ARTICLES

Mediterranean Botany
ISSNe 2603-9109

http://dx.doi.org/10.5209/MBOT.60078

EDICIONES COMPLUTENSE

Genetics and conservation of rare and endemic plants: the case of *Genista sanabrensis* (Fabaceae) in the Iberian Peninsula

Eduardo Cires[1], Mauro Sanna[1], Víctor M. Vázquez[2] & José Antonio Fernández Prieto[1]

Received: 31 October 2017 / Accepted: 20 February 2018 / Published online: 29 june 2018

Abstract. *Genista sanabrensis* Valdés Berm., Castrov. & Casaseca (Fabaceae) is an endemic and rare species of the Northwestern Iberian Peninsula. Despite its limited distribution, the species is locally abundant and therefore not categorized by the IUCN criteria as threatened at the national level. However, comprehensive studies on the genetic diversity and structure of rare and endemic species from Iberian Peninsula are urgently needed to promote effective conservation and management activities. Therefore, we conducted amplified fragment length polymorphism (AFLP), nuclear rDNA (ITS, ETS) and plastid regions (*trnL*, *trnL-F*, *matK*, *rbcL*) analyses to characterize the genetic diversity and variation of this species within and between populations. Our results confirm the monophyly of the species compared to closely related taxa. The presence of insertions/deletions together with point mutations makes the northern populations indispensable in the elaboration of conservation strategies. Genetic diversity was moderate/low, although the survival of these populations at the genetic level shows no signs of being threatened. This study provides important insights into the genetic structure of *G. sanabrensis* with potential applications to its effective conservation.
Keywords: AFLP; conservation strategies; endemism; Fabaceae; genetic diversity; *Genista*; ITS; molecular markers; plastid sequences; population differentiation.

Diversidad genética y conservación de plantas raras y endémicas: el caso de *Genista sanabrensis* (Fabaceae) en la Península Ibérica

Resumen. *Genista sanabrensis* Valdés Berm., Castrov. & Casaseca (Fabaceae) es una especie endémica y rara de la península ibérica noroccidental. A pesar de su distribución limitada, la especie es localmente abundante y, por lo tanto, no está clasificada según los criterios de la UICN como amenazada a nivel nacional. Sin embargo, se necesitan urgentemente estudios exhaustivos sobre la diversidad genética y la estructura de especies raras y endémicas de la Península Ibérica para promover actividades efectivas de conservación y manejo. Por lo tanto, realizamos análisis de polimorfismo de longitud de fragmentos amplificados (AFLP), DNAr nuclear (ITS, ETS) y regiones de plástidos (*trnL*, *trnL-F*, *matK*, *rbcL*) para caracterizar la diversidad genética y la variación de esta especie dentro y entre las poblaciones. Nuestros resultados confirman la monofilia de la especie en comparación con los taxones estrechamente relacionados. La presencia de inserciones/deleciones junto con mutaciones puntuales hace que las poblaciones del norte sean indispensables en la elaboración de estrategias de conservación. La diversidad genética fue moderada / baja, aunque la supervivencia de estas poblaciones a nivel genético no muestra signos de amenaza. Este estudio proporciona información importante sobre la estructura genética de *G. sanabrensis* con posibles aplicaciones para su conservación efectiva.
Palabras clave: AFLP; estrategias de conservación; endemismo; Fabaceae; diversidad genetica; *Genista*; ITS; marcadores moleculares; secuencias de plástidos; diferenciación poblacional.

Introduction

Genetic diversity is one aspect of biological diversity that is extremely important for conservation strategies, especially in rare and narrowly endemic species (Mills & Schwartz, 2005; Tomasello & *al.*, 2015). Preserving the genetic diversity of these plants can significantly strength their long-term survival and evolution in changing environments (Frankham & *al.*, 2002). For instance, rare and endemic plants contribute to biodiversity and help preserve gene pool of local flora (Falk & Holsinger, 1991; Olivieri & *al.*, 2016). In many respects, the biology of rare (and endemic) plants that are locally common is similar to that of widespread congeners. The primary difference is that they are restricted to a particular habitat type or geographical area. That is, rare plants may be locally common but occur in only a few places, or behave in an opposite way, being scarce where

[1] Departamento de Biología de Organismos y Sistemas, Universidad de Oviedo, Área de Botánica. C/ Catedrático Rodrigo Uría s/n, 33071, Oviedo, Spain. Email: cireseduardo@uniovi.es
[2] Real Instituto de Estudios Asturianos. Plaza de Porlier 9, 33003, Oviedo, Spain.

they grow but geographically widespread. Other species also may be both locally scarce and geographically restricted.

Most of the authors agree that genetic diversity is necessary to preserve the long-term evolutionary potential of a species (Falk & Holsinger, 1991). In the last decade, experimental and field investigations have demonstrated that habitat fragmentation and population decline reduce the effective population size. In the same way, most geneticists consider population size as an important factor for maintaining genetic variation (Ellegren & Galtier, 2016; Turchetto & *al.*, 2016). This is very important in fragmented populations because are more vulnerable due to the loss of allelic richness and increased population differentiation by genetic drift (decreases heterozygosity and eventual fixation of alleles) and inbreeding depression (increases homozygosity within populations; Frankham, 2005).

Therefore, knowledge of the genetic variability and diversity within and among different populations of rare and endemic plant species is crucial for their conservation and management (e.g. Cires & *al.*, 2012, 2013; Meloni & *al.*, 2015; Peñas & *al.*, 2016). In this study, we investigated the genetic diversity and structural patterns of *Genista sanabrensis* Valdés Berm., Castrov. & Casaseca (Fabaceae) a northwestern Iberian endemism mainly distributed in Galician-Leonese mountains (Sierra del Teleno, Montes Aquilanos, Sierra de la Cabrera, Sierra Segundera and Peña Trevinca massif), where is very frequent. Out of that area, *G. sanabrensis* has been found in four isolated populations in western Cantabrian Mountains: one in Leonese territories in high Babia (Sierra de Villabandín in the municipality of Cabrillanes; García González & *al.*, 1987), and three in Asturias (Degaña, Somiedo and Cangas del Narcea, although the first of them does not already exist; see Carlón & *al.*, 2010; Fernández Prieto & *al.*, 2014). Despite the rarity and patchy distribution of this endemic taxon, there is no phylogenetic or population genetic study to date.

Here, we report for the first time both approaches, a phylogenetic study based on Sanger sequencing and a genetic variability analysis using amplified fragment length polymorphism (AFLP) markers. The goals of this study were to: i) develop a phylogenetic analysis of *Genista sanabrensis* based on nuclear (ITS, ETS) and chloroplast (*trnL, trnL-F*, *matK*, *rbcL*) DNA sequences; (ii) characterize the level of genetic diversity in *G. sanabrensis*; iii) reveal the distribution of genetic variation within and between the fragmented populations; and finally (iv) discuss possible implications of genetic data for management and conservation in *G. sanabrensis* populations.

