

M. Bosch, L. Sàez, J. Simon & C. Blanché

Karyological study of some E Iberian threatened plants

Abstract

Bosch, M., Sàez, L., Simon, J. & Blanché, C. 2019: Karyological study of some E Iberian threatened plants [In Kamari, G., Blanché, C. & Siljak-Yakovlev, S. (eds), Mediterranean plant karyological data - 29]. – Fl. Medit. 29: 321-328. <http://dx.doi.org/10.7320/FIMedit29.321>

In the framework of cytogenetic studies of rare and threatened plant species of the Catalan Countries and surrounding territories, a karyological study of some taxa – three populations of *Delphinium bolosii* and the only known one of *D. mansanetianum* (*Ranunculaceae*), one of *Hesperis laciniata* subsp. *laciniata* (*Brassicaceae*), and one of *Silene neglecta* (*Caryophyllaceae*) – is presented here. Karyotype microphotographs and corresponding idiograms for most taxa are provided and their karyotype morphology is discussed.

Keywords: CromoCat, Catalan countries, chromosome number, karyotype, threatened plants.

Introduction

Chromosome databases are useful as both a repository of existing knowledge as well as a tool to identify gaps of karyological information. The CromoCat chromosome database gathers data from the Catalan Countries since 1998 (Simon & al. 2011). At present, 215 taxa (4,4 % of the total vascular flora, Simon & Blanché 2016) still remain karyologically unknown.

Thus, we launched a research program to obtain new counts to fill the detected needs, with priority given to, threatened species, endemics and taxa with reported variation in chromosome number. This contribution is oriented to the endemics *Delphinium bolosii* (with two previously detected cytotypes) and *D. mansanetianum* (unknown number) and to two Mediterranean species evaluated as threatened in Catalonia, *Hesperis laciniata* subsp. *laciniata* and *Silene neglecta*, with few previous karyological reports and not counted in our territory.

1968. *Delphinium bolosii* C. Blanché & Molero — $2n = 16$.

- Hs:** Barcelona, Bages, Sant Llorenç del Munt i l'Obac Natural Park, near Mura, footcliff, 41° 40' 58" N 1° 58' 48" E, alt. 552 m, 1 Jul 2014, *M. Bosch* (BCN 113231). – Figs 1A-1B & 2A.
- Lleida, La Noguera, Alòs de Balaguer, screes and understory with strong slope, in N facing cliffs over the river Segre, 41° 54' 37" N 0° 57' 03" E, alt. 305 m, 13 Jun 2007, *M. Bosch, M.C. Martinell, J. Molero & A. Rovira* (BCN 127363). – Figs 1C & 2B.
- Lleida, La Noguera, Presa de Camarasa, cliffs over the river Segre, 41° 54' 14" N 0° 53' 47" E, 465 m, 3 Jul 2008, *M. Bosch & J. Molero* (BCN 53601). – Figs 1D & 2C.

The three surveyed populations share the same chromosomal number of $2n = 16$ (Figs 1A-1D), which is different than the previous reports for this species of $2n = 18$ (Blanché 1991; Simon & al. 1995; Bosch 1999), meaning the coexistence of two cytotypes in this species. It is a very rare phenomenon in the tribe *Delphinieae* only detected in 20 taxa, 4 of them in the genus *Delphinium* (Bosch & al. 2016). In the case of the population near Mura, this number is confirmed in different individuals from two different nuclei, 500 m apart (Figs 1A & 1B).

The karyotype analysis (Figs 2A-2C) shows a formula with 2 pairs of large metacentric or submetacentric chromosomes and 6 smaller subacrocentric ones (Mura: $2n = 2x = 2m\text{-SAT} + 2sm + 10st + 2st\text{-SAT} = 16$; Alòs de Balaguer: $2n = 2x = 2m + 2m\text{-SAT} + 10st + 2st\text{-SAT} = 16$; Presa de Camarasa: $2n = 2x = 2m + 2m\text{-SAT} + 10st + 2st\text{-SAT} = 16$), similar to the general pattern described in *Delphinieae* tribe (Epling & Lewis 1951, Blanché & al. 1997, Bosch & al. 2016). The chromosome size varies from 3.86-14.09 μm .

