The *Cardamine pratensis* (Brassicaceae) group in the Iberian Peninsula: taxonomy, polyploidy and distribution

Judita Lihová[1], Andreas Tribsch[2] & Karol Marhold[1,3]

[1] *Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 14, SK-845 23 Bratislava, Slovak Republic. judita.lihova@savba.sk (author for correspondence); karol.marhold@savba.sk*
[2] *Department of Higher Plant Systematics and Evolution, Institute of Botany, University of Vienna, Rennweg 14, A-1030 Vienna, Austria. andreas.tribsch@univie.ac.at*
[3] *Department of Botany, Charles University, Benátská 2, CZ-128 01 Praha 2, Czech Republic*

The *Cardamine pratensis* group is a taxonomically critical species complex with pronounced karyological and morphological variation. In the present study, representatives from the Iberian Peninsula have been investigated using karyological, morphometric and molecular (amplified fragment length polymorphism) analyses. As a result of this combined approach, three species are recognized in the area studied: *C. pratensis* s.str., *C. crassifolia*, and a herein newly described species, *C. castellana*. For *C. pratensis* s.str., consistent with the pattern known from other European regions, wide variation in chromosome numbers is revealed, consisting of diploid to heptaploid populations in northern and central parts of the Peninsula. Little morphological and genetic differentiation accompanies these cytotypes. Re-evaluation of populations up to now ascribed to *C. crassifolia* reveal two separate taxa: (1) populations from the Eastern Pyrenees, representing typical *C. crassifolia*, and (2) those from central Iberian mountains treated as *C. castellana*. These two diploid taxa differ in several quantitative morphological characters, as well as in morphology of rhizome and basal parts of stem, and they are also distinct in AFLP markers, indicating strong genetic differentiation.

KEYWORDS: AFLPs, *Cardamine castellana*, *Cardamine crassifolia*, chromosome numbers, morphometrics, Portugal, Spain.

INTRODUCTION

The genus *Cardamine* L. (Brassicaceae) comprises several taxonomically critical species complexes, among them the *C. pratensis* group, which shows complicated patterns of morphological and karyological variation (Lövkvist, 1956). It includes several diploid and polyploid closely related taxa distributed throughout most of Europe, occurring also in northern Africa, northernmost North America, and Asia (Schulz, 1903; Lövkvist, 1956; Jones & Akeroyd, 1993; Jalas & Suominen, 1994). The group has been investigated by several authors, especially in northern (Lövkvist, 1956; Dale & Elkington, 1974), central (Urbanska-Worytkiewicz & Landolt, 1974; Landolt, 1984; Marhold, 1994a, b, 1996) and southeastern Europe (Marhold, 1994a, b, 1996; Marhold & Ančev, 1999). Various approaches including karyological analyses, morphometrics, ecological studies, cultivation and crossing experiments have been used in these studies, which helped develop taxonomic concepts for the group.

Most recently, a molecular study on a European-wide scale was performed using several markers (isozymes, RAPD, cpDNA and ITS sequencing) to elucidate the evolutionary history of the *C. pratensis* group (Franzke & Hurka, 2000). Two old lineages, most probably representing (pre)Pleistocene relicts, were revealed, which corresponded to diploid species from the Balkan (*C. penzesii* Ančev & Marhold, *C. rivularis* Schur) and Iberian (*C. crassifolia* Pourr.) Peninsulas. All remaining diploid to polyploid taxa formed a phylogenetically young, derived group showing poor phylogenetic resolution: *C. pratensis* L. s.str. ($2n = 2x–7x$; including *C. udicola* Jord., *C. nemorosa* Lej., *C. latifolia* Lej., *C. rivularis* auct. non Schur), *C. matthioli* Moretti ($2n = 2x$), *C. majovskii* Marhold & Záborský ($2n = 4x$), *C. dentata* Schult. ($2n = 7x–12x$), *C. nymanii* Gand. ($2n = 8x–10x$), and probably also *C. granulosa* All. ($2n = 2x$).

Cardamine pratensis s.str. is the most widespread species of the group in Europe (Jalas & Suominen, 1994), composed of several cytotypes and exhibiting wide morphological variation (Lövkvist, 1956; Marhold, 1996). Despite its heterogeneity, morphological variation is rather continuous, and taxa sometimes segregated and recognized (e.g., *C. pratensis* subsp. *picra* De Langhe & D'hose, *C. pratensis* subsp. *major* Tomšovic, *C. udicola*, *C. nemorosa*, *C. latifolia*) can be hardly distinguished from *C. pratensis* s.str. It seems that diversification of the *C. pratensis* group occurred relatively recently, possibly in postglacial times as proposed by Franzke & Hurka (2000), and that hybridization and polyploidy played a major role in the evolution of this group.

From the Iberian Peninsula, two taxa belonging to the *C. pratensis* group have been reported: *C. pratensis* s.str. and *C. crassifolia* (Jones & Akeroyd, 1993; Rico, 1993; both on the level of subspecies). The former is distributed in northern and central parts of the Peninsula (Rico, 1993), and so far only two records on chromosome numbers, diploid and triploid, both from Portugal, have been reported (Lövkvist, 1956; Queirós, 1973). Although Lövkvist (1956) indicated a distinct position of the Portuguese diploid, no detailed comparative morphological studies with non-Iberian representatives of *C. pratensis* s.str. have been performed so far. *Cardamine crassifolia*, one of the assumed basal taxa of the group (Franzke & Hurka, 2000), is a diploid species described from the Eastern Pyrenees (Pourret, 1788), but reported also from central and western parts of the Pyrenees, and from isolated mountain ranges in central Spain (Lövkvist, 1956; Rico, 1993; Mateo Sanz & al., 1994). It possesses several morphological traits unique within the *C. pratensis* group (creeping rhizome, ascending stem, lack of basal rosette of leaves, presence of stolons) (Lövkvist, 1956; Rico, 1993), although central Iberian representatives and those from the Central Pyrenees have been sometimes regarded as intermediate between *C. crassifolia* and *C. pratensis* s.str. (Rico, 1993).

In this paper we present a combined karyological, morphometric and AFLP-fingerprinting (amplified fragment length polymorphism) study focusing on the Iberian populations of the *C. pratensis* group. The emphasis was put on (1) exploring morphological and karyological variation of Iberian populations of *C. pratensis* s.str., (2) elucidating their relationship to non-Iberian populations, i.e., to test their distinction proposed by Lövkvist (1956), and (3) on resolving the taxonomic position of central Iberian populations ascribed to *C. crassifolia*.

Morphometric analyses have been already successfully applied in previous studies on *Cardamine* species with complex morphological variation, and enabled delimitation of taxa by using a combination of several morphological characters (Marhold, 1992; Marhold, 1996; Lihová & al., 2000). AFLP-fingeprinting is a recently developed (Vos & al., 1995) and already widely used molecular method, suitable for studies on populational and low taxonomic levels (e.g., Hedrén & al., 2001; Zhang & al., 2001; Marhold & al., 2002; Tribsch & al., 2002). As the Iberian Peninsula has been considered one of the major European Pleistocene refugia (Comes & Kadereit, 1998), studies in this area might be significant also from the point of view of the evolutionary history of the *C. pratensis* group and related taxa.

MATERIALS AND METHODS

Plant material. — Twenty-nine populations of *C. pratensis* s.str. were sampled for morphometric evaluation originating from Portugal and several provinces of Spain, to represent wide range of morphological variation. For morphometric analyses, each populational sample consisted of 20–40 individuals; for chromosome numbers 2–7 plants per population (27 populations) were analyzed. Material of *C. crassifolia* used in morphometrics was represented by eight populational samples from the Eastern Pyrenees, and 13 samples from central Iberian mountains (Sierra Segundera, Sierra de Gredos, Sierra de Albarracín, Sierra de Gúdar; Table 1). As some of them were of small population size, fewer individuals were sampled (12–35 plants); in the case of material from Sierra de Albarracín, individuals from three geographically close sites (populations no. 25, 26, 27; altogether 16 plants) were treated together in analyses performed at the populational level, as one populational sample (see morphometric analyses). For each population of *C. crassifolia* chromosome numbers were checked in 2–4 individuals (21 populations). Professor E. Landolt and Professor K. Urbanska (Zürich, Switzerland) provided us with their unpublished results of 15 analyses of chromosome numbers from the Iberian Peninsula, together with voucher specimens. They are published in this paper in order to complete information acquired by us (Table 1).

