

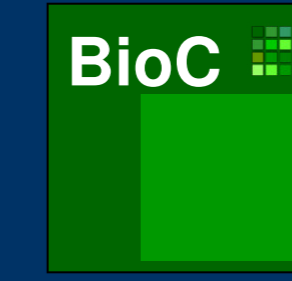
Reinterpretation of *Cynara baetica* based on a principle of integrative species delimitation

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CYNARA BAETICA

Historically described as two different species (*Cynara alba* and *C. hystrix*), but currently they are regarded as two subspecies that were described by Wiklund (1992): *C. baetica* subsp. *baetica* is distributed in southern Spain and *C. baetica* subsp. *maroccana* in northern Morocco.

	<i>C. baetica</i> subsp. <i>baetica</i>	<i>C. baetica</i> subsp. <i>maroccana</i>
Florets color	Whitish	Bright lilac
Involucral bracts		
Color	Pale green	Pale green, tinged with purple
Protrusion	17–30 mm	(20–) 28–32 mm
Spines length	2.5–6 mm	5–10 mm

Table 1: Morphological differentiation between the two described subspecies of *Cynara baetica* based on Wiklund (1992) study.



C. baetica subsp. *baetica*



C. baetica subsp. *maroccana*

AIMS

- Analyse genetic diversity within and between *Cynara baetica* populations
- Infer the phylogeographic pattern of *C. baetica* and the role of Strait of Gibraltar as a barrier
- Test the morphological, genetic and ecological differences between both subspecies
- Provide conservation guidelines

MATERIAL AND METHODS

GENETIC STUDIES: 10 populations from the Iberian Peninsula and 5 from Morocco. Three AFLP primer combinations (*EcoRI*(Vic)-AAG/*MseI*-CAG, *EcoRI*(Vic)-ACG/*MseI*-CTG and *EcoRI*(Vic)-ACA/*MseI*-CAA) and two cpDNA regions (*ycf3-trnS* and *trnS^{GCU}-trnG^{CA}*). 135 individuals in AFLP study and 75 in cpDNA study.

ECOLOGICAL NICHE MODELING: Using MaxEnt with 68 presence records of *C. baetica* (47 for subsp. *baetica* and 21 for subsp. *maroccana*) and 11 variables (nine bioclimatic + pH + altitude). Niche overlap between two subspecies.

MORPHOLOGICAL STUDIES: 72 herbarium specimens (53 from the Iberian Peninsula and 19 from Morocco) - Four characters: two recognized as taxonomically relevant by Wiklund (1992) and two other ones observed in the present study