Material and Methods

Plant material and DNA isolation

Fresh leaves and stems of *Genista sanabrensis* were sampled from five localities in the Iberian Peninsula, representing the fragmented range of the species (Figure 1, Table 1). Moreover, close related species such as *Genista anglica* L., *G. hystrix* Lange, *G. florida* subsp. *polygalaephylla* (Brot.) Cout. and *Cytisus dieckii* (Lange) Fern. Prieto & *al.*, were also included in the study (Table 1). Samples for molecular analyses were dried in silica gel and stored prior to DNA isolation. Total DNA was extracted from approximately 20-30 mg of dried leaf/steam tissue using the DNeasy Plant Mini Kit system (Qiagen), according to the protocol recommended by the manufacturer. DNA concentration was measured by a Beckman-Coulter DU800® spectrophotometer (Fullerton, CA, USA).

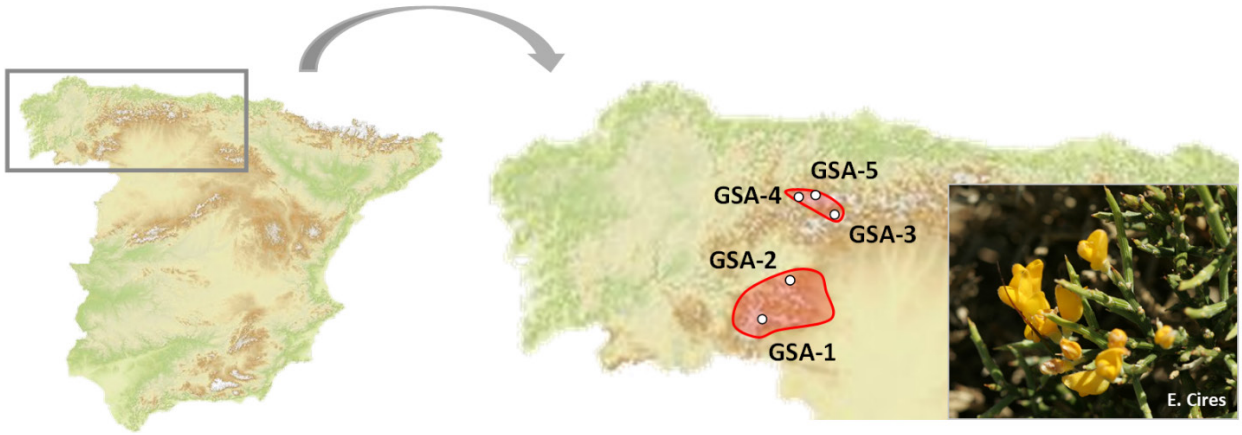


Figure 1. Distribution area of *Genista sanabrensis* (GSA) in the Iberian Peninsula and geographical location of the five populations analyzed.

Table 1. Code, populations and GenBank accessions for DNA sequences of *Genista sanabrensis* (GSA), *G. anglica* (GAN), *G. hystrix* (GHY), *G. florida* subsp. *polygalaephylla* (GPO) and *Cytisus dieckii* (CDI) analysed in the present study. Collector abbreviations: ACR: A. Cerraján Raigoso; AFC: A. Fernández Ceballos; EC: E. Cires; JD: Juan Díaz; MC: M. Ceballos; JAFP: J.A. Fernández Prieto; PV: P. Vázquez; SG: S. García; SRA: S. Rodríguez Ambres; VMV: V.M. Vázquez.

Code	Population	Coordinates	GenBank accession					
			ITS	EST	*trnL*	*trnL-F*	*matK*	*rbcL*
GSA-1a GSA-1b	"De la Laguna de los Peces a la Laguna de las Yeguas, entre los Gorralicos y la Cuesta de Estallarrabos, Sierra de la Cabrera Baja (Zamora, España), 1783 m; *EC, MC & JAFP*".	42°11'20.85"N 6°43'39.72"W	MH000028 MH000029	MH000046 MH000047	MH000064 MH000065	MH000082 MH000083	MH000100 MH000101	MH000118 MH000119
GSA-2a GSA-2b	"Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; *MC, EC & JAFP*".	42°23'36.91"N 6°30'31.07"W	MH000030 MH000031	MH000048 MH000049	MH000066 MH000067	MH000084 MH000085	MH000102 MH000103	MH000120 MH000121
GSA-3a GSA-3b	"Entre el Pico de los Concichones y la Braña del Río, Sierra de Villabandín (León, España), 1898 m; *PV, VMV, EC & JAFP*".	42°54'80.70"N 6°8'30.60"W	MH000032 MH000033	MH000050 MH000051	MH000068 MH000069	MH000086 MH000087	MH000104 MH000105	MH000122 MH000123
GSA-4a GSA-4b	"Altos del Morteiro, Sierra de Caniellas (Asturias, España), 1840 m; *PV, SG, VMV & JAFP*".	42°59'54.67"N 6°31'4.32"W	MH000034 MH000035	MH000052 MH000053	MH000070 MH000071	MH000088 MH000089	MH000106 MH000107	MH000124 MH000125
GSA-5a GSA-5b	"Colláu de la Mochadina, entre el Pico Cogollo y El Cabril (Asturias, España), 1832 m; *ACR, JD, PV, SG & JAFP*".	43°3'20.78"N 6°21'22.06"W	MH000036 MH000037	MH000054 MH000055	MH000072 MH000073	MH000090 MH000091	MH000108 MH000109	MH000126 MH000127
GAN-1	"Quintana y Congosto (León, España), 850 m; *MC & JAFP*".	42°13'59.00"N 6°1'48.68"W	MH000038	MH000056	MH000074	MH000092	MH000110	MH000128
GHY-1	"Entre Corporales y Baillo (León, España), 1387 m; *EC, MC & JAFP*".	42°17'24.45"N 6°27'33.28"W	MH000039	MH000057	MH000075	MH000093	MH000111	MH000129
GHY-2a GHY-2b	"Entre Rabanal Viejo y Santa Marina de Somoza (León, España), 1057 m; *AFC & JAFP".*	42°28'17.35"N 6°14›59.76"W	MH000040 MH000041	MH000058 MH000059	MH000076 MH000077	MH000094 MH000095	MH000112 MH000113	MH000130 MH000131
GHY-3	"Monte de Xestoso, Sena, Ibias (Asturias, España), 930 m; *SRA*".	43°0'22.57"N 6°55'35.96"W	MH000042	MH000060	MH000078	MH000096	MH000114	MH000132
GHY-4	"Unos 700 m hacia el este del Monte de Xestoso, Sena, Ibias (Asturias, España), 937 m; *SRA*".	43°0'15.83"N 6°54'53.17"W	MH000043	MH000061	MH000079	MH000097	MH000115	MH000133
GPO-1	"Subida al Puerto de Ventana, Teverga (Asturias, España), 1410 m; *MC & JAFP*".	42°58'50.05"N 6°52›48.21"W	MH000044	MH000062	MH000080	MH000098	MH000116	MH000134
CDI-1	"Espinilla (Cantabria, España), 930 m; *AFC & JAFP".*	43°1'18.39"N 4°13'48.16"W	MH000045	MH000063	MH000081	MH000099	MH000117	MH000135