AFLP analyses included altogether 114 samples, and the following taxa were represented (Table 1): *C. crassifolia* from the Eastern Pyrenees (7 populations/13 individuals) and from central Spain (Sierra de Gredos, Sierra de Albarracín; 6/12), Iberian *C. pratensis* s.str. (12/31), *C. pratensis* s.str. from France, Italy, Slovakia and Slovenia (7/18), *C. matthioli* (5/10) from Slovenia and Slovakia, *C. majovskii* (5/10) from Slovenia, *C. granulosa* from Piedmont (northern Italy; 2/5), as well as populations of taxonomically uncertain position from central Italy, considered close to *C. granulosa* (4/12). Voucher specimens of all analyses performed in this study are deposited in SAV; only those of chromosome analyses done by Urbanska and Landolt (see Table 1) are in ZT.

For chorological study specimens from the following herbaria were investigated: B, BC, BIO, COI, JACA, M, MA, MACB, MAF, RNG, SALA, SALAF, SANT, ZT (see Appendix).

Karyological analyses. — Chromosome numbers were determined from individuals collected in the field and then cultivated at the Institute of Botany, Slovak Academy of Sciences, Bratislava. Squashes were prepared from root tips treated as described in Marhold & al. (2002), and chromosomes were counted from mitotic metaphase plates.

Table 1. Origin of plant material used in karyological (2*n*), morphometric (Morph) and AFLP analyses. Lu - Portugal, Hs - Spain, It - Italy, Sk - Slovakia, Sl - Slovenia, Fr - France. Data on chromosome numbers labelled with asterisk are taken from Lihová & Marhold (2003); all other data represent new records. Those labelled with pluses were counted by K. Urbanska and E. Landolt, with voucher specimens deposited at ZT. Collectors: EL - E. Landolt, JL - J. Lihová, KM - K. Marhold, KU - K. Urbanska, MP - M. Perný.

Taxon / locality	2*n*	Morph	AFLP
Cardamine crassifolia **Pourr.**			
01 - Hs. Lérida: E Pyrenees, NW of Meranges, Riu Duran, near Refugi J. Folchi Girona, 2320 m, 2 Jul 2001, *JL*	16	×	×
02 - Hs. Lérida: E Pyrenees, N of Meranges, at the lake Estany de Malmiu, 2260 m, 1 Jul 2001, *JL*	16	×	×
03 - Hs. Gerona: E Pyrenees, Núria valley, torrent de Noufonts, 2000 m, 27 Jun 2001, *JL*	16	×	×
04 - Hs. Gerona: E Pyrenees, Núria valley, torrent de Noufonts, 2200-2300 m, 27 Jun 2001, *JL*	16	×	×
05 - Hs. Gerona: E Pyrenees, Núria valley, torrent de Fontalba, 2100 m, 28 Jun 2001, *JL*	16	×	×
06 - Hs. Gerona: E Pyrenees, Núria valley, torrent de Finestreles, 2200 m, 28 Jun 2001, *JL*	16	×	×
07 - Hs. Gerona: E Pyrenees, east of Núria valley, Refugi Manelic, 2000 m, 30 Jun 2001, *JL*	16	×	×
08 - Hs. Gerona: E Pyrenees, between Ribes de Fréser and Camprodon, Rierra d' Abella, 1580 m, 6 Jun 2000, *MP*	-	×	
09 - Fr. 0.5 km SE Porta, 1504 m, 3 Jun 1974, *EL & KU 898*	16^{+}		
10 - Fr. Haute Aude, Vallée de l' Aude, N of Capcir - La Forge, 1400 m, 3 Jun 1974, *EL & KU 893*	16^{+}		
11 - Fr. Haute Aude, Vallée de l' Aude, Capcir, near Barrange de Matemols, ca. 1500 m, 3 Jun 1974, *EL & KU 894*	16^{+}		
12 - Fr. Aude, Foret de Barrés, près de la Pla de Barrés, 1700 m, 3 Jun 1974, *EL & KU 896*	16^{+}		
13 - Fr. En aval du Barrage, Les Bouillouse, dépression de la Bouillousette, 2000 m, 3 Jun 1974, *EL & KU*	16^{+}		
Cardamine castellana **Lihová & Marhold**			
14 - Hs. Asturias: Cordillera Cantabrica, near Puerto de Leitariegos, 1470 m, 25 May 2001, *MP* & al.	16		
15 - Hs. Zamora: Sierra Segundera, at the road from San Martin de Castañeda to the lake Laguna de los Peces, NW of Mt.Gencianal, 1640–1660 m, 24 Jun 2002, *KM*, *P. Bariego Hernández & E. Rico*	16	×	
16 - Hs. Zamora: Sierra Segundera, near the lakes Lagunas Herbosas, SE slopes of mountains Moncalvillo and Moncalvo, 1830-1950 m, 29 Jun 2002, *KM & P. Bariego Hernández*	16	×	
17 - Hs. Zamora: Sierra Segundera, NW of the lake Laguna de los Peces, 1820 m, 25 Jun 2002, *KM*	16	×	
18 - Hs. Zamora: Sierra Segundera, NW of the lake Laguna de Cubillas (= Laguna de la Yegua), 1820-1880 m, 25 Jun 2002, *KM*	16	×	
19 - Hs. Zamora: Sierra Segundera, Arroyo del Fuego, 1620–1555 m, 27 Jun 2002, *KM*	16	×	
20 - Hs. Ávila: Hoyos del Espino, near Hoyos del Collado, 1500 m, 10 May 2000, *KM*; 10 May 2001, *JL*	16	×	×
21 - Hs. Ávila: Hoyos del Espino, road to Plataforma de Gredos, 1460 m, 10 May 2000, *KM*; 10 May 2001, *JL*	16	×	×
22 - Hs. Ávila: Hoyocasero, pinar de Hoyocasero, 1260 m, 10 May 2000, *KM*	16	×	
23 - Hs. Ávila: Santiago del Collado, on the way to Puerto de Peña Negra, 1700 m, 10 May 2000, *KM*; 10 May 2001, *JL*	-	×	×
24 - Hs. Ávila: Becedas, Peña Negra, 1800 m, 27 Jun 2002, *M. Martínez Ortega MO1546 & Muñoz Centeno*	16		
25 - Hs. Guadalajara: Sierra de Albarracín, SW of Alustante, Fuente de los Valles, 1500 m, 14 May 2001, *JL & G. Nieto Feliner*	16	×	×
26 - Hs. Guadalajara: Sierra de Albarracín, S of Orea, Hoz Seca, 1500 m, 14 May 2001, *JL & G. Nieto Feliner*	16	×	×
27 - Hs. Teruel: Sierra de Albarracín, W of Noguera, Barranco de la Peña Aguda, 1400 m, 14 May 2001, *JL & G. Nieto Feliner*	16	×	×
28 - Hs. Teruel: Sierra de Gúdar, E of Puerto de Valdelinares, Cuarto del Prado, 1830 m, 19 Jun 2002, *JL*	16	×	
Cardamine pratensis **L. s.str. (Iberian Peninsula)**			
29 - Lu. Beira Litoral: Fermentelos, near Aveiro, 6 m, 10 Apr 2000, *JL* & al.	16	×	
30 - Lu. Beira Litoral: Reserva Natural de Paul de Arzila, between Arzila and Pereira, 5 m, 10 Apr 2000, *JL* & al.	30	×	×
31 - Lu. Beira Litoral: between Cabecinhas and Calvão, 32 m, 10 Apr 2000, *JL* & al.	30	×	
32 - Lu. Beira Litoral: ca. 50 km N of Coimbra, at the road Mira - Vagos, 5 km of Vagos, 24 May 1972, *EL*	24+		
33 - Lu. Beira Litoral: 2 km N of Vagos, 24 May 1972, *EL*	24+		
34 - Hs. A Coruña: Brión, 80 m, 4 Apr 2000, *JL & I. Pulgar*	30	×	×
35 - Hs. A Coruña: Teo, Rariz, Bouñou, 180 m, 4 Apr 2000, *JL & S. Ortiz*	30	×	
36 - Hs. A Coruña: Portomuoro, river Tambre, 160 m, 6 Apr 2000, *JL & I. Pulgar*	30	×	×
37 - Hs. A Coruña, Ames, Ortoño, near Bertamiráns, 6 Apr 2000, *JL & S. Ortiz*	30	×	
38 - Hs. A Coruña: O Pino, San Miguel de Cerceda, O Castro, 400 m, 7 Apr 2000, *JL & S. Ortiz*	30	×	×
39 - Hs. Pontevedra: Vila de Cruces, Toiriz, river Arnego, 380 m, 5 Apr 2000, *JL & I. Pulgar*	30	×	
40 - Hs. León: Manzanal del Puerto, 1100 m, 9 May 2000, *KM*	30	×	
41 - Hs. Zamora: Pedralba de la Praderia, 980 m, 9 May 2000, *KM*	48	×	
42 - Hs. Segovia: San Miguel de Bernuy, river Duratón, 820 m, 8 May 2000, *KM*	56	×	
43 - Hs. Segovia: Prádena, Villar, 1050 m, 8 May 2000, *KM*	56	×	×
44 - Hs. Segovia: Matabuena, between Soria and Segovia (km. 156), 1150 m, 8 May 2000, *KM*	-		×
45 - Hs. Segovia: Sierra de Guadarrama, between La Granja and Peñalara, 1100-1600 m, 13 May 2001, *JL*	56	×	
46 - Hs. Madrid: Sierra Guadarrama, El Paular, Monasterio de El Paular, 1160 m, 12 May 2001, *JL*	56	×	×
47 - Hs. Cantabria: Luena, Sel de la Carrera, N of Puerto del Escudo, at Emb. del Ebro, 500 m, 7 May 2001, *JL & M. Herrera*	56	×	×
48 - Hs. Cantabria: Pto. de la Magdalena, ca. 200 m from San Pedro del Romeral, 780 m, 7 May 2001, *JL & M. Herrera*	16	×	×
49 - Hs. Cantabria: between Campillo and S. Roque de Riomiera, 750 m, 7 May 2001, *JL & M. Herrera*	32	×	
50 - Hs. Cantabria: Soba, from Revilla to Puerto de Los Tornos, 700 m, 21 May 2001, *MP & M. Herrera*	32	×	
51 - Hs. Álava: Urcabustaiz, Beluntza, 640 m, 6 May 2001, *JL & I. Biurun*	-	×	
52 - Hs. Álava: Villarreal de Álava, Elosu, 560 m, 6 May 2001, *JL & I. Biurun*	48	×	