RESULTS

Code	Population	Coordinates	N _{sp}	Hap.	N _{AFLP}	Private _{frag} (Fixed)	PLP (NL)
IBERIAN PENINSULA							
ES1	Spain: Albacete	38°29'29" N 02°18'47" W	5	1, 2, 3	88	40 (5)	43.6 (92)
	Calar del Mundo	02°18'47" W	5	3	8	4 (1)	20.9 (44)
ES2	Spain: Jaén	38°13'57" N 02°39'48" W	5	1	10	0 (0)	16.6 (35)
	Hornos	02°39'48" W	5	1	9	0 (0)	21.3 (45)
ES3	Spain: Jaén	37°33'58" N 03°52'18" W	5	1	9	0 (0)	21.3 (45)
	Locubin	03°52'18" W	5	1	5	0 (0)	12.3 (26)
ES4	Spain: Córdoba	37°24'08" N 04°17'24" W	5	1	5	0 (0)	12.3 (26)
	Los Villares	04°17'24" W	5	1	10	1 (0)	22.3 (47)
ES5	Spain: Granada	37°22'10" N 02°58'10" W	5	1	10	1 (0)	22.3 (47)
	Gor	02°58'10" W	5	1	10	1 (0)	19.0 (40)
ES6	Spain: Granada	37°08'11" N 03°28'23" W	5	1	10	1 (0)	19.0 (40)
	El Pulche	03°28'23" W	5	1, 2	8	1 (0)	68.2 (144)
ES7	Spain: Málaga	36°59'39" N 04°15'37" W	5	1, 2	8	1 (0)	68.2 (144)
	Alfarnate	04°15'37" W	5	1	10	0 (0)	14.7 (31)
ES8	Spain: Málaga	36°48'13" N 04°51'20" W	5	1	10	0 (0)	14.7 (31)
	Puerto Martínez	04°51'20" W	5	1, 2	8	0 (0)	22.7 (48)
ES9	Spain: Cádiz	36°45'16" N 05°23'39" W	5	1, 2	8	0 (0)	22.7 (48)
	Puerto Boyar	05°23'39" W	5	1	9	3 (0)	16.6 (35)
ES10	Spain: Málaga	36°37'02" N 05°20'32" W	5	1	9	3 (0)	16.6 (35)
	Cortes	05°20'32" W				1 (0.1)	23.5 (49.5)
Mean							
MOROCCO							
MA1	Morocco: Al Hoceïma	35°00'34" N 04°11'22" W	25	4, 5, 6	47	19 (1)	67.3 (142)
	Bni Hadifa	04°11'22" W	5	5	10	1 (1)	55.5 (117)
MA2	Morocco: Ifrane	33°30'16" N 05°01'24" W	5	5	8	2 (0)	71.1 (150)
	Ifrane	05°01'24" W	5	5, 6	9	0 (0)	60.7 (128)
MA3	Morocco: Ifrane	33°02'27" N 05°03'30" W	5	5, 6	9	0 (0)	60.7 (128)
	Col du Zad	05°03'30" W	5	4	10	0 (0)	12.8 (27)
MA4	Morocco: Azilal	32°10'57" N 06°31'16" W	5	4	10	1 (2)	12.8 (27)
	Afourer	06°31'16" W	5	4	10	1 (2)	12.8 (27)
MA5	Morocco: Azilal	31°45'55" N 07°00'45" W	5	4	10	1 (2)	12.8 (27)
	Demnate	07°00'45" W					
Mean						0.8 (0.6)	42.6 (89.8)

Table 2: Code of population, population, coordinates, number of samples used in cpDNA study (N_{sp}), haplotype detected (Hap.), number of samples used in AFLP study (N_{AFLP}), number of private (Private_{frag}) and fixed (Fixed; in parentheses) fragments, percentage of polymorphic loci (PLP) and number of polymorphic loci (NL).