Satellites are located in the short arm of the first metacentric pair and in the short arm of the IV or V subacrocentric pairs (Figs 2A-2C). In the series *Fissa* B. Pawl., where this species is placed (Bosch & al. 2019), the satellites number and pair are reported in slightly variable positions. In the *D. bolosii* population from Rubió (La Noguera, Lleida), with $2n = 18$, satellites have been reported from pairs II, IV and VI, whereas satellites are missing in the reports from the Ulldemolins (Priorat, Tarragona), also with $2n = 18$, and the *D. fissum* subsp. *sordidum* studied populations (Blanché 1991; Bosch 1999). In *D. fissum* subsp. *fissum* French (Bosch 1999) and Greek populations (Constantinidis & al. 1997), satellites appear in one (I) or two (I and V) pairs and in *D. fissum* s.l. from Bulgaria (Koeva-Todorovska 1985), indicated from three pairs (I, IV and VIII or I, V and VIII). In all the surveyed taxa, satellites are reported from the short arms, which is the most abundant case in the tribe (Bosch & al. 2016). Attribution to distinct very similar chromosome pairs (very close in length or symmetry) could be, at least in some cases, a mere artefact, but variation in the total number of satellites (from zero to three in *D. bolosii*, i.e.) can have a more deep evolutionary significance. Notably, this species, with two cytotypes and maximum satellite diversity, also contains the maximum genetic diversity in the whole series *Fissa* and is considered as a genetic reservoir to understand the diversity pattern of this group in the W Mediterranean (Bosch & al. 2019).

Delphinium bolosii is assessed as EN (“Endangered”) (Sàez & al. 2010), but currently under new evaluation to probably lower its threat category.

1969. *Delphinium mansanetianum* Pitarch, Peris & Sanchis — $2n = 16$.

Hs: Aragón, Teruel, Mosqueruela, Masia del Matorrillo, thorny edges of Scots pine clearings, on calcareous rocky soils, 40° 22' 35" N 0° 28' 54" W, alt. 1640 m, 15 Jul 1997, *R. Pitarch* (MA 692377).

The count $2n = 16$ is the first report for this species, which is only known from one location in Teruel (Aragón, Spain). This species belongs to the series *Fissa* of genus *Delphinium* and it is closely related to *D. bolosii* (Bosch & al. 2019), with two different numbers (see previous species reported). It is assessed as CR (“Critically endangered”) (Bosch & al. 2019).

1970. *Hesperis laciniata* All. subsp. *laciniata* — $2n = 12$.

Hs: Lleida, La Noguera, Rubió de Baix, footcliffs and shady herbaceous places below an old tower in a fluvial terrace, 41° 54' 21" N 0° 59' 56" E, 280 m, 9 May 2007, *C. Blanché, M. Bosch & J. Molero* (BCN 47270). – Figs 1E & 2D.

This reported chromosome number of $2n = 12$ (Fig. 1E) confirms the previous counts of Ančev & Peneva (1984) and Ančev & Goranova (1997) from Bulgarian material, Constantinidis & al. (2002), from Greek material, and also with the gametophytic number ($n = 6$) provided by Ruíz de Clavijo Jiménez (1994) from material of Cordoba (S Spain). Constantinidis & Kamari (1994) found the same number for *H. laciniata* subsp. *secundiflora* (Boiss. & Spruner) Breistr., an endemic subspecies of Greece. Dvorak (1966) reported a different number $2n = 14$ for *H. glutinosa* Vis., which is considered a partial synonym of *H. laciniata* (Marhold 2011). Both chromosome numbers ($2n = 12, 14$) occur in *Hesperis* (Rice & al. 2015).

The karyotype analysis showed a formula of $2n = 2x = 8m + 4sm = 12$ chromosomes (Fig. 2D), the same described by *H. laciniata* ssp. *laciniata* (Ančev & Goranova 1997, Constantinidis & al. 2002), but with a small difference in *H. laciniata* subsp. *secundiflora* (Constantinidis & Kamari 1994) where one of the submetacentric pairs is a subacrocentric one. The chromosomes vary in size from 4.08 to 8.16 μm . We have not detected the presence of satellites, observed by Constantinidis & Kamari (1994) and Constantinidis & al. (2002), which mentioned that they are very small. Constantinidis & Kamari (1994) also reported frequent secondary constrictions in the short arms of metacentric chromosomes, but we could not appreciate it in our material.