Table 1. (continued).

Taxon / locality	2*n*	Morph	AFLP
53 - Hs. Guipuzcoa: Anzola, Puerto Descarga, 430 m, 6 May 2001, *JL & I. Biurun*	46	×	
54 - Hs. Guipuzcoa: Régil, between Régil and Pto. de Bidania, 480 m, 6 May 2001, *JL & I. Biurun*	48	×	
55 - Hs. Guipuzcoa: Segura, Otzaurte, 600 m, 22 May 2001, *MP & I. Biurun*	-	×	
56 - Hs. Guipuzcoa: 8 km SE of Irún, Erlaitz, 497 m, 20 May 2001, *MP*	32	×	
57 - Hs. Navarra: Larraín, Sierra de Aralar, 1 km of Baraibar, 600 m, 6 May 2001, *JL & I. Biurun*	46	×	×
58 - Hs. Navarra: Yerri, Sierra de Urbasa, Raso de Bidoiza, S of Pto. de Urbasa, 900 m, 6 May 2001, *JL & I. Biurun*	48	×	×
59 - Hs. Navarra: Quinto Real, 2200 m S of Irurita, 700 m, 8 May 2001, *JL & I. Biurun*	32	×	×
60 - Hs. Navarra: 500 m S of Venta Quemada, Puerta de Velote, 840 m, 5 Apr 1974, *EL 911*	40^{+}		
61 - Hs. Huesca: Central Pyrenees, Alta Ribagorza, NW of Aneto, between Aneto and Refugi de Llauset, 1700 m, 23 Jun 2002, *JL*	16	×	
62 - Hs. Huesca: Valle de Aran, Casau, W of Viella, 5 Jun 1974, *EL & KU 903*	16^{+}		
63 - Fr. Pyrenees, 1 km E of Gourette, Col d'Aubisque, ca. 1450 m, 5 Jun 1974, *EL & KU*	16^{+}		
64 - Fr. Hautes Pyrenees, 4 km N of Col de Peyresourde, 5 Jun 1974, *EL & KU* 904	16^{+}		
65 - Fr. Pyrenees, aux bonnes Crèpes, ca. 3 km E Col d'Aspin, ca. 1400 m, 5 Jun 1974, *EL & KU*	16^{+}		
66 - Fr. Pyrenees, road to Col du Souloz (7 km E of the pass), 5 Jun 1974, *EL & KU*	16, 32^{+}		
67 - Fr. 1.5 km E of La Mongie, at the road to Col du Tourmalet, ca. 1700 m, 5 Jun 1974, *EL & KU*	32^{+}		
68 - Fr. 8 km N of Col des Pourtalet, ca. 1250 m, 5 Jun 1974, *EL & KU 910*	40^{+}		
***Cardamine pratensis* L. s.str. (non-Iberian)**			
69 - Sl. Alpsko območje: between Zg. Stranje and Godič, at the Kamniška Bistrica stream, 430 m, 8 Apr 2001, *JL & T. Bačič*	30*	×	×
70 - Sl. Predalpsko območje: Radomlje, 330 m, 18 Apr 2001, *JL & T. Bačič*	30*	×	×
71 - Sl. Dinarsko obmocje: Notranjsko, Cerkniško jezero, near Otok, 560 m, 17 Apr 2001, *JL & B. Trčak*	44*	×	×
72 - Fr. Grenoble, Arboretum de l'Université J. Fourier, at the river Isère, 5 Apr 2001, *P. Mráz*	30		×
73 - Fr. Dept. Isère, Massif de la Chartreuse, St. Piere-Chartreuse, 1 Apr 2001, *P. Mráz*	30		×
74 - It. Piedmont: Torino, Stupinigi, 250 m, 25 Apr 2001, *JL* & al.	30		×
75 - Sk. Pova ský Inovec Mts., S of Trencianske Jastrabie, Patrovec, 320-340 m, 6 Oct 2001, *MP*	44		×
76 - Sk. Slovenské rudohorie Mts., Mt. Kojšovská hola, 1160 m, 1 Aug 2000, *JL*	30		×
***Cardamine matthioli* Moretti**			
77 - Sl. Subpanonsko območje: Štajerska, between Ptuj and Placar, 240 m, 20 Apr 2001, *JL & B. Frajman*	16*		×
78 - Sl. Subpanonsko območje: Trnovska vas, 250 m, 20 Apr 2001, *JL & B. Frajman*	16*		×
79 - Sl. Predalpsko območje: vicinity of Celje, Šmartinsko jezero lake, 260 m, 20 Apr 2001, *JL & B. Frajman*	16*		×
80 - Sl. Subpanonsko območje: vicinity of Slovenske Konjice, between Tepanje and Pobre , 280 m, 20 Apr 2001, *JL & B. Frajman*	16*		×
81 - Sk. Pova ský Inovec Mts., S of Trencianske Jastrabie, Patrovec, 320–340 m, 6 Oct 2001, *MP*	-		×
***Cardamine majovskii* Marhold & Záborský**			
82 - Sl. Predalpsko območje: Ljubljansko Barje, Blatna Brezovica, 300 m, 17 Apr 2001, *JL & B. Trčak*	32*		×
83 - Sl. Predalpsko območje: vicinity of Ljubljana, Krumperk, 320 m, 18 Apr 2001, *JL & T. Bačič*	32*		×
84 - Sl. Predalpsko območje: vicinity of Ljubljana, Tacen, 320 m, 18 Apr 2001, *JL & T. Bačič*	32*		×
85 - Sl. Predalpsko območje: vicinity of Ljubljana, Topole near Mengeš, 340 m, 18 Apr 2001, *JL & T. Bačič*	32*		×
86 - Sl. Dinarsko območje: Sodra ica, 540 m, 17 Apr 2001, *JL & B. Trčak*	32*		×
***Cardamine granulosa* All.**			
87 - It. Piedmont: Giaveno, Pomeri, 17 May 2002, *JL* & al.			×
88 - It. Piedmont: San Francesco su Avigliana, 620 m, 17 May 2002, *JL* & al.			×
***Cardamine pratensis* s.l., central Italy**			
89 - It. Toscana: Stáffoli, 8 m, 26 Apr 2001, *JL* & al.			×
90 - It. Abruzzo: Gran Sasso, Sorgenti del Vomano, Passo delle Capannelle, 1257 m, 27 Apr 2001, *JL* & al.			×
91 - It. Abruzzo: Lago di Campotosto, Le Serre, 1340 m, 27 Apr 2001, *JL* & al.			×
92 - It. Abruzzo: Gran Sasso, Voltigno, under Mt. Fiore, 1360 m, 27 Apr 2001, *JL* & al.			×

Morphometric analyses. — Morphological characters were measured on herbarium specimens collected from the field, and the analyses were performed in four steps: (1) the whole Iberian material of the *C. pratensis* group was evaluated in order to explore overall patterns of variation, (2) samples of *C. crassifolia* from the Eastern Pyrenees and central Iberian mountains were analyzed comparatively in more detail, (3) different cytotypes found within Iberian *C. pratensis* s.str. were compared to examine the extent of their morphological differentiation, and (4) Iberian populations of *C. pratensis* s.str. were examined relative to those from central Europe.