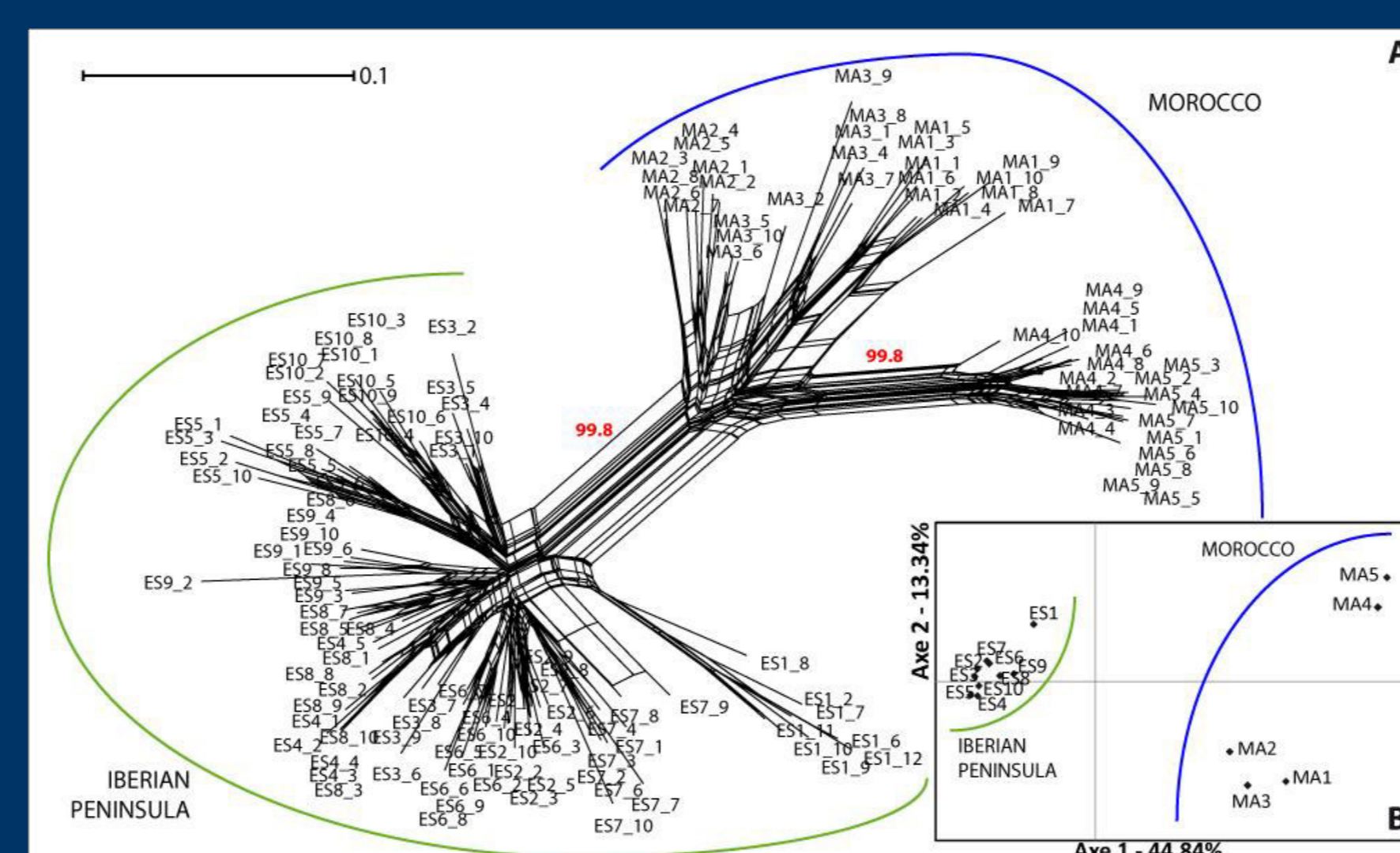
