

## Conservation assessment at a regional level: the study case of *Delphinium fissum* subsp. *sordidum* (Ranunculaceae), an endemic Iberian subspecies with disjunct distribution

Ruben Ramírez-Rodríguez<sup>1</sup>  & Francisco Amich<sup>1</sup> 

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**Abstract.** *Delphinium fissum* subsp. *sordidum* is a restricted endemic subspecies whose core distribution is located in the central-western Iberian Peninsula with a disjunct subpopulation in Sierra Mágina (Jaén Province). This article reports a detailed study of its current distribution, population size and structure, and the threats that it faces. Phytosociological analysis shows two main plant communities where *D. fissum* subsp. *sordidum* develops: *Quercion pyrenaicae* and *Quercion broteroi*. According to the most recent IUCN categories and criteria, the conservation status of *D. fissum* subsp. *sordidum* is “Endangered” (EN) in Castile and Leon. We propose the creation of a Plant Micro-Reserve (PMR) in an enclave with high geological, ecological and conservation values. Medium-long term and individualized monitoring programs are required for all distribution range of *Delphinium fissum* subsp. *sordidum*. An overall Recovery Plan for the subspecies should be considered and implemented, including in-situ and ex-situ conservation measures that guarantee its protection, conservation and recovery.

**Keywords.** Delphinieae; Iberian Peninsula; Castile and Leon; threatened endemic species; IUCN; Plant Micro-Reserve.

### Evaluación del estado de conservación a nivel regional: el caso de *Delphinium fissum* subsp. *sordidum* (Ranunculaceae), un endemismo Ibérico con distribución disyunta

**Resumen.** *Delphinium fissum* subsp. *sordidum* es un endemismo restringido cuyo núcleo principal de distribución se localiza en el centro-oeste de la Península Ibérica, con una subpoblación disyunta en Sierra Mágina (Jaén). Este artículo comprende un estudio detallado de su distribución actual, tamaño y estructura poblacional y de las amenazas a las que se enfrenta. El análisis fitosociológico muestra dos principales comunidades vegetales donde *D. fissum* subsp. *sordidum* se desarrolla: *Quercion pyrenaicae* y *Quercion broteroi*. Según los criterios y categorías más recientes de la UICN, el estado de conservación de *D. fissum* subsp. *sordidum* en Castilla y León es “En Peligro” (EN). Proponemos la creación de una Microrreserva de flora (PMR por sus siglas en inglés) en un enclave con unos valores geológicos, ecológicos y de conservación altos. Los programas de seguimiento a medio-largo plazo e individualizados son necesarios para todo el rango de distribución de *Delphinium fissum* subsp. *sordidum*. Un plan de recuperación general para la subespecie debería de ser considerado e implementado, incluyendo medidas de conservación in-situ y ex-situ que garanticen su protección, conservación y recuperación.

**Palabras clave.** Delphinieae; Península Ibérica; Castilla y León; especies endémicas amenazadas; UICN; Microrreserva de flora.

### Introduction

There are exceptional and unique places in the world that contain high concentrations of endemic plant species (two-thirds of which are exclusive species of priority conservation areas). These are defined as biodiversity hotspots when at least 70% of their primary vegetation has been lost and they contain at least 0.5% or 1500 species of vascular plants as endemics. A total of 34 hotspots were proposed by Mittermeier *et al.* (2004). The Mediterranean basin was described as a hotspot and it contains approximately 25,000 native plant species, of which about 13,000 are endemics. Projected extinctions under climate change scenarios have estimated a potential loss of 3,000 plant species in the Mediterranean basin

(Malcom *et al.*, 2006). The main drivers that produce the natural ecosystem changes and consequently the current biodiversity crisis are: (1) habitat fragmentation (Young & Clarke, 2000); (2) decreased habitat quality (Hooper *et al.*, 2012); (3) introduction of exotic species (Levine *et al.*, 2003) and (4) climate change (Leadley *et al.*, 2010).

During the past 8,000 years the Mediterranean basin, and especially Europe, has suffered the impacts of agricultural activity that have modified habitats, thereby increasing species loss rate (Greuter, 1994). The Iberian Peninsula and the Balearic Islands constitute one of the floristically richest areas of Europe and the Mediterranean basin (Domínguez Lozano *et al.*, 1996) due to its great bioclimatic and biogeographic

<sup>1</sup> Evolution, Taxonomy and Conservation Group (ECOMED), Department of Botany, University of Salamanca. E-37007 Salamanca, Spain. E-mail: rubenrr@usal.es

heterogeneity (Rivas-Martínez *et al.*, 2002) which was determined by geological and historical factors (Médail & Verlaque, 1997; Gomez & Lunt, 2004). The estimated number of vascular plants from mainland Spain and the Balearic Islands is approximately 7,000 taxa (species and subspecies), of which about 1,400 are endemics. Plant species diversity and composition is very similar between Spain and Portugal, sharing approximately 95.4% taxa (Aedo *et al.*, 2013).