H. laciniata is of S. European and N African species, threatened (VU, “Vulnerable”) and very rare in Catalonia (Sàez & al. 2010), our count is from the single confirmed population.

1971. *Silene neglecta* Ten. — $2n = 24$.

Hs: Barcelona, Garraf, Gavà, NW rocks over Ermita de Bruguers church, sandstone rocky soil, 41° 18' 52" N 1° 57' 36" E, alt. 265 m, 6 May 2015, *L. Sáez* (LS-7626, LS: Sáez, personal herbarium). – Figs 1F & 2E.

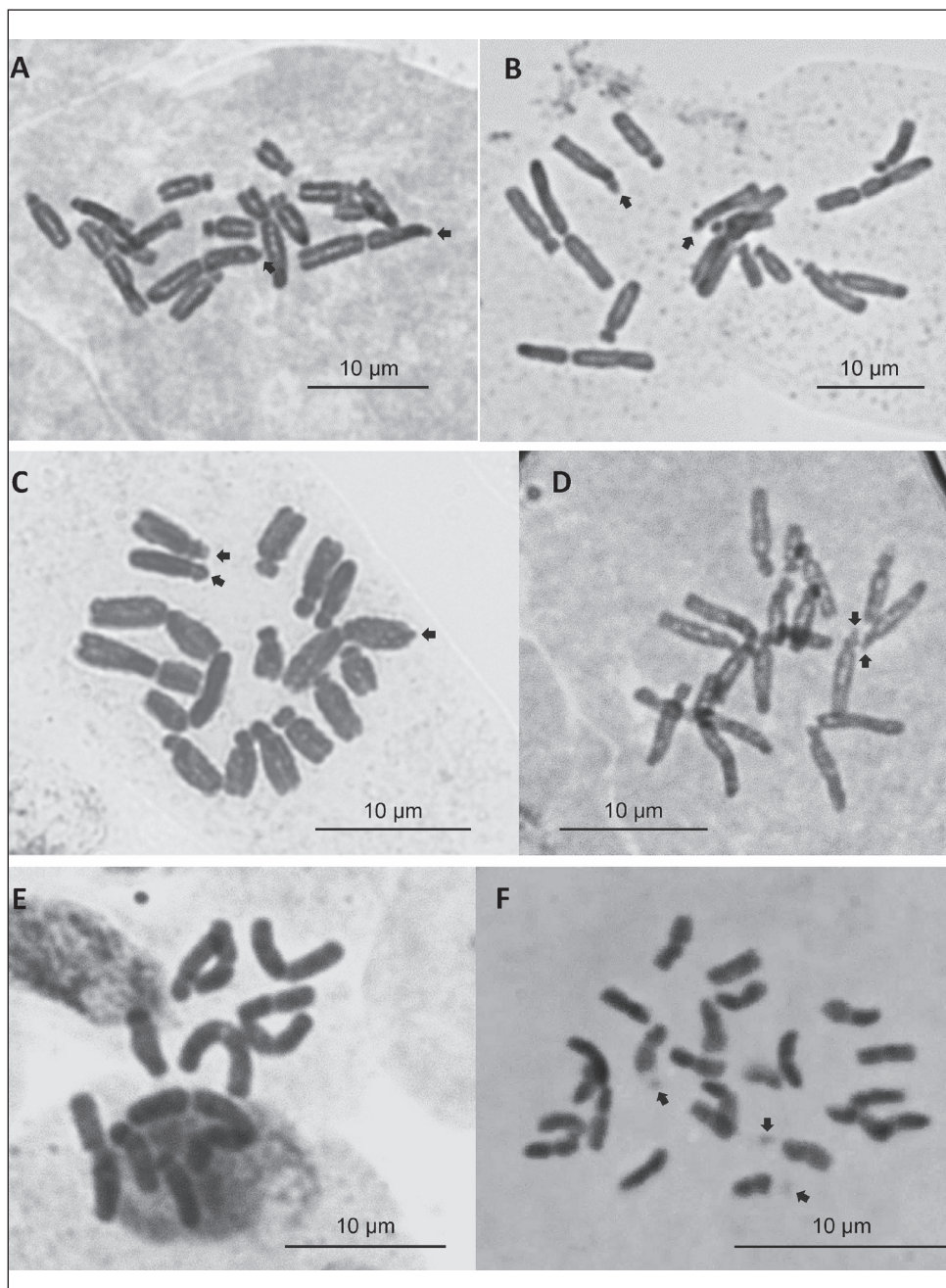


Fig. 1. Microphotographs of somatic metaphase plates of: **A-D**, *Delphinium bolosii*, $2n = 16$: **A & B**, Mura (Hs: Barcelona), **C**, Alòs de Balaguer (Hs: Lleida), **D**, Presa de Camarasa (Hs: Lleida); **E**, *Hesperis laciniata* subsp. *laciniata*, $2n = 12$: Rubió de Baix (Hs: Lleida); **F**, *Silene neglecta*, $2n = 24$: Ermita de Bruguers (Hs: Barcelona). – Arrowheads indicate satellites.