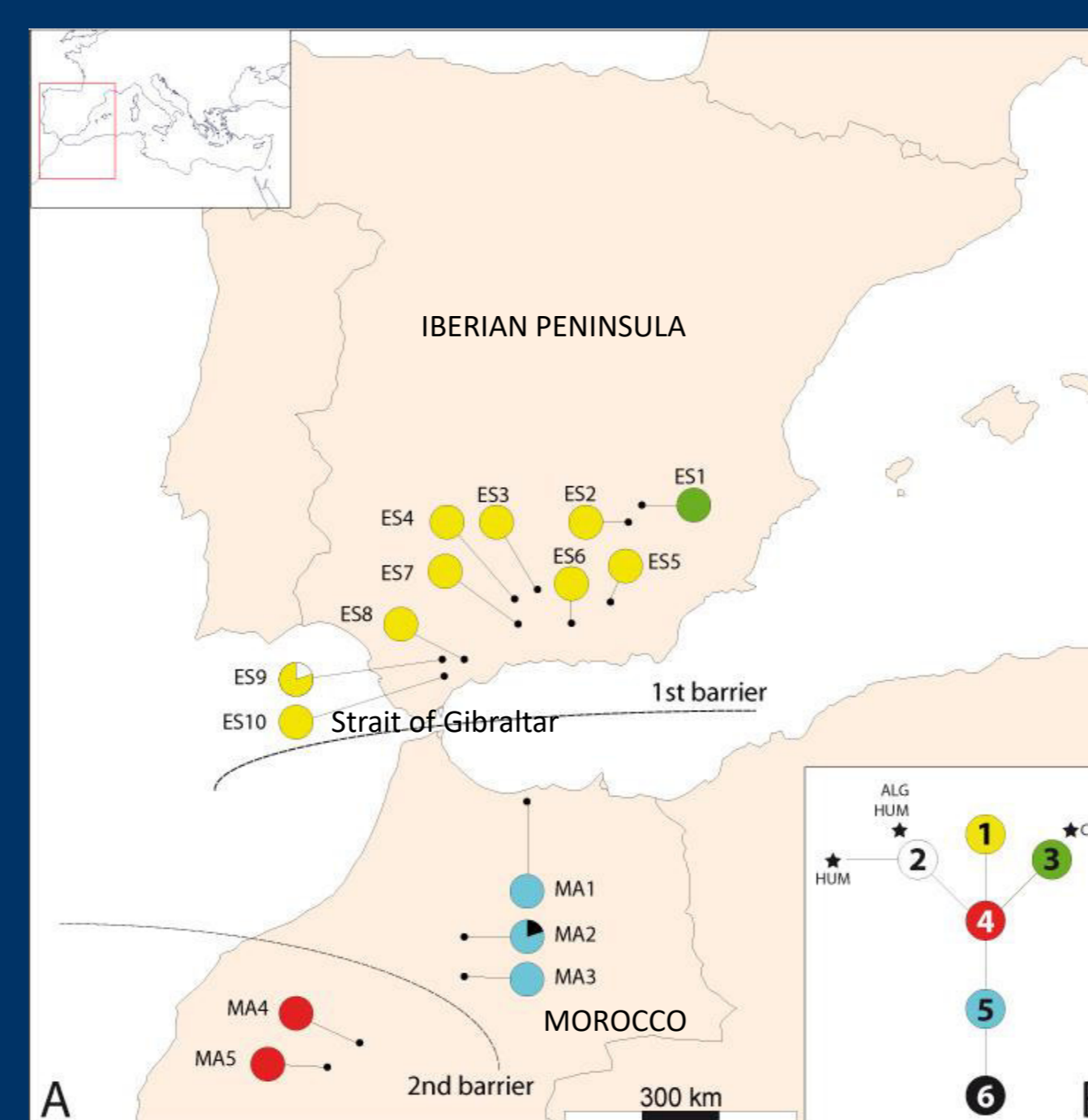