*Delphinium sordidum* Cuatrec. was first recorded in Sierra Mágina (Jaén Province) (Cuatrecasas, 1929), considered as an exclusive endemic species of this mountain range (see also Pawlowski, 1964; Sainz Ollero & Hernández Bermejo, 1981). However, the taxon was not studied in detail in the following years until Amich García *et al.* (1981) pointed out that the morphological characters of dense pubescence in the corolla and widely lanceolate leaf segments were insufficient to differentiate between *D. fissum* Waldst. & Kit. and *D. sordidum* and the latter was thus described as subspecies. Subsequently, Blanché & Molero (1986) confirmed this. *Delphinium fissum* subsp. *sordidum* was included in the Red List of Spanish Vascular Flora 2008 (Moreno, 2008) and the Red Book Adenda 2010 (Bañares *et al.*, 2010) under the category EN B2ab(v)c(iv); C2b, due to its small range, area of occupancy, population size and low number of mature individuals with severe inter-annual fluctuations. In the regional catalogues of threatened flora, it was included in different categories as “In danger of extinction” in the Catalogue of Protected Flora in Castile and Leon (Anon., 2007) and in the Regional Catalogue of threatened species of Castile-La Mancha (Anon., 2001), “Special Interest” in the Regional Catalogue of threatened species of Extremadura (Anon., 2001), and “Vulnerable” in the Andalusian Catalogue of threatened species (Anon., 2012).

At present, 4 sites are located outside the limits of Castile and Leon: (1) in the largest and most important calcareous area in the northeastern Portugal (Mines of Santo Adrião, Caçarelhos, Trás-os-Montes region) (Ramírez-Rodríguez *et al.*, 2017); (2) in Sierra Mágina (Jaén Province) (Cuatrecasas, 1929; Gómez Mercado *et al.*, 1997), (3) in the locality of Hervás (Cáceres Province) (Amich García *et al.*, 1981), and (4) in Sierra de San Vicente (Toledo Province) (García Mateo & Pajarón, 2009). It should be noted that sites in both the locality of Almorchón (Badajoz Province) (Blanché, 1985) and the locality of Piedrabuena (Ciudad Real Province) (Martín-Blanco & Carrasco, 1997) were misidentifications (Carrasco *et al.*, 2003; Martín-Blanco & Carrasco, 2001). Likewise, phylogeographic analysis, using chloroplast DNA markers for Iberian species of the series *Fissa* B. Pawl., revealed that the site located in the Iberian System (Mateo Sanz & Pisco García, 1993) actually corresponds to *Delphinium bolsii* (Ramírez-Rodríguez *et al.*, 2019).

Considering that *Delphinium fissum* subsp. *sordidum* is catalogued as endangered and its core distribution is found in Castile and Leon, the present study aimed at assessing the regional conservation status of the subspecies for this region. To do so, we used both the

most recent IUCN guideline (IUCN, 2017) and the regional guideline (IUCN, 2012a) applying criteria B, C and D. To accurately determine the conservation status of the subspecies, it is necessary taking into account the following data: (1) current distribution range, (2) population size and structure, (3) phytosociological communities, and (4) threat factors. Based on our findings, we suggest adequate medium/long-term conservation measures for improving conservation planning and posit possible future lines of research for achieving a better overall knowledge of the subspecies.

## Materials and methods

### The species

*Delphinium fissum* subsp. *sordidum* is a perennial and rhizomatous hemicryptophyte that develops basal rosettes of palmatisect leaves (3–5 segments split up in linear-lanceolate lobules of 1–4 mm width) with semiamplexicaule petioles. During the blooming period (June–July), it grows a flowering stem, rarely branched in the base and densely pubescent, that forms a single dense inflorescence with 20–80 zygomorphic blue-violet flowers between 22–26 mm long. Lateral sepals are (7)8–9(10) mm long, ciliated, with dense pubescence. Spur is (13)15–16(17) mm and contains two hidden nectaries inside the spurs. Pollination is entomophilous, bumblebees (*Bombus terrestris* (Linnaeus, 1758)) and some butterfly species (especially *Macroglossum stellatarum* (Linnaeus, 1758)) being the most common pollinators (Ramírez-Rodríguez & Amich, 2017). Fructification occurs in the summer months (June–August). Fruits are (3)8–10 mm, glabrous follicles arranged in triplets. When fructification ends, the aerial part dries out and the rhizome remains in dormant state until the following spring, coinciding with the rainy season. Seeds are 2.5–3.5 mm, black, non-winged with 7–8 bands of imbricated membranous scales. Seed dispersal occurs by the combination of boleochory followed by anemochory and zoochory. Its chromosome number is  $2n = 16$  (Rico Hernández *et al.*, 1981).

### Data collection

We compiled historical records of *Delphinium fissum* subsp. *sordidum* in the Iberian Peninsula by consulting specialised scientific literature (Table 1) as well as material collected from our field explorations preserved in the herbarium SALA. *D. fissum* subsp. *sordidum* sites were visited during the 2007–2016 period to check the presence of this subspecies, as well as other sites where it might potentially occur. When these plants were found, we recorded the geographical location (a Garmin e-map GPS was used for georeferencing sites using  $1 \times 1$  km UTM and geographical coordinates), altitude, habitat, phenological data and threats, which were coded using the IUCN Threats Classification Scheme Version 3.2 (IUCN, 2012b).

Table 1. List of *Delphinium fissum* subsp. *sordidum* sites located in Castile and Leon (Spain).

