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

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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.

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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), DNA 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/delecciones 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 genética; *Genista*; ITS; marcadores moleculares; secuencias de plástidos; diferenciación poblacional.

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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 strengthen 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...
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: 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.](image-url)
| Code     | Population                          | Coordinates                          | GenBank accession | Coordinates | GenBank accession |
|----------|-------------------------------------|--------------------------------------|-------------------|-------------|-------------------|
| GSA-1a   | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000030          | 42°23'36.91"N 6°30'31.07"W | MH000030 |
| GSA-1b   | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000031          | 42°23'36.91"N 6°30'31.07"W | MH000031 |
| GSA-1c   | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000032          | 42°23'36.91"N 6°30'31.07"W | MH000032 |
| GSA-1d   | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000033          | 42°23'36.91"N 6°30'31.07"W | MH000033 |
| GSA-2a   | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000034          | 42°23'36.91"N 6°30'31.07"W | MH000034 |
| GSA-2b   | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000035          | 42°23'36.91"N 6°30'31.07"W | MH000035 |
| GSA-3a   | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000036          | 42°23'36.91"N 6°30'31.07"W | MH000036 |
| GSA-3b   | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000037          | 42°23'36.91"N 6°30'31.07"W | MH000037 |
| GSA-4a   | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000038          | 42°23'36.91"N 6°30'31.07"W | MH000038 |
| GSA-4b   | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000039          | 42°23'36.91"N 6°30'31.07"W | MH000039 |
| GSA-5a   | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000040          | 42°23'36.91"N 6°30'31.07"W | MH000040 |
| GSA-5b   | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000041          | 42°23'36.91"N 6°30'31.07"W | MH000041 |
| GAN-1    | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000042          | 42°23'36.91"N 6°30'31.07"W | MH000042 |
| GAN-2    | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000043          | 42°23'36.91"N 6°30'31.07"W | MH000043 |
| GPO-1    | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000044          | 42°23'36.91"N 6°30'31.07"W | MH000044 |
| GPO-2    | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000045          | 42°23'36.91"N 6°30'31.07"W | MH000045 |
| CDI-1    | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000046          | 42°23'36.91"N 6°30'31.07"W | MH000046 |
| CDI-2    | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000047          | 42°23'36.91"N 6°30'31.07"W | MH000047 |
| CDI-3    | Puerto de los Portillinos, Sierra del Teleno (León, España), 1894 m; PV, SG, VMV & JAFP | 42°23'36.91"N 6°30'31.07"W          | MH000048          | 42°23'36.91"N 6°30'31.07"W | MH000048 |
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 were performed using 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 amplification

The AFLP-based PCR was carried out as has been previously described (see Cires & al., 2011). The genomic DNA was digested with EcoRI and MseI restriction enzymes (New England Biolabs Inc.). In the following step, double-strand adapters were ligated to EcoRI and MseI 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 (EcoRI+A and MseI+C), checked by electrophoresis in 1.5% agarose gels and subsequently diluted (1:10) in sterile de-ionised H₂O. Then selective amplifications were performed using EcoRI and MseI 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 EcoRI-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_0), mean effective number of alleles (A_e), observed heterozygosity (H_o), 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 GenAIEx 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

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% |
period of $2 \times 10^5$ and $1 \times 10^5$ 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.

### 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-1) to 75.78% (GSA-1), the mean observed number of alleles per locus ranged from 1.382 (GSA-1) 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 0.277 (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 ($Gst$) was 0.136. Based on the Gst 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).
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 (\( Nm=(1 - F_{ST})/4 F_{ST} \)).

| Level  | N  | PPB         | \( A_o \ ± \ SE \) | \( A_e \ ± \ SE \) | \( H_e \ ± \ SE \) | \( I \ ± \ SE \) | \( A_p \) | \( G_{ST} \) | \( Nm \) |
|--------|----|-------------|---------------------|---------------------|---------------------|-----------------|--------|-----------|-------|
| 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 (Gaudef & 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 *et 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 *et 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).

![Bar plot of population assignment proportions according to Evanno’s statistic (ΔK) for *Genista sanabrensis* based on AFLP. Each individual is represented by a column filled with different colours.](image)

**Figure 4.**

![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.](image)

| 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  |       |      |          |

According to Lence *et 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 *et 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 *et 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 *et 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 | —     |

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**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.