DNA amplification and sequencing

PCR reactions were performed following Fernández Prieto & *al.* (2015). Standard primers were used for amplification and sequencing of the ITS and ETS (Sun & *al.,* 1994; Mahé & *al.*, 2011) and plastid sequences (*trnL*, Taberlet & *al.*, 1991; *trnL-F*, Taberlet & *al.*, 1991; *rbcL*, Olmstead & *al.*, 1992; Fernández Prieto & *al.,* 2013; *matK*, Vere & *al.*, 2012). PCR products were sequenced at the DNA Synthesis and Sequencing Facility Macrogen (Amsterdam, The Netherlands). Sequence data were assembled using MUSCLE (Edgar, 2004) and edited with Geneious 7 (Kearse & *al.*, 2012). International Union of Pure and Applied Chemistry (IUPAC) symbols were used to represent nucleotide ambiguities.

Phylogenetic analyses

Phylogenetic analyses of nuclear and plastid DNA were performed using Maximum Parsimony (MP) and Bayesian Inference (BI) methods. The MP analysis was conducted by a heuristic search with MEGA 7.0 (Kumar & *al.*, 2016) using the Tree-Bisection-Regrafting (TBR) algorithm and the robustness of nodes was inferred from a bootstrap (BS) analysis of 10,000 replicates. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. A Bayesian Markov Chain Monte Carlo method implemented in BEAST 2.3.1 (Bouckaert & *al.*, 2014) were used to estimate BI. The software jModelTest 2.1.7 (Darriba & *al.*, 2012) was executed to select the best-fitting models for DNA substitution for each marker data set according to the Bayesian Information Criterion (BIC). The topology was determined after ten million generations for nuclear data and twenty million generations for plastid data. We visually analyzed the results using Tracer 1.6 (Rambaut & Drummond, 2013) to plot likelihood scores by calculating Effective Sample Sizes (ESS). Trees were summarized by the Maximum Clade Credibility (MCC) method using TreeAnnotator 1.8.2 after discarding of the first 10% of generations as burn-in and visualized using TreeGraph2 2.7.1 (Stöver & Müller, 2010).

Table 2. Main characteristics of *Genista sanabrensis* and related species (*G. anglica*, *G. hystrix*, *G. florida* subsp. *polygalaephylla, Cytisus dieckii*) from DNA sequences.

	ITS	ETS	*trnL*	*trnL-F*	*matK*	*rbcL*
Length range (bp)	599-603	516-517	495-520	376-407	733-739	572-583
Aligned length (bp)	610	518	530	428	739	583
Polymorphic sites	81	96	57	78	31	27
Mean G+C content (%)	58.0%	53.9%	32.0%	23.7%	31.8%	43.5%

AFLP amplification

The AFLP-based PCR was carried out as has been previously described (see Cires & *al.*, 2011). The genomic DNA was digested with *Eco*RI and *Mse*I restriction enzymes (New England Biolabs Inc.). In the following step, double-strand adapters were ligated to *Eco*RI and *Mse*I specific ends by T4 DNA Ligase (Roche Diagnostics). Products of digestion/ligation were checked by electrophoresis in 1.5% agarose. The pre-selective amplification was performed using primers with single selective nucleotides (*Eco*RI+A and *Mse*I+C), checked by electrophoresis in 1.5% agarose gels and subsequently diluted (1:10) in sterile de-ionised H_2O. Then selective amplifications were performed using *Eco*RI and *Mse*I primers with three selective nucleotides (EcoRI-ACG / MseI-CAT, EcoRI-ACT / MseI-CAT, EcoRI-AAC / MseI-CAT, EcoRI-ACG / MseI-CCAC, EcoRI-ACT / MseI-CCAC, EcoRI-AAC / MseI-CCAC). The *Eco*RI-selective primers were 5'-fluorescent labelled. Selective amplification products were submitted to the Fragment Analysis Macrogen (Amsterdam, The Netherlands). The visualization of the AFLP profiles was performed in the capillary sequencer (ABI3730XL) and analyzed with the Peak Scanner 2 software (Applied Biosystems, CA, USA) and RawGeno 2.0-1 (Arrigo & *al.*, 2009) pack of R (R Core Team, 2014).

AFLP data analysis

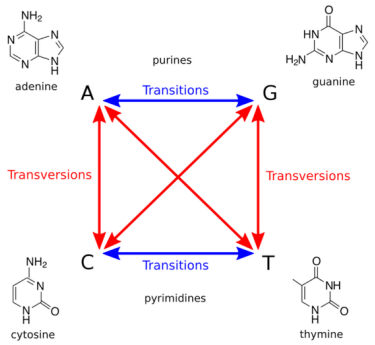
The presence or absence of each band was recorded in a binary data matrix for each individual, assigning a value of 1 or 0 depending on band presence or absence, respectively. The binary data matrix obtained was used to calculate the following parameters assuming Hardy-Weinberg equilibrium: observed number of bands (NB), number of polymorphic bands (NPB), percentage of polymorphic bands (PPB), mean observed number of alleles (A_O), mean effective number of alleles (A_E), observed heterozygosity (H_E), and lastly, Shannon diversity index (I). The hierarchical AFLP frequency distribution was described using the analysis of molecular variance (AMOVA). Furthermore, a principal coordinate analysis (PCoA) was conducted to visualize the genetic relationships among all individual AFLP phenotypes. These AFLP data analyses were performed using GenAlEx 6.5 (Peakall & Smouse, 2006, 2012). To further substantiate the assessment of population genetic structure, a model-based Bayesian inference clustering was run using Structure 2.3 (Pritchard & *al.*, 2000; Falush & *al.*, 2007) with recessive allele model for dominant markers. The analysis assumed an admixture model and uncorrelated allele frequencies between clusters. Five independent runs were carried out for each value of K, ranging from 1 to 10 with a burn-in

period of 2×10^5 and 1×10^5 Markov Chain Monte Carlo replicates after burn-in. The estimated mean logarithmic likelihood of K values and delta K values were calculated to determine an optimal K value (Evanno & *al.*, 2005). To infer the number of genetic groups in our data set, we used Structure Harvester (Earl & vonHoldt, 2012), a website and program for visualizing Structure output and implementing the Evanno method.

Table 3. Sequences characteristics for each molecular marker used in *Genista sanabrensis*. Colour shows the point mutations of DNA, red indicates transversions and blue means transitions of nucleotide bases. R = A or G; W = A or T. Note: *matK* marker is not displayed because no differences were detected.