Subpopulation code	Sites	UTM coordinates	Geographical coordinates	Altitude (m asl)	Source	Voucher
ADR	Salamanca, Aldeadávila de la Ribera, Los Ceños	29TQF0168	41° 14.551'N 6° 35.692'W	480	Ramírez– Rodríguez & Amich (2014)	SALA 152998
PDR	Salamanca, Aldeadávila de la Ribera, Corporario, Playa del Rostro	29TQF0168	41° 14.608'N 6° 35.839'W	390	Present work	SALA 107219
MAS	Salamanca, Masueco de la Ribera, Cascada del Pinero	29TQF0267	41° 13.790'N 6° 34.876'W	480	Present work	SALA 160042
VDA	Salamanca, Villarino de los Aires, Teso de San Cristóbal	29TQF1472	41° 16.718'N 6° 26.422'W	660	Amich García <i>et al.</i> (1981)	SALA 21458, 26026, 63768
VIL	Ávila, Villatoro, Puerto de Villatoro	30TUK1789	40° 31.927'N 5° 9.280'W	1400	Ramírez– Rodríguez <i>et al.</i> (2016)	SALA 159347
FER	Zamora, Fermoselle, riberas del Río Duero	29TQF1274	41° 17.690'N 6° 27.458'W	470	Sánchez Rodríguez (1986)	No voucher
LIN	Salamanca, Linares de Riofrío, Las Honfrías-base del Pico Cervero	30TTK4797	40° 35.376'N 5° 58.899'W	1250	Rico Hernández (1985)	SALA 28920, 109622
BEJ	Salamanca, Béjar, Tranco del Diablo	30TTK6274	40°23.319'N 5°48.063'W	810	Present work	SALA 137545, 159346

### Population size and structure

Population size was estimated by direct counting of all potentially reproductive individuals of each subpopulation, except for those not found (PDR and FER), marking them with coloured ribbons in the flowering stems. Mature individuals were considered if they developed flowering stems. To count the vegetative individuals, we visited VDA, ADR, VIL and MAS two months before the blooming period (April–May) and conducted a census in 2016, preventing the double counting of closely vegetative individuals. In either case, we estimated a counting error of approximately 10%. Each individual was assigned to one of the three different life-cycle stages, as indicated for *Delphinium bolosii* C. Blanché & Molero, a closely related species (Bosch *et al.*, 2009): (1) vegetative individuals, (2) reproductive individuals with a height less or equal to 1 m, and (3) reproductive individuals with a height of more than 1 m. Given the difficulty in counting the individual seedlings and recognizing whether the development of new individuals sprung from the growth of seedlings or were resprouts of dormant individuals already established, we decided not to include them in this study. To simplify the evaluation of the minimum viable population (MVP), we used guide values, following the indications offered by Blanca & Marrero (2003).

### Calculation of demographic parameters

We calculated the finite growth rate ( $\lambda$ ) of those subpopulations for which we have sufficient sampling data (i.e. VDA, ADR and BEJ). Finite growth rate ( $\lambda$ ) is defined as the ratio between the number of individuals recorded in a given year and the number of individuals in the previous year. This rate regards the population growth as stable, without considering other factors such as density, stochasticity and migration.

### Habitat

IUCN guidelines take into account the reduction of habitat quality during the assessment process (IUCN, 2017). Thus, the study of vegetation was performed according to the methodology proposed by Braun-Blanquet (1964).

### Threat category according to IUCN guidelines

In accordance with the IUCN regional guidelines (IUCN, 2012a) and the most recent guidelines for their application (IUCN, 2017), the regional conservation status of *Delphinium fissum* subsp. *sordidum* was assessed using criteria B, C and D. We calculated the extent of occurrence (EOO), defined as the minimum area that can be drawn to encompass all the known sites of occurrence of a taxon with exclusion of vagrancy cases, by using the Minimum Convex Polygon method (MCP) (Figure 1). Likewise, the area of occupancy (AOO), defined as the area within the EOO that is occupied by a taxon, was calculated superimposing a 2 × 2 km grid on population locations (Gargano, 2011).

### Results

#### Current distribution

The current distribution of *Delphinium fissum* subsp. *sordidum* in the Iberian Peninsula is depicted in Figure 1. Its core distribution is located in the central-western Iberian Peninsula, especially in Castile and Leon. At present, a total of eight subpopulations are located in the Ávila, Zamora and Salamanca provinces, six of which belong to the last one (Figure 1). We report one new subpopulation (MAS) included in the Arribes del Duero Natural Park and confirm four already known subpopulations (ADR, VDA, BEJ and VIL) (Table 1). LIN was only detected in 2014 and PDR and FER were not confirmed during the study period.