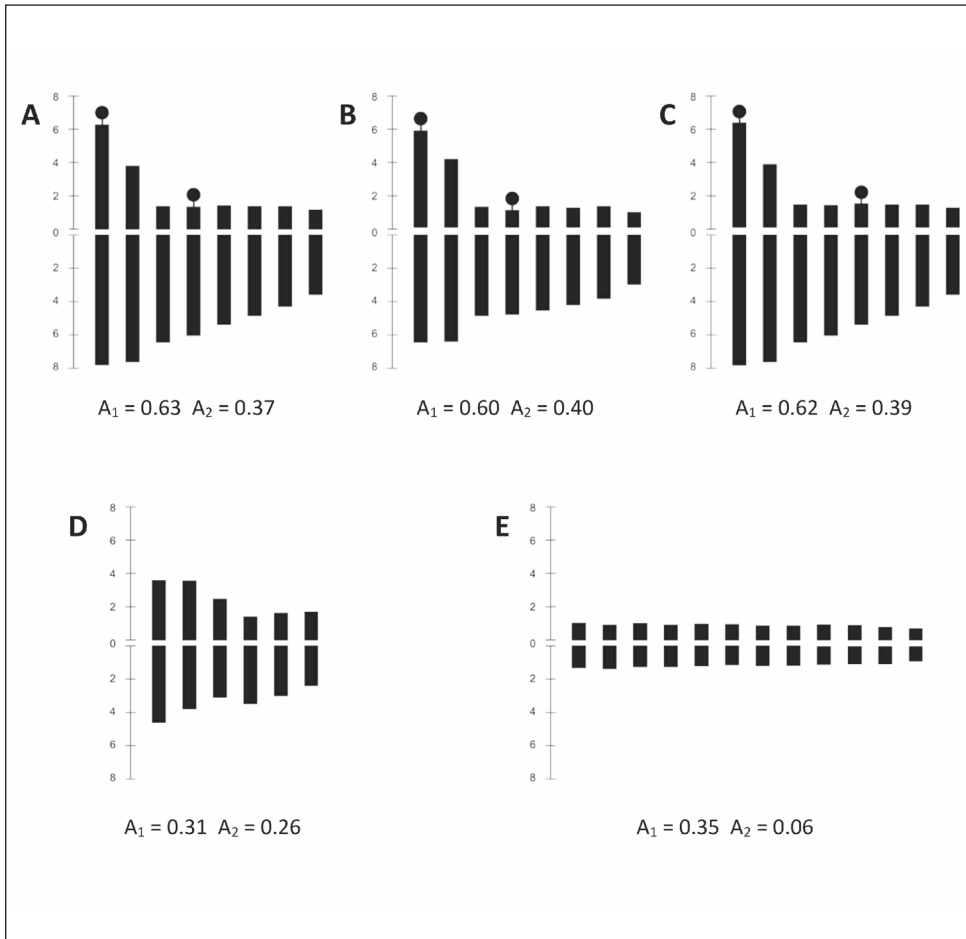


Fig. 2. Haploid idiograms obtained by measuring at least 5 good mitotic metaphases, 2 hours after pretreatment with hidroxiquinoleine 0.002 M. Asymmetry indices A_1 and A_2 are calculated following Romero (1986). **A-C**, *Delphinium bolosii*: **A**, Mura (Hs: Barcelona), **B**, Alòs de Balaguer (Hs: Lleida) & **C**, Presa de Camarasa (Hs: Lleida); **D**, *Hesperis laciniata* subsp. *laciniata*: Rubió de Baix (Hs: Lleida); **E**, *Silene neglecta*: Ermita de Bruguers (Hs: Barcelona). – Scale bars = 8+8 μm .

The count of $2n = 24$ (Fig. 1F) is the first report of this species for the Iberian Peninsula. This taxon has been misunderstood and confused with *Silene nocturna* L., although several reproductive characters (petals shape and colour, seed size, shape and seed-coat surface, among others) allow separating both species (Talavera 1990; Pignatti 2019). This population is the only known one in Iberian Peninsula, and locally considered severely threatened: Iberian evaluation as EN (“Endangered”) (Sàez 2018). The species is mainly distributed in Italy and N Africa, with some cited populations in S France although not confirmed. This count coincides with the most frequent number of the genus *Silene* (Rice & al. 2015).

The karyological study reveals a chromosomic formula of $2n = 2x = 24m = 24$ chromosomes (Fig. 2E), varying in size from 1.59 to 2.34 μm , which are very similar between them and very symmetrical (all are metacentric chromosomes). Presence of satellites is detected (at least 3 pairs) but it is hard to assign due to the similarity of chromosomes in size and symmetry.

The revision by Peruzzi & Carta (2013) of the *S. nocturna* group in Italy (including the first count for *S. neglecta*) gives the same chromosome number ($2n = 24$) for the components of this group: *S. capraria* Sommier (= *S. nocturna* subsp. *capraria* (Sommier) Peruzzi & Carta), *S. neglecta* and *S. nocturna*, with