Sample	Molecular marker and position of change																			
	ITS	*trnL*												*trnL-F*						
	140	241	242	243	244	245	246	247	277	278	279	280	281	21	96	148	149	150	151	280	298
GSA-1a	C	T	A	T	A	T	A	C	-	-	-	-	-	-	T	-	-	-	-	C	T
GSA-1b	C	T	A	T	A	T	A	C	-	-	-	-	-	T	T	-	-	-	-	C	T
GSA-2a	C	-	-	-	-	-	-	-	-	-	-	-	-	-	**C**	A	T	A	T	-	G
GSA-2b	C	-	-	-	-	-	-	-	-	-	-	-	-	-	**C**	A	T	A	T	-	G
GSA-3a	C	T	A	T	A	T	A	C	-	-	-	-	-	T	T	-	-	-	-	C	T
GSA-3b	C	T	A	T	A	T	A	C	-	-	-	-	-	T	T	-	-	-	-	C	T
GSA-4a	**A**	T	A	T	A	T	A	C	-	-	-	-	-	T	T	-	-	-	-	C	T
GSA-4b	C	T	A	T	A	T	A	C	A	T	A	T	T	T	T	-	-	-	-	C	T
GSA-5a	C	T	A	T	A	T	A	C	-	-	-	-	-	T	T	-	-	-	-	C	T
GSA-5b	C	T	A	T	A	T	A	C	-	-	-	-	-	T	T	-	-	-	-	C	T

	rbcL														
	244	360	435	536	539	540	541	542	543	544	545	546	547	548	549
GSA-1a	R	A	W	G	-	-	-	-	-	-	-	-	-	-	-
GSA-1b	R	**G**	T	G	-	-	-	-	-	-	-	-	-	-	-
GSA-2a	R	A	T	**C**	A	T	T	G	C	A	A	T	T	C	C
GSA-2b	R	A	T	**C**	A	T	T	G	C	A	A	T	T	C	C
GSA-3a	R	A	T	G	-	-	-	-	-	-	-	-	-	-	-
GSA-3b	R	A	W	G	-	-	-	-	-	-	-	-	-	-	-
GSA-4a	R	A	T	G	-	-	-	-	-	-	-	-	-	-	-
GSA-4b	G	A	T	G	-	-	-	-	-	-	-	-	-	-	-
GSA-5a	R	**G**	T	G	-	-	-	-	-	-	-	-	-	-	-
GSA-5b	R	**G**	T	G	-	-	-	-	-	-	-	-	-	-	-



Results

Phylogenetic analyses

The characteristics of the nuclear (ITS, ETS) and plastid (*trnL, trnL-F, matK, rbcL*) sequences used here for the samples of *Genista sanabrensis* and related species are summarized in Table 2 and 3. The phylogenies estimated using MP and BI analyses of nuclear and plastid sequences are well-resolved and highly consistent one with another (Figure 2). In both cases, *Genista sanabrensis* appears as a well-supported monophyletic clade (100% BS, 100% PP for nDNA; 88% BS, 100% PP for cpDNA).

AFLP polymorphism

The six selected primers generated a total of 1222 bands for the 103 *Genista sanabrensis* samples. The number of bands and the percentage of polymorphic bands produced by each primer varied (Table 4). A summary of the genetic diversity for each of the five populations is given in Table 5. Moderate/low levels of genetic diversity were found: the percentage of polymorphic bands ranged from 68.66% (GSA-4) to 75.78% (GSA-1), the mean observed number of alleles per locus ranged from 1.382 (GSA-4) to 1.516 (GSA-1) while the mean effective number of alleles per locus ranged from 1.139 (GSA-5) to 1.288 (GSA-4). The Nei's gene diversity ranged from 0.105 (GSA-5) to 0.174 (GSA-4), and the Shannon's information index ranged from 0.188 (GSA-5) to 0.276 (GSA-2). At species level, moderate/low levels of genetic diversity were revealed (H_E = 0.164 and I = 0.277). The genetic differentiation between the populations (*G*st) was 0.136. Based on the *G*st value, the level of gene flow (*Nm*) was estimated as 1.580. These results indicated low rate of gene flow among populations and low differentiation between extant populations.

Analysis of molecular variance revealed that 18.00% of the genetic variation was partitioned between populations and 82.00% was observed within populations (Table 6). These results indicated low genetic variation levels among the five populations analysed. Principal Coordinate Analysis (PCoA) did not show a clear separation of groups, although the samples tend to be aggregated according to each population (Figure 3). The first three axes of PCoA explained 18.04, 4.43 and 2.48% of the total variation respectively. Genetic identity (I) and genetic distance (D) among populations varied from 0.922 to 0.995 and from 0.005 to 0.081, respectively (Table 7). In the Structure analysis based on the delta K values, K=2 was found to represent an optimal clustering of individuals (Figure 4).