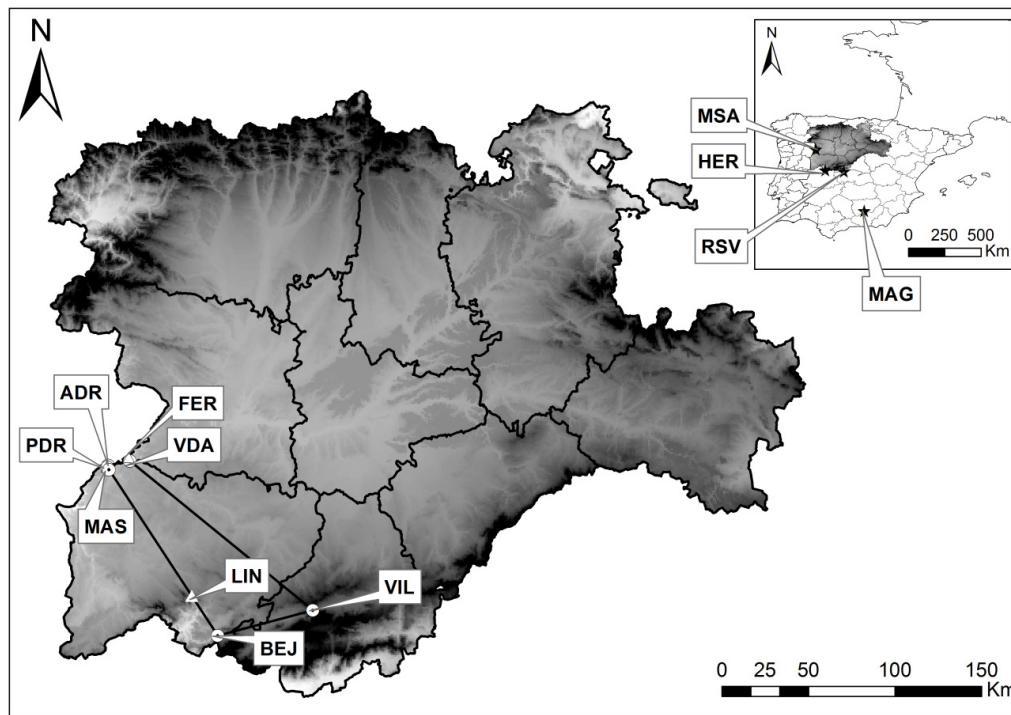


Figure 1. Location of Castile and Leon within Iberian Peninsula which is situated in the southwestern Europe. The distribution map of *Delphinium fissum* subsp. *sordidum* shows the Extent of Occurrence (EOO) calculated by the Minimum Convex Polygon (MCP). The circle symbol indicates the confirmed subpopulations and the triangle symbol the unconfirmed subpopulations located in Castile and Leon. The rest of subpopulations are represented by a star symbol completing the whole distribution range of the study subspecies.

### Threat factors

The actual and potential threat factors that might have a negative effect for the conservation of the subspecies are compiled in Table 2 and listed below:

- I. 1.3. The creation of an artificial beach and the construction of new picnic sites in the enclave known as “Playa del Rostro” affected PDR until its disappearance. In this sense, recreational activities (6.1) such as hiking, running, walking or bullfight exhibitions cause a significant decrease in habitat extent and habitat quality. Garbage and solid waste (9.4) is a direct consequence of human activities.
- II. 2.3. Although the number of cattle is not so large at present, it still exerts a noteworthy pressure on the habitat, affecting its extent and quality.

Herbivory can become a serious threat for some subpopulations (e.g. LIN and VIL). Soil nitrification, a consequence derived from the presence of livestock, causes deep changes in the habitat, favouring the growth of nitrophilous communities such as *Pruno-Rubion ulmifolii* O. Bolós 1954 in VIL and *Onopordetalia acanthii* Br.-Bl. & Tüxen ex Klika & Hadač 1944 in VDA (problematic native species (8.2)). Likewise, and relative to this threat, we observed signs that individuals had been trodden down and the soil had been turned over, attributed to the presence of wild animals such as wild boar and roe deer in ADR, and rabbits in VDA.

- III. In regard to potential threats that can occur in all sites, we report plant collecting (5.2) and fire (7.1.1).

Table 2. Threats detected in each studied subpopulation of *Delphinium fissum* subsp. *sordidum* (see Table 1 for abbreviations).

Threats/Subpopulations	ADR	PDR	MAS	VDA	VIL	FER	LIN	BEJ
Threat 1.3: Tourism and recreation areas	.	+	.	.	.	.	.	.
Threat 2.3: Livestock farming	.	.	.	.	+	.	+	.
Threat 5.2: Gathering terrestrial plants	+	+	+	+	+	+	+	+
Threat 6.1: Recreational activities	.	+	+	+	.	.	.	+
Threat 7.1.1: Increase in fire frequency/intensity	+	+	+	+	+	+	+	+
Threat 8.2: Problematic native species	+	.	.	+	+	.	.	.
Threat 9.4: Garbage and solid waste	.	+	.	.	.	+	.	.

## Population size and structure

The evolution in the number of reproductive individuals during the study period for each subpopulation is shown in Table 3. Final totals of 73, 2, 169, 0, 7 and 6 reproductive individuals were counted for the six studied subpopulations of *Delphinium fissum* subsp. *sordidum* confirmed in 2016. The number of reproductive individuals experienced an important inter-annual variability during the study period (Figure 2). The highest density occurred in BEJ and VDA, which corresponded to 65.8% and 28.4% respectively, from a total of 257 reproductive individuals counted in 2016 (Table 3).

Table 3. Evolution in the number of reproductive individuals during the corresponding study period for each subpopulation of *Delphinium fissum* subsp. *sordidum*.