chromosome sizes ranging from 0.5 to 2.5 μm . The karyotype analysis of Italian populations of *S. neglecta* (from Tuscan Archipelago) is very similar in length and symmetry to our population, also confirming longer chromosomes in *S. neglecta* than in *S. capraia*. However, no satellites are reported for any of the Italian studied populations. The same number $2n = 24$, similar karyotype and absence of satellites are reported for *S. nocturna* subsp. *boullui* (Rouy & Foucaud) Gamisans in Gamisans & D. Jeanmonod (Bachetta & al. 2014) and for another close species of the same group, as the Turkish endemic *S. muradica* Schischk., also with similar chromosome lengths (1.50 - 2.77 μm) and symmetry (all pairs are median type) (Martin & al. 2019). Thus, the three pairs with satellites seem to characterize the Catalan population. In relatively related species, we have found only a report of one pair (I) of satellites in the $2n = 24$ common European annual *S. gallica* (Bari 1973).

Acknowledgements

To Chaimae Bouhriba, Sandra Candela and Anna Rovira, for her help in laboratory work and to Julià Molero and Ricard Pitarch, for advice and help in plant collection. To Rural Agents of La Noguera and Guards of Sant Llorenç del Munt i l'Obac Natural Park for seed collection. To Generalitat de Catalunya, Departament de Medi Ambient, for collection authorization of *Delphinium bolosii* (legally listed in D. 172/2008, de 26 d'agost, de creació del Catàleg de flora amenaçada de Catalunya).

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Address of the authors:

Maria Bosch¹, Llorenç Sàez², Joan Simon¹ & Cèsar Blanché¹,

¹BioC-GReB & IRBio, Laboratori de Botànica, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Catalonia (Spain). E-mail: mariabosch@ub.edu

²GReB, Departament de Biologia Vegetal, Biologia Animal i Ecologia, Universitat Autònoma de Barcelona, Bellaterra, Catalonia (Spain) & Societat d'Història Natural de les Balears, Illes Balears (Spain).