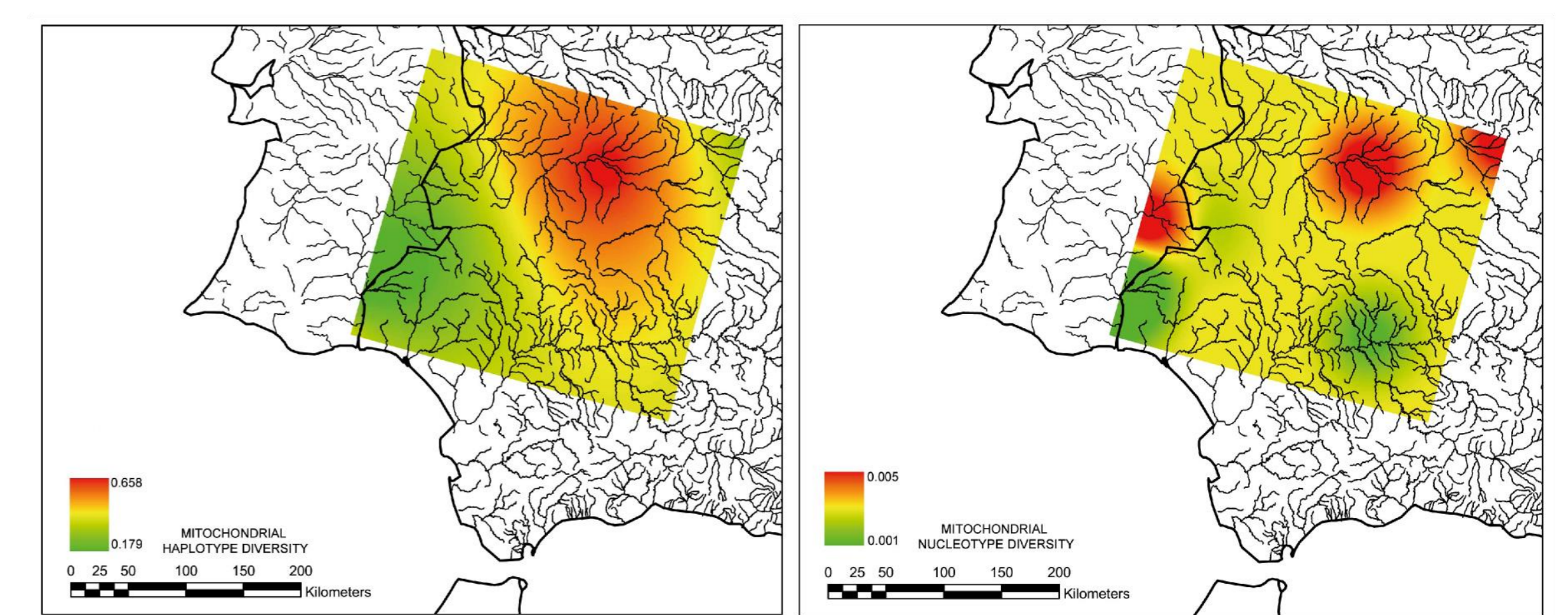


Fig. 2 – Distribution of the haplotype (H) and nucleotide diversity (π) for the *cytb* gene throughout the sampling area.