(1) Iberian representatives of the *C. pratensis* group. The following ten quantitative characters were measured: length of petals, width of petals, length of sepals, length of longer and shorter filaments, number of stem leaves, number of segments on the stem leaf nearest the midpoint of the leafy part of stem, number of segments on the middle stem leaf, number of lateral inflorescences (longer than 1 cm), and number of leaflets of basal leaves. Floral organs were attached to adhesive tape when fresh, and size characters were measured on them after drying. In addition, six ratios were computed (based on measurement on the stem leaf nearest the midpoint of the leafy part of stem): length of stem up to the lowest

peduncle of flower or fruit/length of stem leaf (HP1/LL2), length of stem leaf/number of leaf segments (LL2/NS2), length of terminal leaf segment/width of terminal leaf segment (LTS/WTS), length of lateral leaf segment/width of lateral leaf segment (LLS/WLS), length of terminal leaf segment/length of lateral leaf segment (LTS/LLS), and length of stem leaf/length of terminal leaf segment (LL2/LTS). As the size of vegetative organs is largely influenced by environmental factors (see e.g., Lövkvist, 1956; Dale & Elkington, 1974), these characters were used only for computing ratios. The characters evaluated in this study were selected following our previous studies on *Cardamine* species (Marhold, 1996; Lihová & Marhold, 2003) and initial field observations, aiming to include the characters that might have discriminatory value.

Cluster analysis (UPGMA, unweighted pair-group method using arithmetic averages; Everitt, 1986) and principal component analysis based on a correlation matrix (PCA; Sneath & Sokal, 1973; Krzanowski, 1990) were performed using populations characterized by mean values as OTUs (operational taxonomic units, i.e., objects). Prior to clustering, data were standardized by zero mean and unit standard deviation, and Euclidean distance was used for computing pairwise similarities between OTUs.

(2) *Cardamine crassifolia*. PCA, and subsequently canonical discriminant analysis (CDA) and non-parametric classificatory discriminant analysis (Klecka, 1980) were performed on individuals as OTUs and populations of *C. crassifolia* from two main distributional areas, the Eastern Pyrenees and central Iberian mountains, as groups. The same character set was used as in part (1). PCA was performed to reduce the overall variation in 16 examined characters into three uncorrelated variables (components), and to depict morphological relationships among individual specimens. CDA, which by weighting characters maximizes differences between groups, was conducted to reveal the extent of morphological separation suggested by PCA and analyses in part (1). To determine characters mostly contributing to that separation, total canonical structure expressing correlation of the characters with the canonical axis was computed. In the classificatory discriminant analysis, which assesses the percentage of OTUs classified correctly into predicted groups, discriminant function was determined by the cross-validation procedure using k-nearest-neighbours (k = 11). In this procedure, classification criterion is based on n-1 individuals (n = total number of individuals) and then applied to classify the individual left out. Descriptive statistical parameters of the measured characters (mean, median, standard deviation and percentiles) for populations from the Pyrenees and central Iberian mountains were also computed, and those of most discriminating characters are presented in the form of box-plots. Besides the quantitative characters measured, also qualitative ones were observed and recorded for each specimen. These included characters on rhizome (thickness, growth orientation) and on basal parts of stem (ascending vs. erect stem, presence of stolons, presence or absence of basal rosette), as the following character combination, thin, horizontally creeping rhizome, ascending stem and lack of rosette, has been considered diagnostic for typical *C. crassifolia* from the Pyrenees (Lövkvist, 1956; Rico, 1993).

(3) Iberian *C. pratensis* s.str. CDA on both individuals and populations as OTUs and non-parametric classificatory discriminant analysis were performed, with various cytotypes revealed (see Results) as groups. The aim of these analyses was to explore whether particular cytotypes can be distinguished morphologically. The characters evaluated were those included in (1), plus the number of segments on the third stem leaf (numbered from the stem base up), as this character was used in previous studies on the *C. pratensis* group (Marhold, 1996; Lihová & Marhold, 2003). Because basal leaves are sometimes lacking on plants at flowering stage, the character number of leaflets of basal leaves had to be omitted from analyses performed at the individual level, but was included when evaluating populations. The values of measured characters (mean, interquartile range and 10/90 percentiles) scored for different cytotypes were compared to obtain detailed insights into their variation.

(4) Iberian vs. central European *C. pratensis* s.str. For their morphological comparison, all quantitative characters (qualitative differences were not observed) used in previous studies on the *C. pratensis* group from the Carpathians and Pannonia (Marhold, 1996) and from Slovenia (Lihová & Marhold, 2003) were included: size of flowers [for exact list of floral characters see (1)], number of leaves, number of segments on the third stem leaf, and number of lateral inflorescences. Central European populations were represented by four ploidy levels: $2n$ = 30, 38, 44 and two diploid ($2n$ = 16) morphological types called *C. rivularis* auct., and "*ucranica* type" [for details and origin of samples see Marhold (1996) and Lihová & Marhold (2003)]. PCA and UPGMA cluster analysis were performed using 29 Iberian and 45 central European populations as OTUs.

All morphometric analyses were done by using the SAS 8.2 package (SAS Institute, 2000), except for cluster analyses which were run using SYN-TAX 2000 (Podani, 2001).

AFLP analyses. — Total DNA was extracted from silica gel-dried leaves following the CTAB extraction protocol by Doyle & Doyle (1987) with minor modifications. The AFLP procedure (Vos & al., 1995) was the same as described in detail in Schönswetter & al. (in press). DNA restriction with the endonucleases *Eco*RI and *Mse*I and ligation to double stranded adapters were performed in one step. After that, preselective amplification with primers with a one selective base extension followed. On a basis of a primer test with 15 different primer combinations, three primer pairs were chosen for selective amplification: *Eco*RI -AAG-(HEX), *Mse*I -CTG; *Eco*RI -ATC-(6-FAM), *Mse*I -CAG; *Eco*RI -AGC-(NED), *Mse*I -CTG. The amplified AFLP fragments were electrophoresed and detected in an ABI Prism 377 sequencer, and then analyzed with GeneScan® software (PE Applied Biosystems). Presence or absence of fragments ranging from 70 to 500 bp were scored for each sample (only well scorable, unambiguous fragments were analyzed) and transferred into a binary matrix using GenoGrapher (version 1.6.0, © Montana State University 1999; http://hordeum.msu.montana.edu/genographer/). Distribution of AFLP fragments across the studied taxa was extracted from the binary data matrix. For each taxon (or geographically isolated group of populations), the total number of AFLP fragments, mean number of fragments per individual, number of exclusive (present in a given taxon only, but not necessarily in all its samples) and diagnostic fragments (present in all samples of a taxon and absent from all other taxa) were calculated. Principal coordinate analysis (PCoA) based on pairwise similarities computed with Jaccard's coefficient was performed using SYN-TAX 2000 (Podani, 2001). Neighbour-joining tree using Nei & Li (1979) genetic distance was generated by the TREECON program (version 1.3b; Van de Peer & De Wachter, 1994) with bootstrap option (2,000 replications). In addition, analysis of molecular variance (AMOVA; Arlequin, version 2.000, Schneider & al., 2000) was calculated from a matrix of squared Euclidean distances to estimate population differentiation in *C. pratensis* s.str. and *C. crassifolia* (including populations from the Eastern Pyrenees and those from central Iberian mountains). Total genetic variance was partitioned into levels of individuals within populations, among populations, and among groups of populations. Various groupings of populations (with two to nine groups), either based on geographic regions or ploidy level were tested. Population sample no. 44 (see Table 1) of unknown ploidy level was omitted from the AMOVAs. In all cases, 10,000 permutations were run to obtain F-statistics.