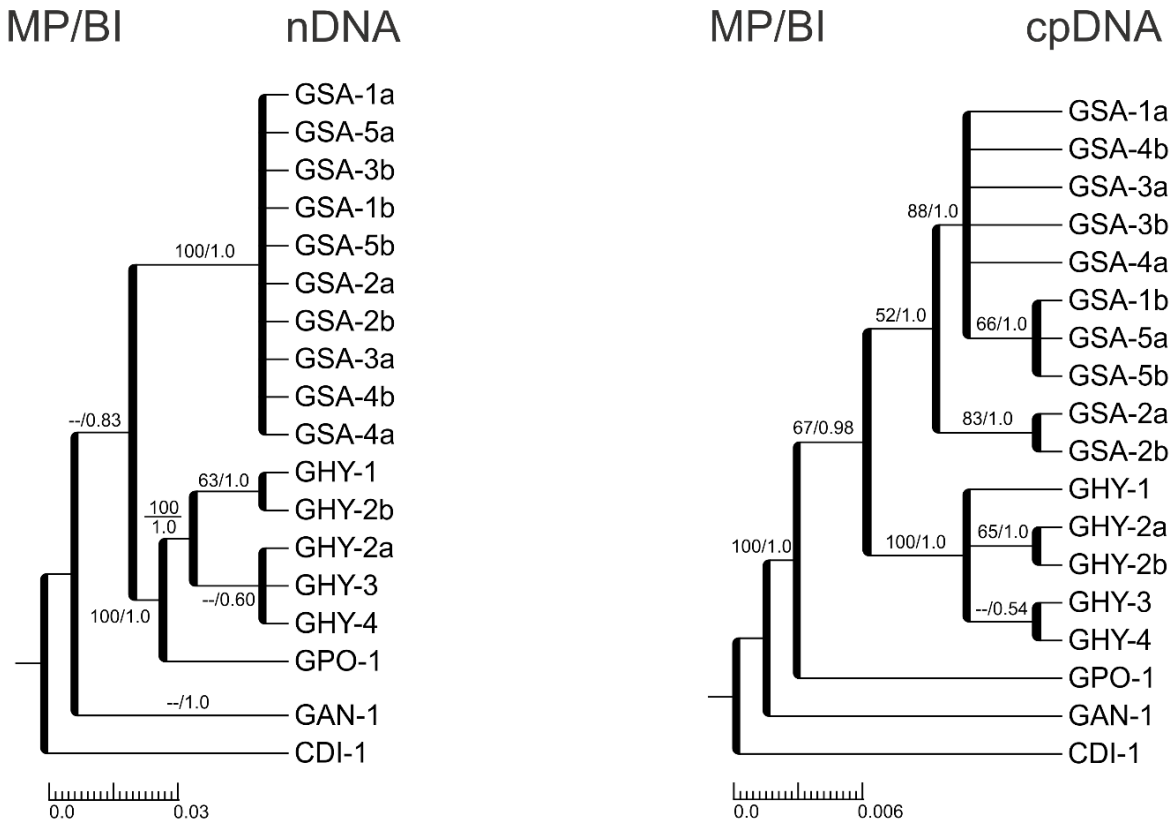


Figure 2. Phylogenetic trees derived from the analysis of Maximum Parsimony (MP; MEGA 7.0) and Bayesian Inference (BI; BEAST 2.4.6) in populations of *Genista sanabrensis* based on nuclear (ITS+ETS) and plastid (*trnL+trnL-F+matK+rbcL*) sequences. Along branches, bootstrap values (>50%; 1000 replicates) of MP and BI respectively. Note: *Genista sanabrensis* (GSA), *G. anglica* (GAN), *G. hystrix* (GHY), *G. florida* subsp. *polygalaephylla* (GPO), *Cytisus dieckii* (CDI).

Table 4. Pairs of primers used for AFLP amplification of *Genista sanabrensis* and summary of amplified bands. NB: number of bands; NPB: number of polymorphic bands; PPB: the average percentage of polymorphic bands.

Primer pairs	NB	NPB	PPB (%)
*Eco*RI-ACG / *Mse*I-CAT	196	150	76.30
*Eco*RI-ACT / *Mse*I-CTT	308	227	73.64
*Eco*RI-AAC / *Mse*I-CAT	197	156	79.19
*Eco*RI-ACG / *MseI*-CCAC	165	110	66.42
*Eco*RI-ACT / *MseI*-CCAC	184	138	74.78
*Eco*RI-AAC / *MseI*-CCAC	172	112	65.12
Total	1222	893	

Discussion

Nature is having a hard time where human activities, global environmental changes, habitat loss and species extinction often lead to a loss of biodiversity. For example, habitat fragmentation and population decline could reduce the effective population size and threaten the viability of the target species (Falk & Holsinger, 1991). Many biologists argue that establish correct conservation strategies minimizing biodiversity loss (Hamrick & Godt, 1996; Marchese, 2015) and a good example is conserve geographically-rare species (Vázquez & Gittleman, 1998).

Programs to conserve rare and endemic plants (usually these two characteristics are associated with endangered species) should take into account the use of molecular markers because can contribute to the setting of conservation priorities (Frankham & *al.*, 2004; Höglund, 2009). Recent studies (Vane-Wright & *al.* 1991; Nee & May, 1997) argues that used a phylogenetic approach is essential to guarantee the maintenance of high levels of biological diversity

in the future. Unfortunately, limited information is available regarding the population genetics of rare, endemic, threatened or endangered species. Endemic (and rare) plants with narrow distribution range have been analyzed traditionally within the framework of the theoretical predictions of small populations. In these taxa, the lowest population genetic diversity levels are expected, and many study cases confirm such predictions (Gitzendanner & Soltis, 2000; Cole, 2003; Solórzano & *al.*, 2016).

Table 5. Genetic diversity in *Genista sanabrensis* determined by AFLP markers at population level. Population codes are as shown in Table 1. Abbreviations are: N: sample size; PPB: percentage of polymorphic bands; A_O: observed mean number of alleles per locus; A_E: effective mean of alleles per locus; H_E: expected heterozygosity; I: Shannon diversity index; A_P: number of private alleles; A_D: number of discriminating alleles; G_{ST}: coefficient of genetic differentiation among populations; *Nm*: gene flow (Nm=(1 - FST)/4 FST).

			AFLP						
Level	N	PPB	$A_O \pm$ SE	$A_E \pm$ SE	$H_E \pm$ SE	$I \pm$ SE	A_P	G_{ST}	*Nm*
Populations									
GSA-1	21	75.78	1.516±0.025	1.179±0.006	0.129±0.004	0.223±0.005	39		
GSA-2	21	74.14	1.489±0.025	1.273±0.009	0.172±0.005	0.276±0.007	36		
GSA-3	21	75.61	1.512±0.025	1.213±0.007	0.149±0.004	0.251±0.006	38		
GSA-4	20	68.66	1.382±0.026	1.288±0.010	0.174±0.005	0.273±0.007	27		
GSA-5	20	70.62	1.412±0.026	1.139±0.005	0.105±0.003	0.188±0.005	42		
Average		72.96	1.462	1.218	0.146	0.242	36.4		
Species	103	100	2.000 ± 0.000	1.239±0.007	0.164±0.004	0.277±0.006	182	0.136	1.580

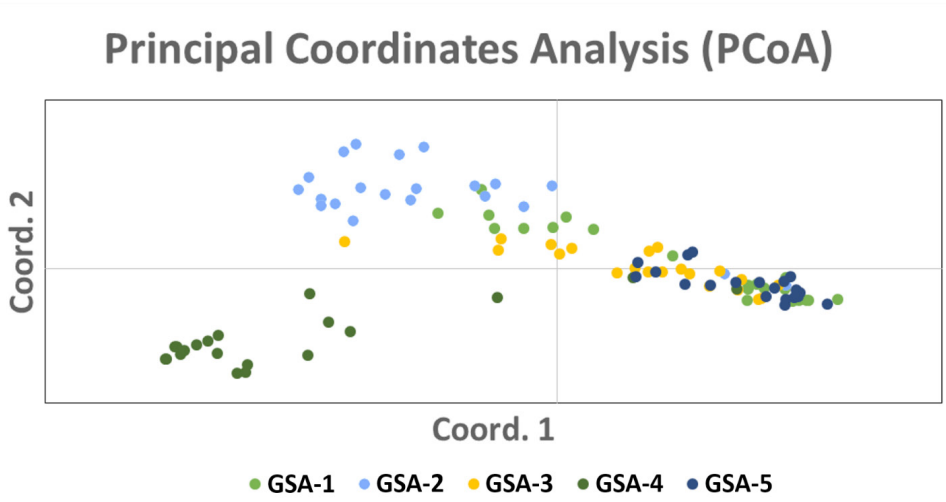


Figure 3. Principal Coordinates Analysis (PCoA) from five populations of *Genista sanabrensis* based on the correlation matrix of presence/absence of AFLP fragments.