Figure 3: (A) Neighbor-Net graph of 135 individuals of *Cynara baetica* based on 211 AFLP loci computed with SplitsTree. In red, bootstrap values. (B) PCoA based on Nei's genetic distance among the studied populations of *C. baetica*.

Genetic studies

Figure 1: (A) Location and haplotype of the 15 studied populations of *Cynara baetica*. (B) Statistical parsimony network of *C. baetica* haplotypes (indicated by numbers). Stars indicate three closely related species (ALG = *C. algarbiensis*; CAR = *C. cardunculus*; HUM = *C. humilis*). Dotted line are barriers to gene flow detected by the Barrier software, with AFLP and cpDNA markers.

Morphological studies

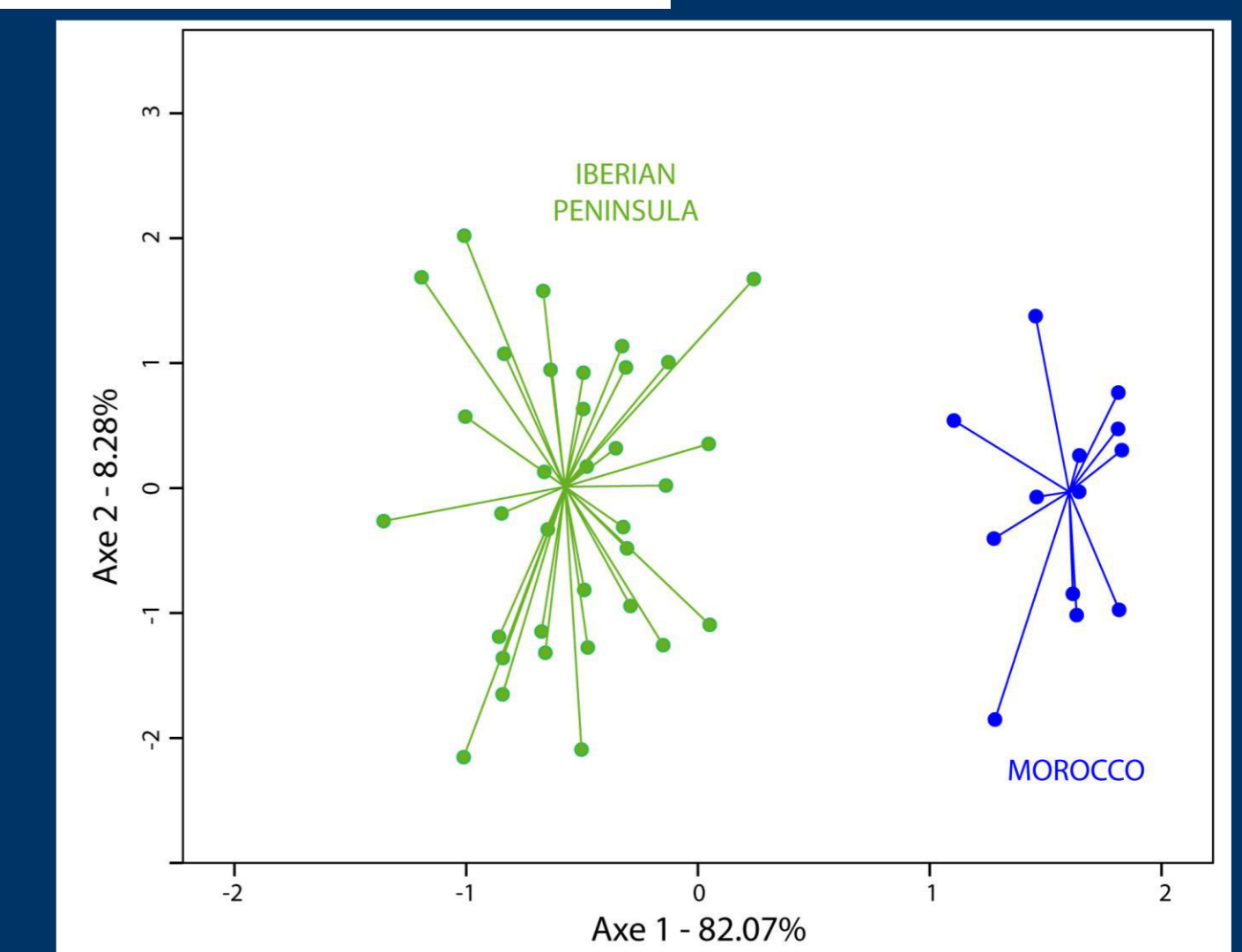


Figure 2: Scatterplot of the first two axes from the PCA for the 72 individuals studied of *Cynara baetica*.