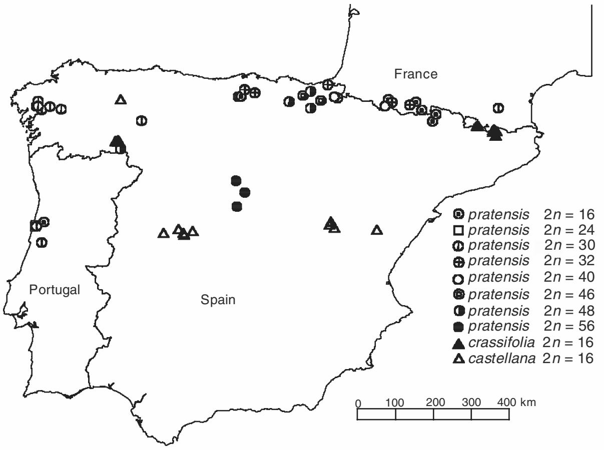

Fig. 1. Map showing distribution of different cytotypes of *C. pratensis* s.str. in the Iberian Peninsula, the investigated populations of *C. crassifolia* in the Eastern Pyrenees, and *C. castellana* from the central Iberian mountains.

RESULTS

Karyological analyses. — All populations previously classified as *C. crassifolia* from the Pyrenees and central Iberian mountains were found to be diploid with $2n = 16$ (Table 1). For *C. pratensis* s.str. remarkable karyological variation was revealed (Table 1, Fig. 1). Diploid to heptaploid populations, including also dysploid ones, were found across its distribution in the Iberian Peninsula. Diploids ($2n$ =16) were recorded from Beira Litoral region in Portugal, from the Basque country (central part of northern Spain), and from the Central Pyrenees. Two cytotypes at the tetraploid level, namely $2n = 30$ and $2n = 32$ were observed, the former one concentrated in Galicia (NW Spain) and Portugal (but found also on the northern slopes of the Eastern Pyrenees), the latter in the Basque country and Central Pyrenees. In a geografically small area in Beira Litoral one diploid ($2n = 16$) and one hypotetraploid ($2n = 30$) population was found by us, in addition to two triploid ($2n = 24$) counts recorded by Urbanska and Landolt (Table 1). Two pentaploid populations with $2n = 40$ were recorded from the Western and Central Pyrenees. At the hexaploid level, two cytotypes with $2n = 46$ and 48 were found. Populations with $2n = 56$ were determined from central (prov. Madrid, Segovia) and northern Spain (prov. Cantabria).

Morphometric analyses. — (1) Iberian representatives of the *C. pratensis* group. Both the PCA (Fig. 2) and cluster analysis (figure not shown) performed on the whole Iberian material gave similar results. Two main groups, corresponding to *C. pratensis* s.str. and *C. crassifolia* as previously classified, were resolved, clearly separated along the first axis in the ordination graph. Only one population of *C. pratensis* s.str. from the Central Pyrenees (population no. 61, see Table 1) was unexpectedly placed among samples of *C. crassifolia*. All floral and leaf characters (excluding ratios) contributed almost equally to division along the first axis, as seen from the eigenvector values (Table 2). *Cardamine*

Table 2. Eigenvectors expressing correlation of characters with principal components (axis 1, axis 2, axis 3) in morphometric analysis of Iberian populations of the *C. pratensis* group (*C. pratensis* s.str., *C. crassifolia* from the Eastern Pyrenees and *C. castellana* from central Iberian mountains).

Character	Axis 1	Axis 2	Axis 3
length of petals	0.314	-0.226	0.014
width of petals	0.304	-0.221	0.451
length of sepals	0.313	-0.157	0.020
length of longer filaments	0.275	-0.309	0.015
length of shorter filaments	0.274	-0.299	0.050
number of stem leaves	0.266	0.265	0.064
number of segments on the stem leaf nearest the midpoint of stem	0.317	0.185	0.080
number of segments on the middle stem leaf	0.327	0.110	0.093
number of lateral inflorescences	0.175	0.177	0.160
number of leaflets of basal leaves	0.304	0.224	0.008
HP1/LL2	0.163	0.351	0.073
LL2/NS2	-0.017	-0.463	0.013
LTS/WTS	0.090	0.167	0.666
LLS/WLS	0.088	-0.175	0.634
LTS/LLS	-0.189	0.210	0.269
LL2/LTS	0.284	0.227	-0.161

Table 3. Morphometric analyses of *C. crassifolia* from the Eastern Pyrenees and *C. castellana* from central Iberian mountains. PCA - eigenvectors showing correlation of characters with principal components (axis 1, axis 2); CDA - total canonical structure expressing correlation of characters with the canonical axis.

	PCA		
Character	Axis 1	Axis 2	CDA
length of petals	0.458	0.121	0.634
width of petals	0.438	0.038	0.936
length of sepals	0.388	0.124	0.487
length of longer filaments	0.442	0.034	0.702
length of shorter filaments	0.333	0.110	0.251
number of stem leaves	-0.116	0.295	-0.375
number of segments on the stem leaf nearest the midpoint of stem	-0.161	0.313	-0.241
number of segments on the middle stem leaf	-0.109	0.404	-0.140
number of lateral inflorescences	-0.011	0.142	0.032
number of leaflets of basal leaves	-0.226	0.261	-0.435
HP1/LL2	0.111	0.085	0.297
LL2/NS2	0.024	-0.265	-0.165
LTS/WTS	-0.038	0.433	-0.127
LLS/WLS	-0.006	0.435	-0.092
LTS/LLS	-0.111	-0.032	-0.204
LL2/LTS	-0.118	-0.252	-0.240

pratensis s.str. has larger flowers, and higher number of leaves, leaf segments and leaflets on basal leaves when compared to *C. crassifolia*. Populations of *C. crassifolia* were placed in two partially overlapping groups, referring to the samples from the Eastern Pyrenees on one side, and those from central Iberian mountains on another.

(2) *Cardamine crassifolia* from the Eastern Pyrenees and central Iberian mountains. In PCA of individual plants, two groups corresponding to the samples from the Pyrenees and central Iberian mountains, respectively, were revealed, although with many specimens in overlapping positions (Fig. 3). Floral characters showed the highest correlation with the first axis (Table 3, PCA). In CDA distinct separation between individuals from these two areas was achieved (Fig. 4), based on floral characters, as indicated by PCA, and on the number of leaflets of basal leaves (see total canonical structure in Table 3, CDA). As can be seen also from the mean values and interquartile ranges of these characters (Fig. 5), Pyrenean plants have generally larger petals, longer filaments, and lower number of leaflets of basal leaves. Classificatory discriminant analysis also showed good extent of separation, as 96–97% of the specimens were correctly classified. Most of the misclassified plants came from the population in Sierra de Gúdar (population no. 28; Table 1), due to slightly longer filaments, fewer stem leaves and leaflets of basal leaves possessed by these individuals. Except for the differences in quantitative characters recovered between Pyrenean and central Iberian populations, we observed also pronounced qualitative differences. While plants from the Pyrenees have an exclusively thin, horizontally creeping rhizome, ascending stem, and basal leaves not forming a rosette, those from central Iberian mountains differ in this respect. Both plants with horizontal and erect rhizome (or intermediate one) can be found, often at least the upper part of the rhizome is thickened (and sometimes bearing multiple flowering stems), basal leaves either form or do not form

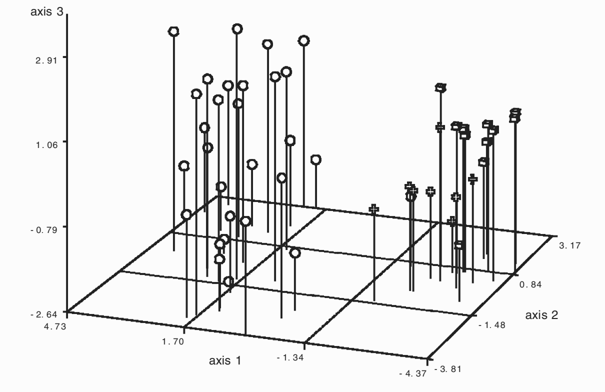

Fig. 2. Principal component analysis of Iberian populations of the *C. pratensis* group based on 16 morphological characters (see Table 2). Circles, *C. pratensis* s.str.; crosses, *C. crassifolia* from the Eastern Pyrenees; cubes, *C. castellana* from central Iberian mountains. First three axes explain 51.78%, 18.28% and 10.30% of total variation.

Table 4. Total canonical structure expressing correlation of morphological characters with canonical axes in discriminant analysis of Iberian *C. pratensis* s.str. on the populational level. Groups defined as different cytotypes.