Plant genetic diversity is spatially structured at different scales (e.g. geographical areas, populations, or among neighbouring individuals), and therefore, management schemes for conservation often require an understanding of population dynamics and knowledge of relative levels of genetic diversity, within- and among-population, in order to focus efforts on specific populations needing recovery (Engelhardt & *al.*, 2014; Peñas & *al.*, 2016; Turchetto & *al.*, 2016). Our study of the genetic structure of *Genista sanabrensis* has important implications for the conservation and management of this narrowly distributed and rare species. Genetic differentiation among populations and regions of *G. sanabrensis* was moderate/low, which could be interpreted as the result of recent allopatric fragmentation. The results obtained for both types of DNA sequences (nuclear and plastid) confirm the monophyly of the species. Moreover, the presence of indels (insertions/deletions) together with point mutations makes the northern populations (i.e. Asturian populations) essentials for the elaboration of future conservation strategies. The AFLP (H_E = 0.164) genetic diversity levels found for *G. sanabrensis* is comparable to those reported for other rare and/or endemic plants studied with this method [i.e. *Astragalus cremnophylax* (Travis & *al.*, 1996), *Cochlearia pyrenaica* (Cires & *al.*, 2011), *Edraianthus serpyllifolius* (Surina & *al.*, 2011), *Eryngium alpinum* (Gaudeul & *al.*, 2000), *Juniperus cedrus* (Rumeu & *al.*, 2014) or even long-lived trees such as *Juniperus thurifera* (Terrab & *al.*, 2008)].

The IUCN does not hold any information for *Genista sanabrensis*, but it does appear in listings at the regional level (see for example Red List of the Leon Flora; see Llamas & *al.,* 2003). However, applying an endemicity index, threat and rarity (PriCon index), together with other criteria (i.e. restricted distribution, protection at local and national level or fragility related with habitat rarity and habitat loss), Acedo & *al.* (2011) consider *G. sanabrensis* as a priority taxon for the conservation of their populations.

European dry heathland constituting habitat types of community interest in Spain, and there are characterized by the presence of typically Eurosiberian species, such as *Erica cinerea* L., *Daboecia cantabrica* (Huds.) K. Koch and *Ulex europaeus* L., as well as *Calluna vulgaris* (L.) Hull. At its upper altitudinal limits, these heaths include other species such as, *Juniperus communis* L., *Genista carpetana* Leresche ex Lange and the study taxon here presented *G. sanabrensis* (Ojeda, 2009).

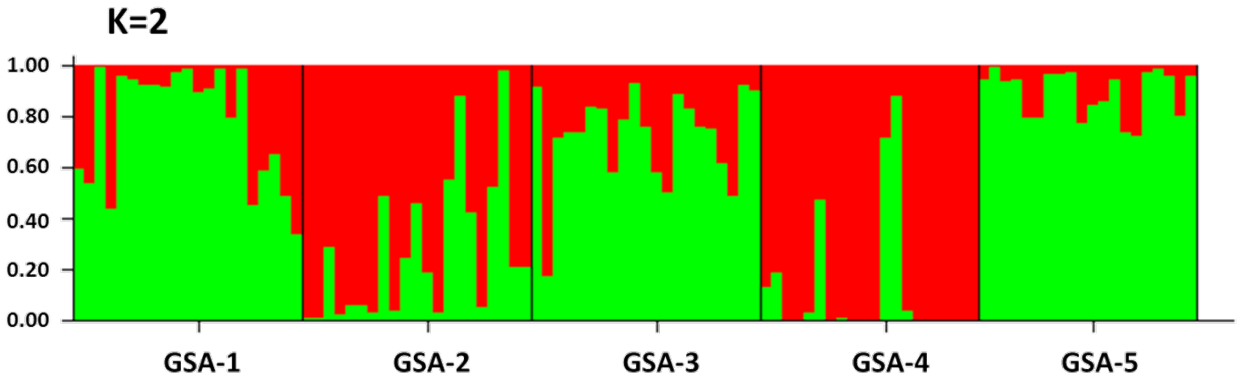


Figure 4. Bar plot of population assignment proportions according to Evanno's statistic (ΔK) for *Genista sanabrensis* based on and AFLP. Each individual is represented by a column filled with different colours.

Table 6. Analysis of molecular variance among and within five populations of *Genista sanabrensis* based on AFLP data. Abbreviations are: df degree of freedom; SS sum of squares; MS mean of squares; VC variance component; % total variation contributed by each component; *P* value* of fixation index after 9999 random permutations.

		AFLP				
Source of variation	df	SS	MS	VC	%	*P* value*
Among populations	4	2768.77	692.191	27.55	18	<0.001
Within populations	98	12214.69	124.640	124.64	82	<0.001
Total	102	14983.45		152.19	100	

According to Lence & *al.* (2010), the present state of conservation of the species *G. sanabrensis* is favourable with an optimum habitat of silicate creeping juniper (association *Genisto sanabrensis-Juniperetum nanae* Fern. Prieto 1983). The accompanying species are very few, highlighting *Juniperus communis* subsp. *nana* Syme, *Vaccinium myrtillus* L. and *Calluna vulgaris* (L.) Hull. Potential threat factors are grazing (although its spinous morphology protects it well against the herbivores), burning and/or scrub cleaning. As mentioned by several authors (e.g. Scherr & McNeely, 2008; Tucker & *al.*, 2017), we need to go toward a common, modern and broader vision of biodiversity conservation. The increasing availability of molecular data and the recent advances in software and phylogenetic methods will enhance even more the use of phylogenetic information to better characterize and describe biodiversity patterns (Roquet & *al.*, 2013).

In the present work, we show the use of phylogenies for rare taxa as guides in the selection of conservation areas to guarantee maximum biological diversity. We conclude that *Genista sanabrensis* is not globally threatened given its distribution range, the ecology and the conservation status of its populations. According to the IUCN (2017) criteria, it should be considered a species of Least Concern (LC). Nevertheless, it is protected in some parts of its distribution area owing to its local rarity. Since most management practices have been directed toward habitat preservation, *Genista sanabrensis* does not appear to be in immediate danger despite its reduced distribution and habitat fragmentation. The genetic diversity suggests that the species is not at high risk of extinction due to genetic factors. Because the main threats to *G. sanabrensis* is habitat fragmentation, *in situ* conservation should be especially aimed at controlling the general reduction of human impact on the populations. A highly fragmented structure of the habitat will limit the dispersal capacity of the seeds and will cause demographic isolation of the different populations (Olivieri & *al.*, 2016). We hope that these results will convince conservation biologists that genetic data for a rare species are very informative establishing global and/or regional conservation strategies and that the use of phylogenies in ecology is increasingly common and has broadened our understanding of biological diversity.