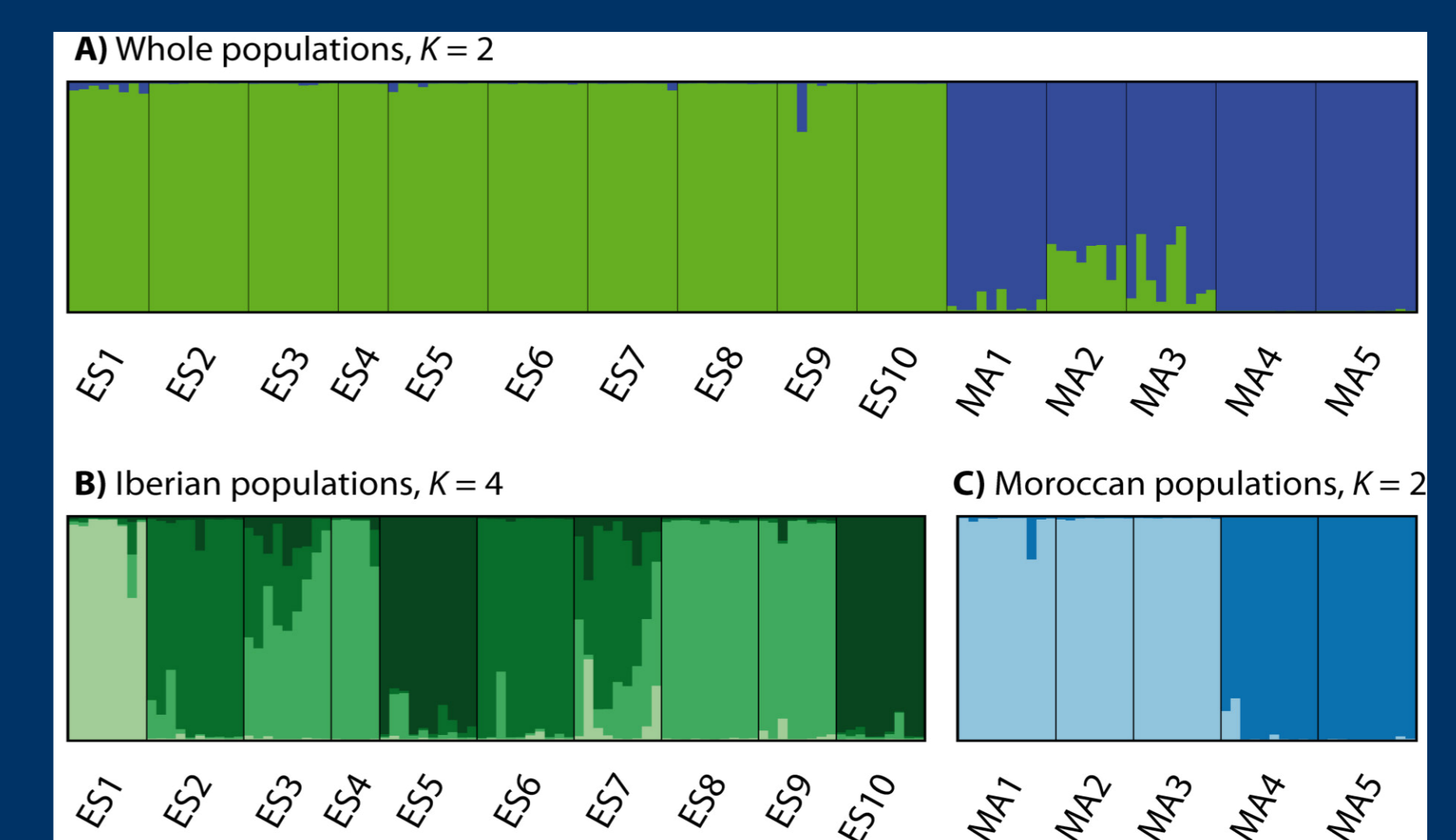


Figure 4: The most optimal K results using Bayesian clustering analyses with Structure. (A) Using whole populations of *Cynara baetica* (K = 2). (B) Using only Iberian populations (K = 4). (C) Using only Moroccan populations (K = 2).