Character	Axis 1	Axis 2	Axis 3
length of petals	-0.343	0.114	0.489
width of petals	0.417	0.215	0.272
length of sepals	-0.022	0.421	0.427
length of longer filaments	0.280	-0.017	0.452
length of shorter filaments	-0.343	0.065	0.431
number of stem leaves	0.513	0.151	-0.186
number of segments on the third stem leaf	0.603	0.271	-0.026
number of segments on the stem leaf nearest the midpoint of stem	0.638	0.337	0.041
number of segments on the middle stem leaf	0.551	0.263	0.081
number of lateral inflorescences	0.230	-0.173	0.141
number of leaflets of basal leaves	0.689	0.117	0.127
HP1/LL2	0.561	0.218	-0.337
LL2/NS2	-0.6645	-0.030	0.172
LTS/WTS	0.334	-0.254	0.314
LLS/WLS	-0.254	-0.269	0.406
LTS/LLS	0.553	-0.440	0.106
LL2/LTS	0.677	0.264	-0.056

Table 5. Distribution of AFLP fragments generated by three primer combinations across the investigated taxa of the *C. pratensis* group. N_{IND} = number of analyzed individuals; N_{TF} = total number of fragments; N_{AF} = average number of fragments per individual (± standard deviation); N_{EF} = number of fragments present exclusively in the taxon (but not necessarily present in all its samples); N_{DF} = number of fragments diagnostic for the taxon (i.e., fragments restricted to the taxon, present in all samples).

Taxon	N_{IND}	N_{TF}	N_{AF}	N_{EF}	N_{DF}
C. pratensis s.str.	52	132	46 (±4.40)	39	-
C. pratensis s.str., Iberian	31	106	46 (±4.79)	16	-
C. pratensis s.str., non-Iberian	21	107	46 (±3.88)	12	-
C. crassifolia	13	64	47 (±3.67)	14	-
C. castellana	12	63	44 (±3.01)	15	2
C. castellana (Sierra de Gredos)	6	46	42 (±1.52)	7	3
C. castellana (Sierra de Albarracín)	6	51	47 (±0.75)	6	5
C. matthioli	10	59	42 (±1.85)	6	-
C. majovskii	10	64	44 (±2.45)	4	-
C. granulosa	5	58	45 (±3.96)	6	1
C. pratensis s.l. (central Italy)	12	65	45 (±3.94)	15	4

a true basal rosette, and both ascending and erect stems can be observed (Fig. 6). We found very few plants (12 out of 268 examined) having the same combination of these characters, as is typical for plants from the Pyrenees.

(3) Iberian *C. pratensis* s.str. In CDA performed on populations as OTUs and six cytotypes as groups, populations with 2*n* = 48 and 46 were separated along the first canonical axis (most strongly correlated with the number of segments on stem leaves, number of leaflets of basal leaves and ratios LL2/NS2, LL2/LTS; Table 4) on one side, and remaining populations on another, with diploids in an intermediate position (Fig. 7). Heptaploid, hypotetraploid and tetraploid populations were separated from each other along the second axis, most correlated with the length of sepals and ratio LTS/LLS. However, when CDA was performed on individual plants, different cytotypes were found largely intermingled in one dense group, showing only slight shifts along the axes (figure not shown). Similarly, in the classificatory discriminant

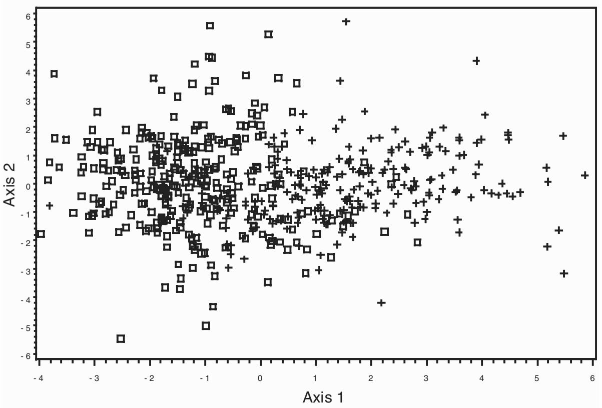

Fig. 3. Principal component analysis of individuals of *C. crassifolia* (crosses) from the Eastern Pyrenees and of *C. castellana* (squares) from central Iberian mountains based on 16 morphological characters (see Table 3, PCA). First two axes explain 24.31% and 14.43% of total variation.

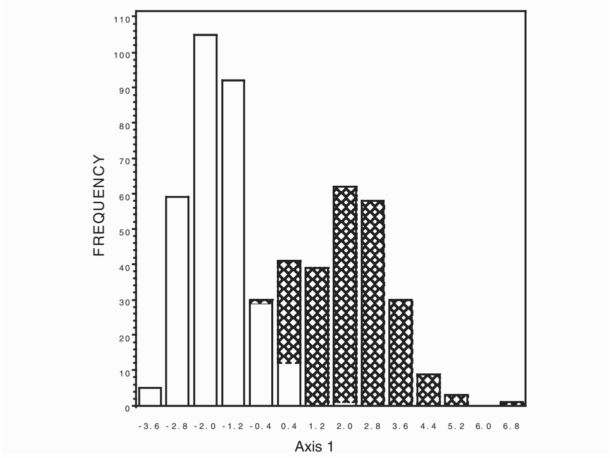

Fig. 4. Histogram of canonical discriminant analysis performed on individuals of *C. crassifolia* (hatched) from the Eastern Pyrenees and *C. castellana* (white) from central Iberian mountains based on 16 morphological characters (see Table 3, CDA).

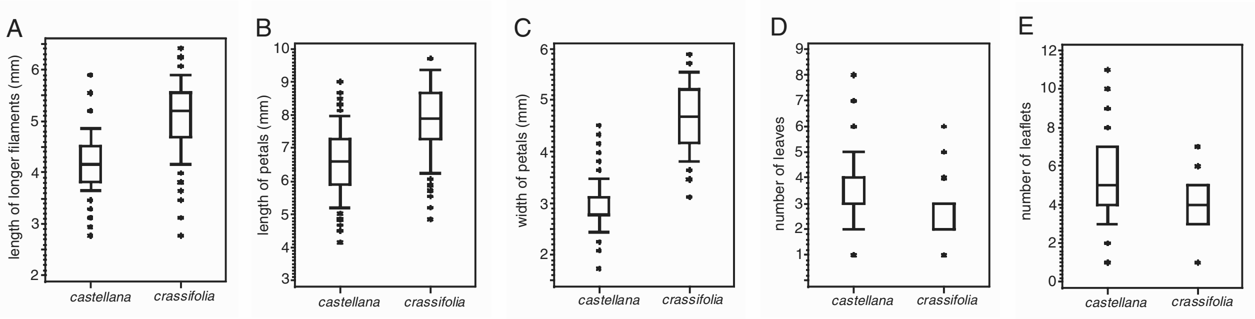

Fig. 5. Variation in selected morphological characters of *C. crassifolia* and *C. castellana*. Rectangles define 25 and 75 percentiles; horizontal lines show median; whiskers are from 10 to 90 percentiles; asterisks show extreme values. A, max. length of longer filaments (mm); B, length of petals (mm); C, width of petals (mm); D, number of stem leaves; E, number of leaflets of basal leaves.

analysis, the percentages of plants correctly classified into predicted groups (= cytotypes) were low, ranging from 39.62% for plants with $2n = 32$, to 87.76% for those with $2n = 46$. The latter cytotype, however, was represented by only two populations, and thus the value can be somewhat biased. When comparing the values (mean, median, percentiles) of the measured characters, increase in length of petals and length of filaments can be observed from diploid to heptaploid levels (Fig. 8). In addition, hypotetraploids shifted with respect to lower number of leaves and segments on leaves, and diploids had the shortest sepals. Still, for all characters examined, large overlap among cytotypes was evident (Fig. 8).