Table 7. Nei's measures of genetic distance (above diagonal) and genetic identity (below diagonal) of *Genista sanabrensis* populations.

	GSA-1	GSA-2	GSA-3	GSA-4	GSA-5
GSA-1	—	0.038	0.009	0.068	0.005
GSA-2	0.968	—	0.032	0.038	0.044
GSA-3	0.991	0.968	—	0.064	0.010
GSA-4	0.934	0.963	0.938	—	**0.081**
GSA-5	**0.995**	0.957	0.990	0.922	—

Acknowledgements

We thank Alicia Cerraján Raigoso, Antón Fernández Ceballos, Juan Díaz García, María Ceballos, Pablo Vázquez, Susana Garcia Díaz and Salvador Rodríguez Ambres, for providing plant material and help during collecting. We would like to thank Candela Cuesta Moliner (Área de Fisiología Vegetal, Universidad de Oviedo) for her help and critical reading of the manuscript. This research was funded by the "Consejería de Desarrollo Rural y Recursos Naturales, Principado de Asturias (CN-16-020)".

References

Acedo, C., Lence, C., Molina, A., Alonso, A. & Llamas, F. 2011. Aplicación del Índice PriCon, de Prioridad de Conservación, a la flora del municipio de. Ponferrada (León, España). Act. IX Col. Int. Bot. Pir. Cant.: 7-20.

Arrigo, N., Tuszynski, J.W., Ehrich, D., Gerdes, T. & Alvarez, N. 2009. Evaluating the impact of scoring parameters on the structure of intra-specific genetic variation using RawGeno, an R package for automating AFLP scoring. BMC Bioinformatics 10(1): 1.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.H., Xie, D., Suchard, M., Rambaut, A. & Drummond, A.J. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLOS Comput. Biol. 10(4): e1003537.

Carlón, L., González del Valle, J.M., Laínz, M., Moreno Moral, G., Rodríguez Berdasco, J.M. & Sánchez Pedraja, O. 2010. Contribuciones al conocimiento de la flora cantábrica, VIII. Doc. Jard. Bot. Atl. (Gijón) 7: 1-95.

Cires, E., Cuesta, C. & Fernández Prieto, J.A. 2012. Conservation genetics of the endangered endemic Ranunculus cabrerensis subsp. muniellensis (Ranunculaceae) in the Northwest of Spain. Bol. Ci. Nat. RIDEA 52: 117-134.

Cires, E., Cuesta, C. & Fernández Prieto, J.A. 2013. Genetic diversity and structure in fragmented populations of the endangered species Ranunculus cabrerensis (Ranunculaceae): implications for conservation. Biologia 68(1): 30-40.

Cires, E., Samain, M.-S., Goetghebeur, P. & Fernández Prieto, J.A. 2011. Genetic structure in peripheral Western European populations of the endangered species Cochlearia pyrenaica (Brassicaceae). Plant Syst. Evol. 297(1-2): 75-85.

Cole, C.T. 2003. Genetic variation in rare and common plants. Annu. Rev. Ecol. Evol. Syst. 34: 213-237.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9(8): 772.

Earl, D.A. & vonHoldt, B.M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4(2): 359-361.

Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32(5): 1792-1797.

Ellegren, H. & Galtier, N. 2016. Determinants of genetic diversity. Nat. Rev. Genet. 17: 422–433.

Engelhardt, K.A.M., Lloyd, M.W. & Neel, M.C. 2014. Effects of genetic diversity on conservation and restoration potential at individual, population, and regional scales. Biol. Conserv. 179: 6-16.

Evanno, G., Regnaut, S. & Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14: 2611-2620.

Falk, D.A. & Holsinger K.E. (Eds.). 1991. Genetics and conservation of rare plants. Oxford Univ. Press, New York.

Falush, D., Stephens, M. & Pritchard, J.K. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol. Ecol. Notes 7: 574-578.

Fernández Prieto, J.A. 1983. Aspectos geobotánicos de la Cordillera Cantábrica. An. Jard. Bot. Madrid 39(2): 489-513.

Fernández Prieto, J.A., Arjona, J.M., Sanna, M., Pérez, R. & Cires, E. 2013. Phylogeny and systematics of Micranthes (Saxifragaceae): an appraisal in European territories. J. Plant Res. 126(5): 605-611.

Fernández Prieto, J.A., Cires, E., Bueno Sánchez, A., Vázquez, V.M. & Nava Fernández, H.S. 2014. Catálogo de las plantas vasculares del Principado de Asturias. Doc. Jard. Bot. Atlántico (Gijón) 11: 7-267.

Fernández Prieto, J.A., Molero Mesa, J., Pérez Súarez, M., Sanna, M., Muñoz Díaz, J.M. & Cires, E. 2015. Are they different species or vicariant elevational races of the same species? The case of an Iberian endemic plant, Ranunculus bupleuroides (Ranunculaceae). Phytotaxa 239(3): 242-252.

Frankham, R. 2005. Stress and adaptation in conservation genetics. J. Evol. Biol. 18: 750-755.

Frankham, R., Ballou, J.D. & Briscoe, D.A. 2002. Introduction to conservation genetics. Cambridge Univ. Press, Cambridge.

Frankham, R., Ballou, J.D., Briscoe, D.A. & McInnes, K.H. 2004. A Primer of conservation genetics. Cambridge Univ. Press, Cambridge.

García González, M.E., Puente, E., López Pacheco, M.J., Herrero, L. & Penas, A. 1987. De plantis legionensibus. Notula X. Stud. Bot. Salamanca 6: 103-108.

Gaudeul, M., Taberlet, P. & Till-Bottraud, I. 2000. Genetic diversity in an endangered alpine plant, Eryngium alpinum L. (Apiaceae), inferred from amplified fragment length polymorphism markers. Mol. Ecol. 9: 1625-1637.

Gitzendanner, M. & Soltis, P.S. 2000. Patterns of genetic variation in rare and widespread plant congeners. Am. J. Bot. 87: 783-792.

Hamrick, J.L. & Godt, M.J.W. 1996: Conservation genetics of endemic plant species. In: Avise, J.C. & Hamrick, J.L. (Eds.). Conservation genetics: case histories from nature. Chapman and Hall, New York, pp. 281-304.

Höglund, J. 2009. Evolutionary conservation genetics. Oxford University Press, Oxford.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12): 1647-1649.

Kumar, S., Stecher, G. & Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33: 1870-1874.

Lence, C., Alfaro, E. & Acedo, C. 2010. Genista sanabrensis. Fichas del catálogo de Flora protegida en Castilla y León: Documento técnico de difusión restringida para la Junta de Castilla y León. Junta de Castilla y León.

Llamas, F., Acedo, C., Alonso, R., Lence, C., del Río, S. & Fernández. A. 2003. Flora leonesa amenazada. Acta Bot. Barcinon. 49: 53-66.