Subpopulation	Years										Percentage
	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	
Villarino de los Aires (VDA)	39	42	68	41	173	76	195	249	41	73	28.4
Aldeadávila de la Ribera, Los Ceños (ADR)							3	17	17	2	0.8
Béjar (BEJ)								113	57	169	65.8
Linares de Riofrío (LIN)							1	0	0	0	0
Villatoro (VIL)									1	7	2.7
Masueco de la Ribera (MAS)										6	2.3
									Total	257	100

## Calculation of demographic parameters

The finite rates of population growth ( $\lambda$ ) were calculated from Table 3 data and indicate the increased/decreased population trend in percentages (Table 4).

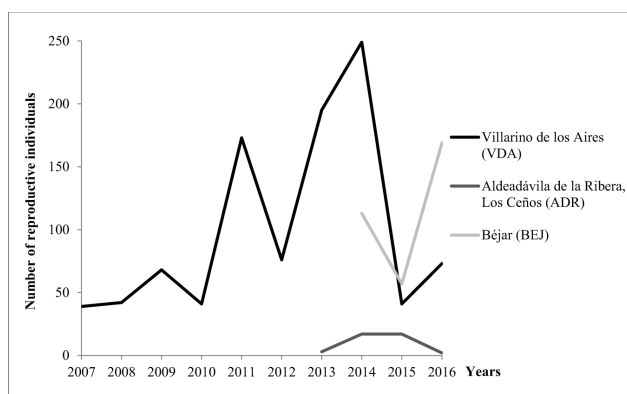


Figure 2. Variation in the number of reproductive individuals of *Delphinium fissum* subsp. *sordidum* for the subpopulations of Villarino de los Aires (VDA), Aldeadávila de la Ribera, Los Ceños (ADR) and Béjar (BEJ).

## Habitat

In this study a total of nine relevés and 93 taxa from Salamanca, Zamora and Ávila provinces were recorded (Table S1). The main habitat was constituted by

The population structure was studied for four subpopulations in 2016, and they displayed a similar composition (Figure 3). Thus, vegetative individuals clearly dominated the population structure (on average 79.25%). The rest of the composition consisted of reproductive individuals  $> 1$  m. (19.95%) and reproductive individuals  $\leq 1$  m. (0.8%) (Figure 3).

The MVP is estimated to be around 150–200 individuals, since it is a perennial species growing in climactic and serial habitats, with a lifespan of less than 25 years. These values are in accordance with the criteria previously indicated and characteristic features described for *Delphinium fissum* subsp. *sordidum*.

Despite the number of reproductive individuals of the three subpopulations having fluctuated significantly from year to year, all of them maintained a positive mean value and slightly improved in the overall trend (Table 4).

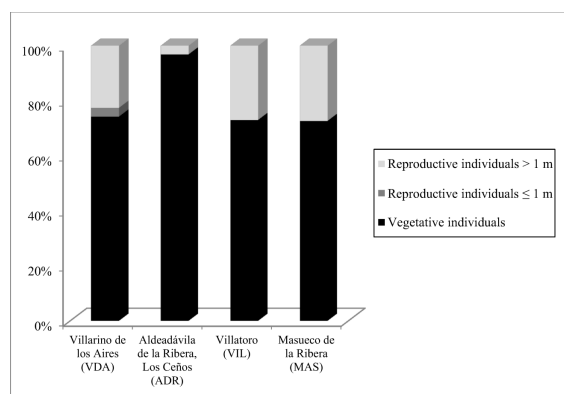


Figure 3. Population structure (percentages of vegetative individuals, reproductive individuals with a height less or equal than 1 m and reproductive individuals with a height more than 1 m) of four subpopulations of *Delphinium fissum* subsp. *sordidum*.

*Quercus pyrenaica* Willd. forests, sometimes intermixed with *Castanea sativa* Mill. forests, mainly in acidic soils referable to the alliance *Quercion pyrenaicae* Rivas-Goday ex Rivas-Martínez 1964 (Relevés 1–2). Occasionally, as occurred in VDA (Relevé 3),

this subspecies grows in clearings within *Quercion pyrenaicae* forests, seeking refuge from insolation under trees and shrubs; on the other hand, habitat modification, caused by livestock for example, has changed the species composition of the *Quercion pyrenaicae* due to habitat nitrification favouring the growth of sub-nitrophilous or/and nitrophilous communities. Thus, *Pruno spinosae-Rubion ulmifolii* corresponded to the first replacement stage of the alliance *Quercion pyrenaicae* in VIL (relevé 4). In relation to ADR, MAS and FER subpopulations (relevés 5–9) they presented typical species composition of the acidophilous marcescent forest, belonging to the suballiance *Quercenion broteroi* Rivas-Martínez 1987 and characteristic of the Lusitan-Duriensean biogeographic sector, included in the alliance *Quercion broteroi* Br.-Bl., P. Silva & Rozeira 1956 em. Rivas-Martínez 1975 corr. V. Fuente 1986.

### Current regional conservation status

The distribution range of *Delphinium fissum* subsp. *sordidum* in Castile and Leon is quite fragmented. In the study area, the EOO covered an approximate area of 3896.37 km<sup>2</sup> and AOO an approximate area of 20 km<sup>2</sup>.