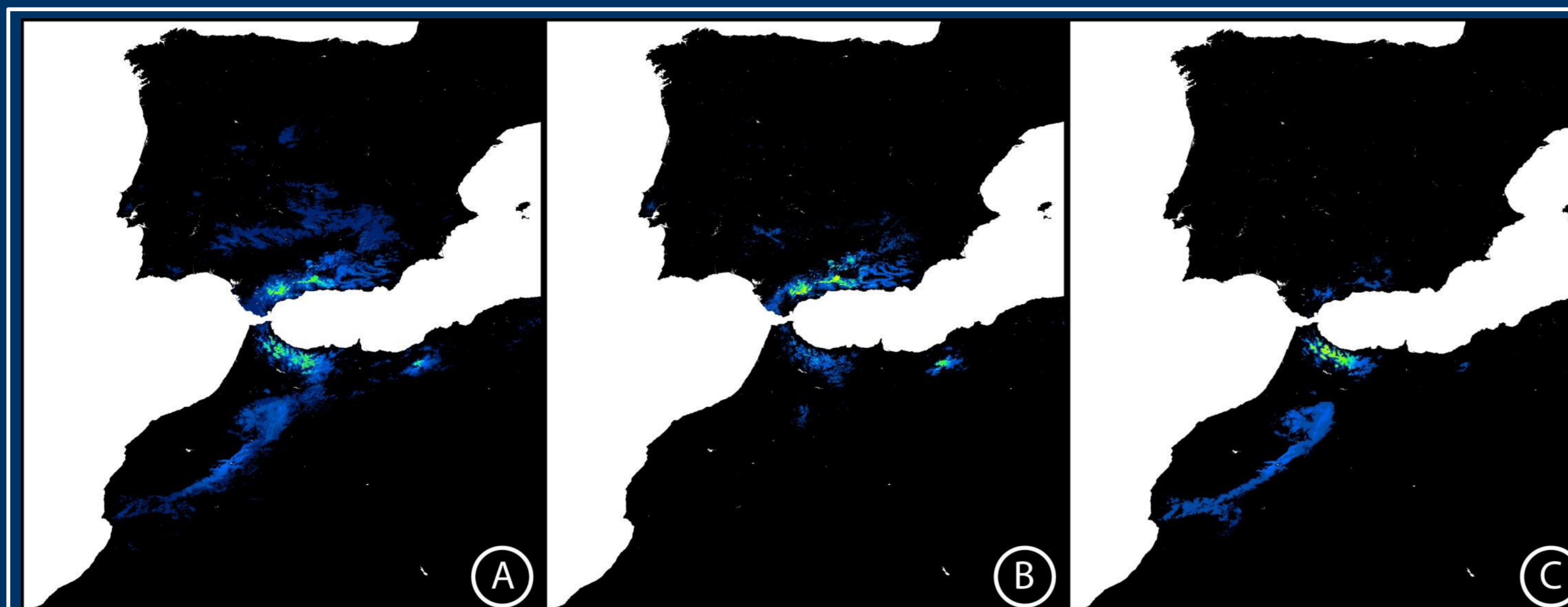
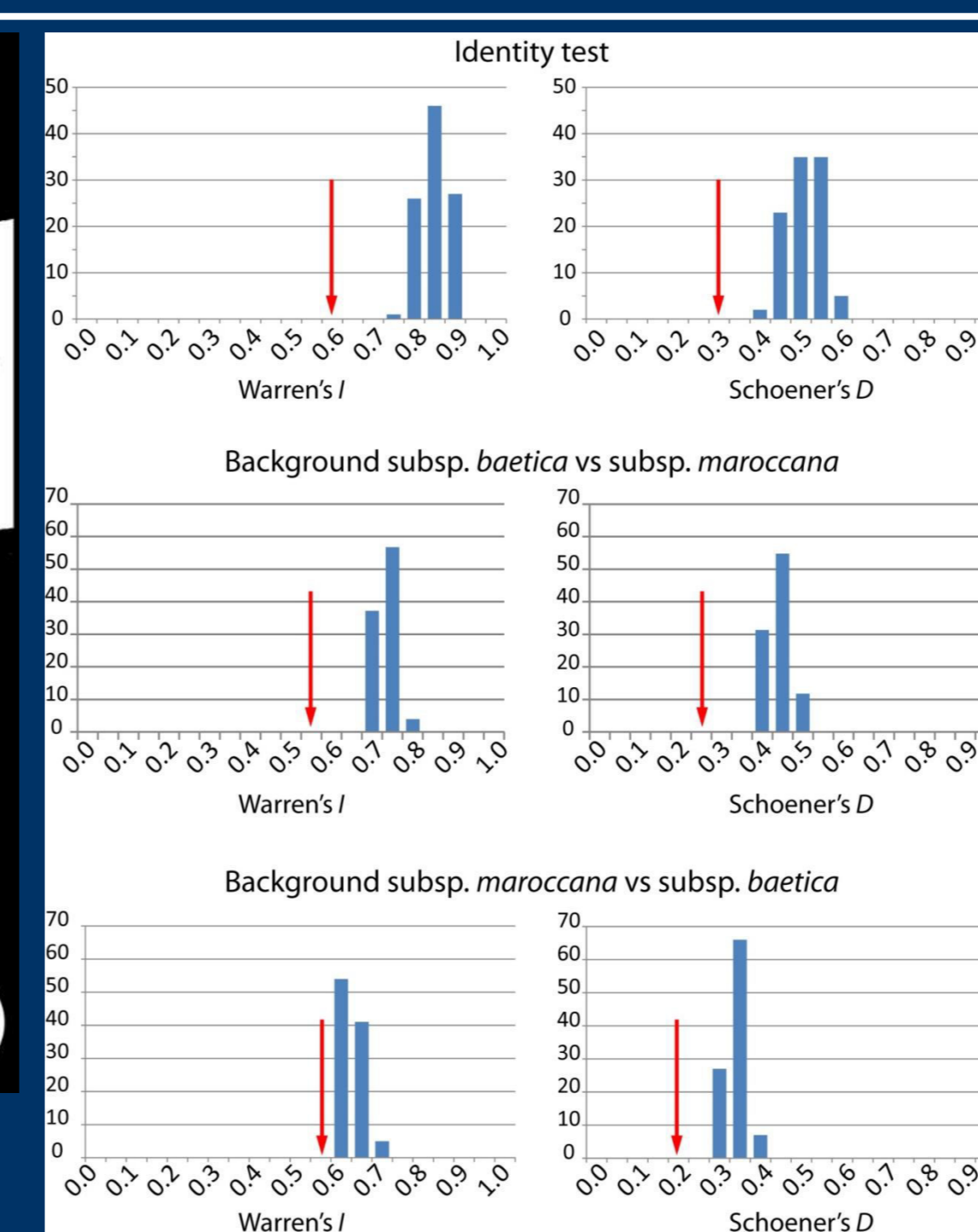