Mahé, F., Pascual, H., Coriton, O., Huteau, V., Perris, A.N., Misset, M.T. & Aïnouche, A. 2011. New data and phylogenetic placement of the enigmatic Old World lupin: Lupinus mariae-josephi H. Pascual. Genet. Resour. Crop Ev. 58(1): 101-114.

Marchese, C. 2015. Biodiversity hotspots, a shortcut for a more complicated concept. Global Ecol. Conserv. 3: 297-309.

Meloni, M., Reid, A., Caujapé-Castells, J., Soto, M., Fernández-Palacios, J.M. & Conti, E. 2015. High genetic diversity and population structure in the endangered Canarian endemic Ruta oreojasme (Rutaceae). Genetica 143(5): 571-580.

Mills, M. & Schwartz, M. 2005. Rare plants at the extremes of distribution: broadly and narrowly distributed rare species. Biodivers. Conserv. 14: 1401-1420.

Nee, S. & May, R.M. 1997. Extinction and the loss of evolutionary history. Science 278: 692-694.

Ojeda, F., 2009. Brezales secos europeos. In: Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España. Pp. 66. Min. Med. Amb. Med. Rur. Mar., Madrid.

Olivieri, I., Tonnabel, J., Ronce, O. & Mignot, A. 2016. Why evolution matters for species conservation: perspectives from three case studies of plant metapopulations. Evol. Appl. 9: 196-211.

Olmstead, R.G., Michaels, H.J., Scott, K.M. & Palmer, J.D. 1992. Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of rbcL. Ann. Mo. Bot. Gard. 79: 249-265.

Peakall, R.O.D. & Smouse, P.E. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6(1): 288-295.

Peakall, R.O.D. & Smouse, P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28: 2537-2539.

Peñas, J., Barrios, S., Bobo-Pinilla, J., Lorite, J. & Martínez-Ortega, M.M. 2016. Designing conservation strategies to preserve the genetic diversity of Astragalus edulis Bunge, an endangered species from western Mediterranean region. PeerJ 4: e1474.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

R Core Team. 2014. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/.

Rambaut, A. & Drummond, A.J. 2013. Tracer (Ver. 1.6). http//beast.bio.ed.ac.uk/Tracer. Accessed 15 June 2017.

Roquet, C., Thuiller, W., Lavergne, S., 2013. Building megaphylogenies for macroecology: taking up the challenge. Ecography 36: 13-26.

Rumeu, B., Vargas, P., Jaén-Molina, R., Nogales, M., Caujapé-Castells, J. 2014. Phylogeography and genetic structure of the threatened Canarian Juniperus cedrus (Cupressaceae). Bot. J. Linn. Soc. 175: 376-394.

Scherr, S. & McNeely, J.A. 2008. Biodiversity conservation and agricultural sustainability: towards a new paradigm of "eco-agriculture" landscapes. Philos. T. Roy. Soc. B 363: 477-494.

Solórzano, S., Arias, S. & Dávila, P. 2016. Genetics and conservation of plant species of extremely narrow geographic range. Diversity 8(4): 31.

Stöver, B.C. & Müller, K.F. 2010. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. BMC Bioinformatics 11(1): 1.

Sun, Y., Skinner, D.Z., Liang, G.H. & Hulbert, S.H. 1994. Phylogenetic analysis of Sorghum and related taxa using internal transcribed spacers of nuclear ribosomal DNA. Theor. Appl. Genet. 89: 26-32.

Surina, B., Schönswetter, P. & Schneeweiss, G.M. 2011. Quaternary range dynamics of ecologically divergent species (Edraianthus serpyllifolius and E. tenuifolius, Campanulaceae) within the Balkan refugium. J. Biogeogr. 38: 1381-1393.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17(5): 1105-1109.

Terrab, A., Schönswetter, P., Talavera, S., Vela, E. & Stuessy, T.F. 2008. Range-wide phylogeography of Juniperus thurifera L., a presumptive keystone species of western Mediterranean vegetation during cold stages of the Pleistocene. Mol. Phylogenet. Evol. 48: 94-102.

Tomasello, S., Álvarez, I., Vargas, P. & Oberprieler, C. 2015. Is the extremely rare Iberian endemic plant species Castrilanthemum debeauxii (Compositae, Anthemideae) a 'living fossil'? Evidence from a multi-locus species tree reconstruction. Mol. Phylogenet. Evol. 82: 118-130.

Travis, S.E., Manchinski, J. & Keim, P. 1996. An analysis of genetic variation in Astragalus cremnophylax var. cremnophylax, a critically endangered plant, using AFLP markers. Mol. Ecol. 5: 735-745.

Tucker, C.M., Cadotte, M.W., Carvalho, S.B., Davies, T.J., Ferrier, S., Fritz, S.A., Grenyer, R., Helmus, M.R., Jin, L.S., Mooers, A.O., Pavoine, S., Purschke, O., Redding, D.W., Rosauer, D.F., Winter, M. & Mazel, F. 2017. A guide to phylogenetic metrics for conservation, community ecology and macroecology. Biol. Rev. 92(2): 698-715.

Turchetto, C., Segatto, A.L.A., Mäder, G., Rodrigues, D.M., Bonatto, S. & Freitas, L.B. 2016. High levels of genetic diversity and population structure in an endemic and rare species: implications for conservation. AoB Plants 8: plw002.

IUCN 2017. The IUCN Red List of Threatened Species. Version 2017-3. http://www.iucnredlist.org.

Vane-Wright, R.I, Humphries, C.J., Williams, P.H. 1991. What to protect? - Systematics and the agony of choice. Biol. Conserv. 55: 235-254.

Vázquez, D.P. & Gittleman, J.L. 1998. Biodiversity conservation: does phylogeny matter? Curr. Biol. 8(11): R379-R381.

Vere, N. De, Rich, T.C.G, Ford, C.R., Trinder, S.A, Long,Ch., Moore, C.W. Satterthwaite, D., Davies, H., Allainguillaume, J., Ronca, S., Tatarinova, T., Garbett, H., Walker, K., Mike J. & Wilkinson, M.J. 2012. DNA barcoding the native flowering plants and conifers of Wales. PLoS One 7(6): e37945.

Floristic appendix

The list below contains the ranks and accepted authorship of the taxa mentioned in the text, when they do not coincide with those recognised in Flora Iberica (Castroviejo & als. (Eds.), 1986-2015) and in Flora Europaea (Tutin & al. (Eds.), Cambridge 1964-1980, 2010).

Cytisus dieckii (Lange) Fern.Prieto & al. (2017) = *Cytisus cantabricus* sensu auct., non (Willk.) Rchb.f. & Beck in Rchb.

Genista florida subsp. *polygaephylla* (Brot.) Cout. = *Genista florida* L.

Juniperus communis subsp. *nana* Syme in Sm. = *Juniperus communis* subsp. *alpina* (Suter) Čelak.