A number of subpopulations occur largely in Castile & Leon (8) and remain isolated from each other. Extreme fluctuations in the number of reproductive individuals have been observed from year to year (Figure 2). Likewise, the subspecies was faced with several threats identified during the study period (Table 2). In accord with IUCN criteria the extent of occurrence is estimated to be lower than 5000 km<sup>2</sup>; the area of occupancy is estimated to be lower than 500 km<sup>2</sup>; the number of mature individuals is lower than 2500, with less than 250 mature individuals in each subpopulation, thus the assigned conservation status of *D. fissum* subsp. *sordidum* in Castile and Leon is Endangered (EN) [B1ab(iii)c(iv)+2ab(iii)c(iv); C2a(i)b; D1].

When applying the regional criteria its category has not suffered any change up or down, in spite of the suitable conditions and good habitat conservation in Castile and Leon, due to: (1) the mode of pollen and seed dispersal of the subspecies, (2) the lack of habitat connectivity and (3) the distance that exists between the subpopulations. Such characteristics suggest that effective gene flow between the subpopulations is almost non-existent, and consequently they are isolated from each other.

Table 4. Finite rates of population growth calculated for the subpopulations of Villarino de los Aires (VDA), Aldeadávila de la Ribera (ADR) and Béjar (BEJ).

Years	2007/08	2008/09	2009/10	2010/11	2011/12	2012/13	2013/14	2014/15	2015/16	Mean ± SD
λ VDA	1.08	1.62	0.6	4.22	0.44	2.57	1.28	0.16	1.78	1.53 ± 1.25
Increase/decrease population size	+8%	+62%	-40%	+322%	-56%	+157%	+28%	-84%	+78%	
λ ADR							5.7	1	0.12	2.27 ± 3
Increase/decrease population size							+470%	0%	-88%	
λ BEJ								0.5	2.96	1.73 ± 1.74
Increase/decrease population size								-50%	-196%	

### Discussion

When assessing the conservation status of taxa with disjunct distribution, such as for *Delphinium fissum* subsp. *sordidum*, it is necessary to take into account that the current techniques proposed by the International Union for Conservation of Nature (IUCN) overestimate the extent of occurrence (EOO) and, therefore, their degree of threat is underestimated (de Castro Pena *et al.*, 2014). With this in mind we determined the regional conservation status of the subspecies with disjunct distribution, selecting as our study area the Autonomous Community of Castile and Leon since it contains most of the known populations.

In this study we provide one new finding (MAS) for *Delphinium fissum* subsp. *sordidum* (Table 1). Although several authors (Amich *et al.*, 2004; Sardinero, 2004) have intensely surveyed the territories where the subspecies grow, we believe that in light of these findings it is necessary to continue with the fieldwork in these sites (Arribes del Duero Natural Park and Central System) and other potentially suitable areas for the subspecies (Rus *et al.*, 2018). Species distribution modelling (SDM) is a useful tool to identify potential

habitats and make more efficient use of our economic and human resources.

The main threats that affect *Delphinium fissum* subsp. *sordidum* are grazing, tourism and recreational activities, the last being the usual threat for several species in the Iberian Peninsula (Ballantyne & Pickering, 2013). In this sense, the extinction of the PDR subpopulation is clearly attributed to new construction of picnic sites, new roads and paths, and a remarkable increase in the number of tourists who take the river cruise (see Table 2). Trampling by hikers or visitors is an important threat derived from tourism and recreational activities (Rossi *et al.*, 2009), including impacts related to changes in soil ecology (Light & MacConaill, 2007).

In recent years, a significant increase in wild animals was detected in the study area, which can become a serious threat for *Delphinium fissum* subsp. *sordidum* through deteriorating habitat conditions in several subpopulations. Rabbits favour the growth of nitrophilous communities dominated by *Carduus tenuiflorus* (*Onopordetalia acanthii*) in an important area of VDA. Consequently, plant species composition was altered and replaced for other nitrophilous plant species that compete for resources with the subspecies

and may thus decrease the number of individuals. Furthermore, wild boar and roe deer occasionally uproot and trod down some individuals and turn over the soil, reducing the habitat quality and population size in ADR. On the other hand, the presence of domestic herbivores observed in LIN and VIL has negative biological and ecological consequences for the individuals of *D. fissum* subsp. *sordidum*, reported also for RSV (García Mateo & Pajarón, 2009) and MAG (Gómez Mercado *et al.*, 1997). Firstly, they eat the basal leaves and floral scape affecting the development and the subsequent fruiting of the damaged plants. Secondly, livestock cause the nitrification of soils and consequently the development of nitrophilous communities referable to *Pruno-Rubion ulmifolii*. Opposite effects have been described for *Rubus ulmifolius*. As a positive effect, the species is considered as nitrophilous shrub which can grow forming a barrier that protect individuals of *D. fissum* subsp. *sordidum* from a possible herbivory (Ramírez-Rodríguez *et al.*, 2016). In addition, the subspecies is very sensitive to extreme hot and dry periods (Blanché, 1991; Bosch, 1999), therefore *R. ulmifolius* might maintain shady conditions, and thus the humidity in the growing area of the subspecies. In contrast, some authors (Oostermeijer *et al.*, 1988; Orellana *et al.*, 2008) reported negative effects on plant pollination due to the presence of *Rubus* species.