Figure 5: MaxEnt distribution map showing the environmental suitability for *Cynara baetica* (A), *C. baetica* subsp. *baetica* (B), and *C. baetica* subsp. *maroccana* (C). Warmer colors depict better environmental suitability.



Ecological niche modelling

Model	AUC±sd	Total predicted area (km ²)	Main variables
<i>Cynara baetica</i>	0.977 ± 0.006	107,963	pH > bio18 = bio19
subsp. <i>baetica</i>	0.980 ± 0.010	31,726	pH > bio18 > bio19
subsp. <i>maroccana</i>	0.986 ± 0.009	35,826	bio19 > altitude = bio18

Table 3: Predicted potential distribution of *Cynara baetica*, model performance, and mean climatic variables for all ENM used in this study. The three most important variables for each model are included.

Figure 6: Niche overlap values (arrows) for Warren's I and Schoener's D indices compared to a null distribution. In all cases, the similarity score (red arrow) is lower than the null hypothesis of niche equivalency, indicating that the environmental niches are not equivalent.

CONCLUSIONS

Both nuclear and plastid data analyses suggest the presence of two separate lineages within *Cynara baetica*, each one in each side of the Strait of Gibraltar. In addition, ecological niche modelling and morphological analyses confirm these differences.

According to these results, a reinterpretation of the taxonomical treatment for these taxa is suggested and the best way is to return to the ancient treatment: one species in the Iberian Peninsula and another one in Northern Africa, and redefine the two subspecies proposed by Wiklund (1992) as *Cynara baetica* (Spreng.) Pau instead of *C. baetica* subsp. *baetica*, and *C. hystrix* Ball instead of *C. baetica* subsp. *maroccana* due to these two species were described and accepted with these names.

Finally, two evolutionary significant units (ESUs; Waples, 1991) were proposed, one per each taxon, and two management units (MUs; Moritz, 1994) are defined inside each ESU due to haplotypes endemic to restricted areas represent singular genetic variants that may have evolved separately from each other and, therefore, they deserve particular conservation efforts: ES1, ES2–ES10, MA1–MA3 and MA4–MA5.