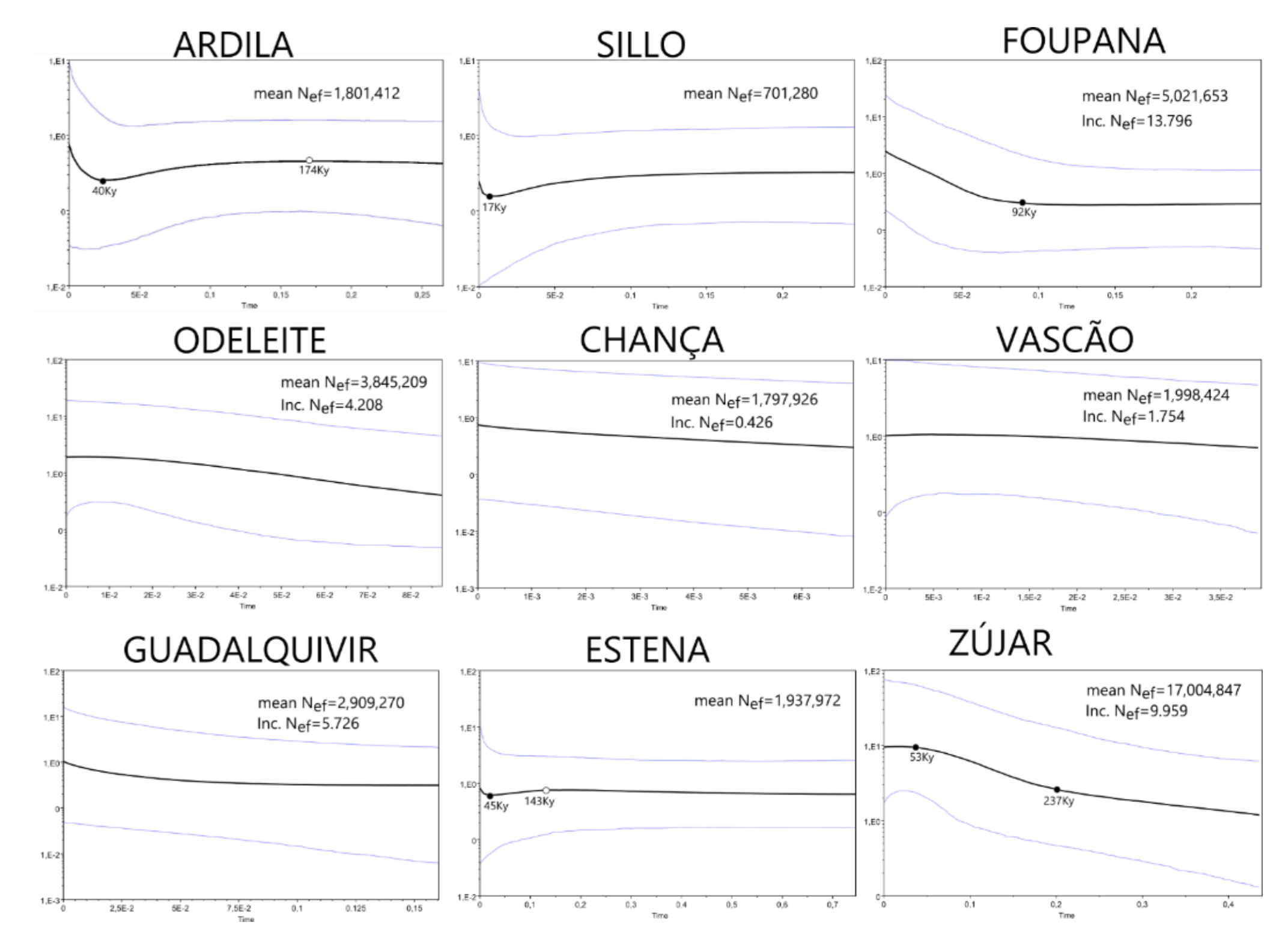


Fig. 3 – Bayesian skyline plots. Changes in effective population size (N_e millions of individuals on a log scale; Y-axis) are depicted over time (in million years; X-axis) based on *cytb* sequence data, assuming a mutation rate of 0.0033 per My and a generation time of 1 year (Materials and Methods). Black central lines represent the median values of effective population size N_e , while the blue lines represent the 95% highest posterior density of the N_e estimates. Present day N_e mean values (mean N_e) and the increasing rate of the N_e mean values for expanding populations (Inc. N_e) given by the ratio between N_e at the present and the ancient N_e estimate) are presented. The beginning of expansion and contraction of the median N_e values are represented, respectively, by black and white dots.

MAIN CONCLUSIONS

- ❖ marked population structure, with very restricted or absent gene flow
- ❖ even populations from different tributaries of the same sub-basin exhibited highly significant Φ_{ST} values
- ❖ moderate to high genetic diversity
- ❖ latitudinal trend of decreasing diversity from the east to the west
- ❖ Pleistocene westwards colonization route
- ❖ *A. hispanica* populations should be managed independently, aiming to preserve adaptive diversity and evolutionary processes across its geographic range