(4) Iberian vs. central European *C. pratensis* s.str. In the cluster analysis of Iberian and central European populations of *C. pratensis* s.str., Iberian populations did not group together, but were found scattered in all three main clusters (Fig. 9). The population from the Central Pyrenees (no. 61, see Table 1), which showed a somewhat isolated position relative to other Iberian populations, clustered here together with central European populations of $2n = 30$ and 38, and the diploid *C. rivularis* auct. type. Similarly, in PCA no separation between Iberian and non-Iberian populations was seen (figure not shown). Also in the qualitative characters colour of petals and indumentum of rachis of basal leaves, no differences

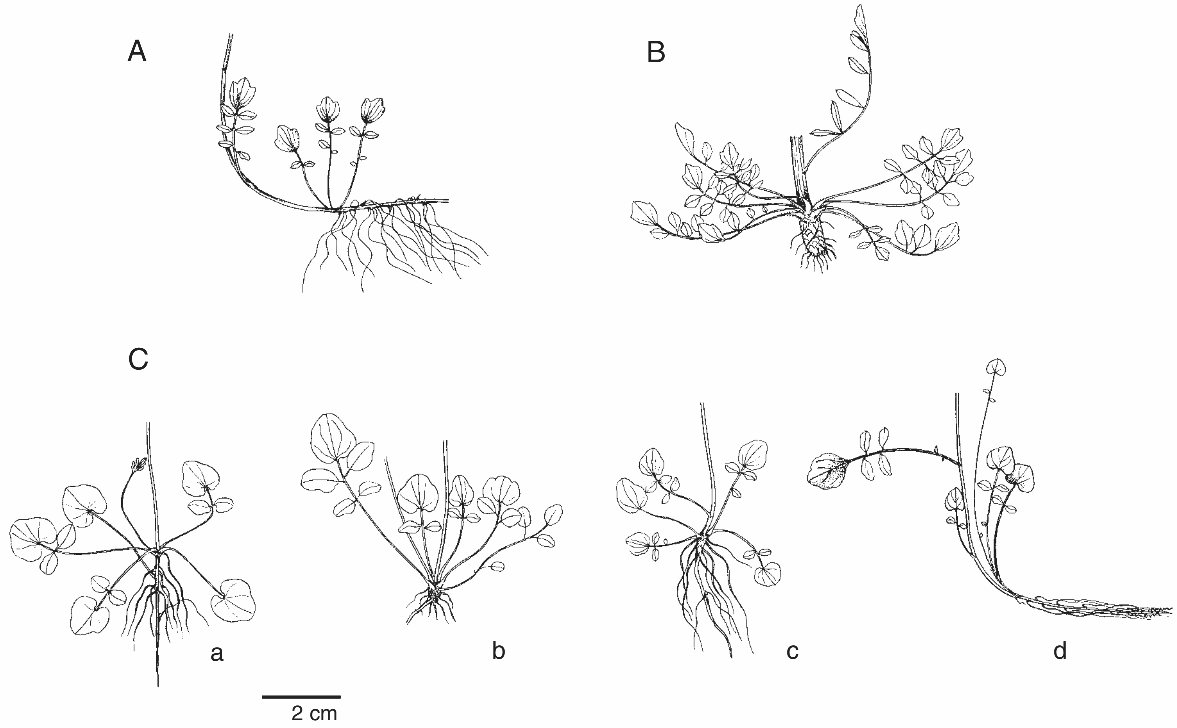

Fig. 6. Morphology of rhizome and basal parts of stem in the studied Iberian taxa. A, *C. crassifolia* (Eastern Pyrenees); B, *C. pratensis* s.str.; C, *C. castellana* (central Iberian mountains), a–d show variation range in this species.

Table 6. Analysis of molecular variance (AMOVA) of AFLP phenotypes in *C. pratensis* s.str. (a) Partitioning of the overall variation into two levels (within and among populations), and (b)–(f) into three levels with different groupings; (b) nine geographic regions: Madrid & Segovia provinces (central Spain), A Coruña province (NW Spain), Cantabria province (N Spain), Navarra province (N Spain), central Portugal, SE France, Piedmont (NW Italy), Slovakia, Slovenia; (c) cytotypes 2*n* = 16, 30, 32, 44, 46, 48, 56; (d) Iberian populations only, five geographic regions: Madrid & Segovia provinces (central Spain), A Coruña province (NW Spain), Cantabria province (N Spain), Navarra province (N Spain), central Portugal; (e) Iberian cytotypes only 2*n* = 16, 30, 32, 46, 48, 56; (f) Iberian and non-Iberian populations. Population numbers as given in Table 1 are in brackets. d.f. = degrees of freedom; *** = $P<0.001$; ** = $P<0.05$.

Grouping [population number]	Source of variation	d.f.	Sum of squares	Variance components	% of total variance
(a) [30,34,36,38,43,46-48,57–59,69–76]	among populations	18	432.43	7.20	56.65***
	within populations	30	165.17	5.51	43.35***
(b) [43,46],[34,36,38],[47,48],[57-59],30,[72,73], 74,[75,76],[69–71]	among groups	8	274.64	3.39	26.14***
	among populations	10	157.78	4.06	31.35***
	within populations	30	165.17	5.51	42.51***
(c) 48,[30,34,36,38,69,70,72,73,74,76],59,[71,75], 57,58,[43,46,47]	among groups	6	173.68	1.01	7.79**
	among populations	12	258.75	6.44	49.73***
	within populations	30	165.17	5.51	42.48***
(d) [43,46],[34,36,38],[47,48],[57-59],30,	among groups	4	134.05	3.64	30.55***
	among populations	6	82.71	3.57	29.91***
	within populations	17	80.17	4.72	39.54***
(e) 48,[30,34,36,38],59,57,58,[43,46,47]	among groups	5	150.92	3.70	30.98***
	among populations	5	65.85	3.52	29.51***
	within populations	17	80.17	4.72	39.51***
(f) [30,34,36,38,43,46-48,57–59],[69–76]	among groups	1	63.04	1.69	12.51***
	among populations	17	369.38	6.32	46.75***
	within populations	30	165.17	5.51	40.73***

Table 7. Analysis of molecular variance (AMOVA) of AFLP phenotypes in *C. crassifolia* and *C. castellana*. (a) Partitioning of the overall variation into two levels (within and among populations), and (b)–(d) into three levels with different groupings; (b) *C. crassifolia* (Eastern Pyrenees), *C. castellana* (Sierra de Gredos, Sierra de Albarracín); (c) *C. crassifolia* Núria region, *C. crassifolia* Meranges region, *C. castellana* Sierra de Gredos, *C. castellana* Sierra de Albarracín; (d) *C. castellana* Sierra de Gredos, *C. castellana* Sierra de Albarracín. Population numbers as given in Table 1 are in brackets. d.f. = degrees of freedom; *** = $P<0.001$; ** = $P<0.05$; n.s. = not significant.

Grouping [population number]	Source of variation	d.f.	Sum of squares	Variance components	% of total variance
(a) [01–07,20,21,23,25–27]	among populations	11	311.56	12.69	86.84***
	within populations	13	25.00	1.92	13.16***
(b) [01–07],[20,21,23, 25–27]	among groups	1	174.14	12.84	62.77**
	among populations	10	137.42	5.69	27.83***
	within populations	13	25.00	1.92	9.40***
(c) [03–07],[01,02],[20,21,23],[25-27]	among groups	3	263.28	13.45	77.53***
	among populations	8	48.28	1.97	11.38***
	within populations	13	25.00	1.92	11.09***
(d) [20,21,23],[25-27]	among groups	1	70.17	10.81	77.34$^{n.s.}$
	among populations	4	21.33	2.17	15.51**
	within populations	6	6.00	1.00	7.16***

were found, as Iberian populations have reddish-violet flowers and rachis glabrous (mostly) or with patent hairs, as is typical for *C. pratensis* s.str. Although some Iberian populations had strongly thickened tuberous rhizomes, not observed among central European populations, this feature was neither correlated with any other morphological character, nor geographically, since such plants were found in Portugal, in Spanish provinces A Coruña, Pontevedra, Guipuzcoa and Segovia, and in diploid to heptaploid populations.

AFLP analyses. — In 114 individuals of the *C. pratensis* group investigated, 219 scorable fragments were amplified by three primer combinations. Nine fragments were monomorphic, and 19 fragments were restricted to single samples. The number of fragments amplified per taxon (or group of populations) varied

Table 8. Morphological characters distinguishing *C. pratensis* s.str., *C. crassifolia* (Eastern Pyrenees) and the newly described *C. castellana* from central Iberian mountains. For quantitative characters, mean values with 5 and 95 percentiles in parentheses are given.