Plant collecting of remarkable or/and rare species is a potential threat. However, botanists have generally been careful with collecting activities, and these botanical tasks have had very positive results for the conservation of species, providing timely and accurate information (Aedo *et al.*, 2015). Likewise, fire is also a potential threat that may occur most frequently in areas with high sun exposure and in grassland and southwest facing forest clearings, for example in VDA and in the only Portuguese locality, MSA (Ramírez-Rodríguez *et al.*, 2017). During the summer of 2006 a fire affected VDA, although its population fitness recovered in subsequent years. Another fire took place in the surroundings of FER as well in the summer of 2017, affecting the *Quercenion broteroi* forests.

The MVP for this subspecies is estimated at approximately 150–200 individuals, according to the characteristics of the subspecies already mentioned. Only VDA and BEJ had equal or greater numbers of individuals than the MVP. The remaining populations are more prone to suffer demographic and genetic stochasticity (Schemske *et al.*, 1994). Simulation studies showed that demographic stochasticity is relevant in very small populations ( $N < 50$ ) (Lande, 1993), being likely to be affected ADR, MAS and VIL subpopulations.

The distribution range of *Delphinium fissum* subsp. *sordidum* in Castile and Leon is fragmented, and in general the subpopulations remain isolated from each other. Effective gene flow between subpopulations depends on both effective pollen and seed dispersal (Petit *et al.*, 1993) and habitat connectivity. The subspecies has a great dependence on insect pollination, *Bombus terrestris* and *Macroglossum stellatarum* being the main pollinators (Ramírez-Rodríguez & Amich,

2017). Whereas the foraging distance of *B. terrestris* was estimated to range from 800 to 1,750 m (Osborne *et al.*, 2008), the maximum foraging distance of *M. stellatarum* was estimated at over 32 km (Stockhouse, 1973). This means that pollen can move long distances. Likewise, long-distance seed dispersal events can occur sporadically by wind and via domestic and wild herbivores (M. Melendo, pers.com.). As such, long-distance gene flow can occur between subpopulations.

To identify and understand the spatial and temporal variation in the population dynamic of *Delphinium fissum* subsp. *sordidum* from year to year it is crucial to know the environmental factors that produce these changes and influence the critical stages of the subspecies (see Rus *et al.*, 2018). As such, the noteworthy decrease in the number of reproductive individuals observed in VDA and BEJ during 2015 (Figure 2) can be explained by the lack of summer precipitation, giving rise to drought conditions during the blooming period of the subspecies.

During our fieldwork, we observed the same vegetative individuals in identical positions. This phenomenon can be explained due to the low recruitment rate and long establishment, since the individuals of *Delphinium fissum* subsp. *sordidum* have a long life-span (can live over 10 years). The reproductive strategy of the subspecies has evolved to favour vegetative spread, assured by a rootstock bank mechanism. The rhizome remains in a latent state, dormant until environmental conditions become favourable, as occurs in the closely related species *D. pentagynum* subsp. *formenterianum* (López-Pujol *et al.*, 2003) and *D. bolosii* (Orellana *et al.*, 2007). A significant percentage of individuals (70–75%) was observed in a vegetative stage (Figure 3), limiting the number of mates in a subpopulation in a given year, although this might increase the opportunities for crossing between genetically different individuals of the subpopulations, creating positive effects on genetic diversity and population fitness.

According to our results, *Delphinium fissum* subsp. *sordidum* occurred in heterogeneous areas, integrated in different types of plant communities. Two main phytosociological behaviours in Castile and Leon can be clearly distinguished: on the one hand, the relevés 1–4 (Table S1) belong to the alliance *Quercion pyrenaicae* or its first replacement stage (*Pruno spinosae-Rubion ulmifolii*) due to the presence of domestic herbivores (VIL). On the other hand, the following relevés (5–9) are included within the alliance *Quercion broteroi*, although we should perform more detailed phytosociological studies in these complex territories located in the Arribes del Duero Natural Park. It should be pointed out that the current westernmost subpopulation (MSA) is found on south-facing slopes in holm-oak forests referable to the suballiance *Paeonio broteroi-Quercenion rotundifoliae*, belonging to the alliance *Quercion broteroi* (Ramírez-Rodríguez *et al.*, 2017). The subspecies grows in shady and humid areas, especially on north-facing slopes under shrubs and trees, seeking organic matter produced by defoliation and refuge to mitigate the effects of insolation and to prevent water loss by evapo-

transpiration. The same behaviour was reported for the MAG subpopulation where it is integrated in thorny deciduous shrubs, pulviniform shrubs and grassland (Gómez-Mercado *et al.*, 1997).

As Médail & Quézel (1999) suggested, in order to obtain a better knowledge of plant diversity in plant-rich territories, a small scale approach is required. The Plant Micro-Reserve (PMR) network, with areas ranging from 20 to 200 ha, is the best way to encompass a large share of regional plant biodiversity (Laguna, 2001; Laguna

*et al.*, 2004; Anon., 2007) and protect plant-rich spots in the Iberian Peninsula (Gomez-Campo & Herranz-Sanz, 1993). We proposed the creation of a PMR in the Cascada del Pinero site (MAS subpopulation), which covers an area of 1,900 m<sup>2</sup> (0.19 ha) (Figure 4). Furthermore, the PMR is included in the larger protected area (Arribes del Duero Natural Park) as recommended by Araújo *et al.* (2007). Olive and grape farming areas, which are typical due to the mesoclimatic conditions (Calonge Cano, 1990), encompass to the PMR.

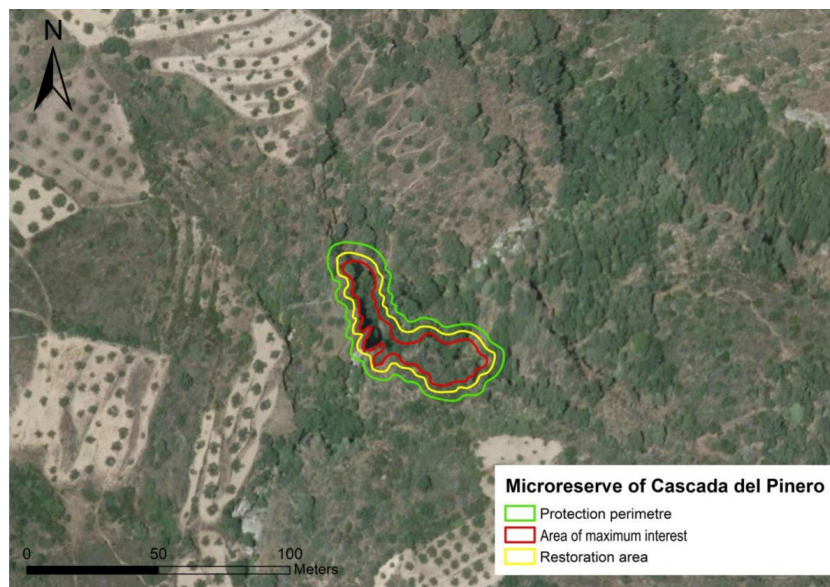


Figure 4. Proposal of Plant Micro-reserve in the enclave of Cascada del Pinero.

The area proposed for the creation of a PMR has exceptional geological and ecological characteristics and combines different habitats such as termophilous mixed forest of *Celtis australis* L., *Acer monspessulanum* L., *Pistacia terebinthus* L. and *Fraxinus angustifolia* Vahl together with rupicolous habitats where characteristic vegetation develops, belonging to the Lusitan-Duriensean synendemism *Phagnalo saxatilis*-*Antirrhinetum lopesianii* Bernardos, Crespi, Aguiar, F.J. Fernández and Amich 2004. The enclave is crossed by hiking paths and it might therefore be subject to threats related to recreational activities, especially during late spring-early summer seasons coinciding with the blooming periods of several rare, endemic, threatened and of interest species. As such, we found two species (*Antirrhinum lopesianum* Rothm. and *Delphinium fissum* subsp. *sordidum*) listed in the Red List of Spanish Vascular Flora 2008 (Moreno, 2008) as Endangered (EN) and in the Catalogue of Protected Flora in Castile and Leon (Anon., 2007) as “In danger of extinction”. Moreover, one of the three localities of *Adiantum capillus-veneris* L. in the Salamanca Province has been reported here (Ramírez-Rodríguez & Amich, 2014), growing on the rock faces of Ordovician gneisses with carbonated intercalations (Rodríguez-Alonso *et al.*, 2004). Other interesting species are *Clematis campaniflora* Brot., endemic to the southwestern quadrant of the Iberian Peninsula, and *Celtis australis*, whose forests have a high conservation value, on a European level, conferring uniqueness to the landscape.

Medium-long term and individualized monitoring programs for all subpopulations of *Delphinium fissum* subsp. *sordidum* are required to perform matrix analysis in order to know its population dynamic. Some interesting aspects should be surveyed in the future: (1) the dormant period; (2) behaviour and survival of seedlings, and (3) the different variables that affect the recruitment rate (e.g. herbivory, decrease in pollen transfer and seed production, soil characteristics, environmental variables, plant competition and resource availability). In relation to the genetic diversity of *D. fissum* subsp. *sordidum*, Orellana *et al.* (2007) reported that it was low. Therefore, seeds should be collected and stored in the germplasm bank at the University of Salamanca in order to preserve their genetic diversity and evolutionary potential, to perform germination tests and make reinforcement, restoration and reintroduction works in the future tasks if necessary.

In short, we believe that it is necessary to implement an overall Recovery Plan for *Delphinium fissum* subsp. *sordidum*, not only at regional level in the Guadalajara Province (DOCM, 2002) but also at peninsular level. This Recovery Plan should contain in-situ conservation measures (to locate priority sites for surveys of potentially unknown subpopulations; identify and reduce the threat factors; limit herbivore access to subpopulations and establish defined protected areas with marked trails for recreational users) and ex-situ conservation



measures (to store seeds in germplasm banks) that guarantee the protection and conservation of *D. fissum* subsp. *sordidum*. We stress the importance for all level of government to understand the benefits of funding and investing in conservation programs for species recovery, as well as the involvement of politicians in sustainable economic and nature restoration projects.

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## Supplementary Material

**Table S1.** Relevés recorded in the localities where *Delphinium fissum* subsp. *sordidum* was collected.