Character	*C. crassifolia*	*C. castellana*	*C. pratensis* s.str.
Habitus	always single stem (never caespitose), often forming cushions	sometimes with multiple stems (caespitose), but not forming cushions	sometimes with multiple stems (caespitose), but not forming cushions
Rhizome			
length and width	elongated, always equally thin	elongated or shortened, mostly thickened at least in the upper part, very rarely entirely thin	shortened, strongly thickened
growth orientation	always horizontally creeping	erect, oblique, rarely horizontal	always erect, never creeping
branching	usually branched along its length	simple, if branched then in upper part only	simple, if branched then in upper part only
Stem			
growth orientation	always ascending	ascending to erect	always erect
Basal rosette	absent (short internodes between basal leaves always retained)	present or absent (short internodes between basal leaves sometimes retained)	always present
Number of stem leaves	(2–)3(–4)	(2–)4(–6)	(2–)5(–10)
Number of pairs of leaflets on basal leaves	1–2	1–4	2–9(–10)
Petals			
length (mm)	(5.9–)8.1(–10.4)	(5.0–)6.5(–8.3)	(9.4–)13.3(–16.7)
width (mm)	(3.5–)4.7(–5.9)	(2.2–)2.9(–3.6)	(5.9–)7.9(–9.7)
shape	obovate to broadly obovate	narrowly obovate	obovate to broadly obovate

from 46 in populations from Sierra de Gredos (central Spain) to 132 in *C. pratensis* s.str. Except for *C. pratensis* s.str. with the largest amount of samples analyzed, the highest numbers of exclusive fragments were recorded in *C. crassifolia* (14 fragments), central Iberian populations (15), and in taxonomically uncertain populations from central Italy (15). In the latter two, and also *C. granulosa* (Piedmont, northern Italy), diagnostic fragments were found as well (Table 5). When comparing the average number of fragments amplified per individual in different taxa and cytotypes, only small differences were found. With the exception of samples of Iberian diploid *C. pratensis* s.str. (no. 61), which showed only 36 (± 2.1) fragments per genotype, in all other samples, including diploid *C. crassifolia* and *C. granulosa*, 42–51 fragments were scored. The number of fragments in *C. pratensis* s.str. was correlated with ploidy level (Spearman correlation coefficient $r_S = 0.764$, $P = 0.05$). However, the highest value was not found in the samples of $2n = 56$, but in those of $2n = 44$ (51 ± 1.9 fragments).

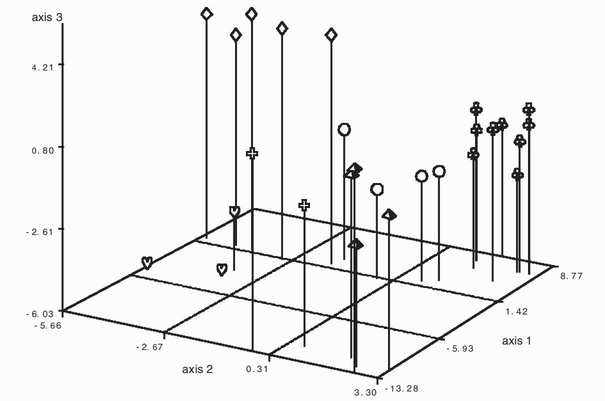

Fig. 7. Canonical discriminant analysis of Iberian *C. pratensis* s.str. with groups defined as different cytotypes and populations as OTUs, based on 17 morphological characters (see Table 4). Hearts, 2*n* = 16; clubs, 2*n* = 30; circles, 2*n* = 32; crosses, 2*n* = 46; pyramids, 2*n* = 48; diamonds, 2*n* = 56. First three axes explain 73.93%, 10.90%, and 8.83% of total variation.

In PCoA based on Jaccard's coefficient, several groups of related AFLP phenotypes can be recognized (Fig. 10). Samples of *C. pratensis* s.str. both from the Iberian Peninsula and other parts of Europe formed one dense group, without further division in the ordination of the first three coordinates. Diploid *C. matthioli* and tetraploid *C. majovskii* were placed close to each other and to *C. pratensis* s.str., even with some samples intermingled. *Cardamine crassifolia* from the Eastern Pyrenees and central Iberian mountains formed two distinctly separated groups corresponding to their geographic origin. Samples of *C. granulosa* from Piemont were different from those from central Italy, but this Italian taxon will not be discussed here in more detail, as it is being evaluated elsewhere, including also its morphology (Lihová & al., submitted b). In the PCoA ordi-

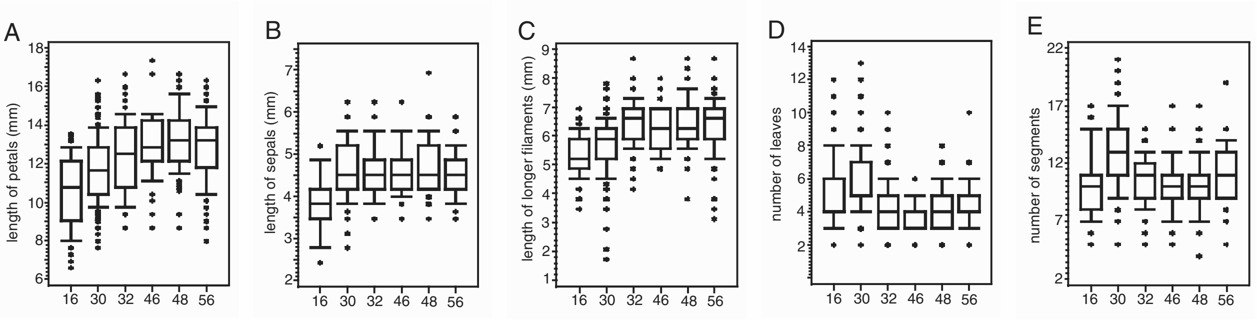

Fig. 8. Variation in selected morphological characters compared among different cytotypes (2*n* = 16, 30, 32, 46, 48, 56) of *C. pratensis* s.str. from the Iberian Peninsula. Rectangles define 25 and 75 percentiles; horizontal lines show median; whiskers are from 10 to 90 percentiles; asterisks show extreme values. A, length of petals (mm); B, length of sepals (mm); C, length of longer filaments; D, number of stem leaves; E, number of segments on middle stem leaves.

nation including only *C. pratensis* s.str., *C. matthioli* and *C. majovskii* (graph not shown), slight separation among samples of *C. pratensis* s.str. was achieved, showing correlation with ploidy level and geographic origin. Galician hypotetraploids and central Iberian heptaploids (prov. Segovia) formed two groups separated along the second axis, with other Iberian and central European samples in-between. The neighbour-joining tree based on genetic distance of Nei & Li produced similar structure as PCoA (Fig. 11). The group consisting of *C. pratensis* s.str., *C. matthioli* and *C. majovskii* is rather heterogeneous, as is evident from the bootstrap value of 57%, but at the same time it shows low resolution, only samples from the same population and those from geographically close localities clustering with higher support. *Cardamine crassifolia* from the Pyrenees formed one well supported group (100% bootstrap value), as well as that from central Iberian mountains (92%), but they both received only moderate support as one group (69%). Such clustering is consistent with a rather high number of fragments exclusively recorded (although not present in all accessions) for each of them, 14 fragments for the Pyrenean and 15 for the central Iberian samples (Table 5). When considering both as one entity, only three such fragments were scored.

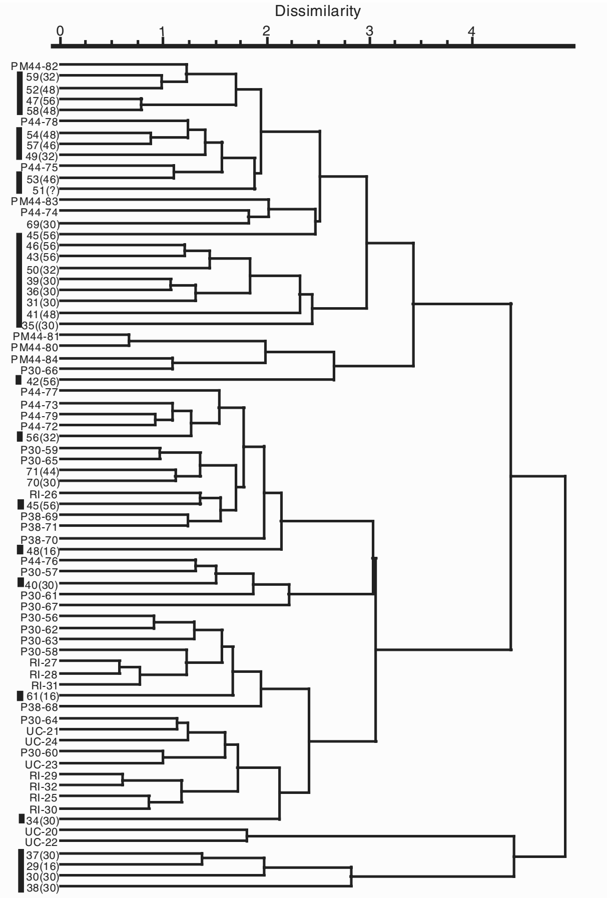

Fig. 9. Cluster analysis of Iberian and Central European populations of *C. pratensis* s.str. based on eight morphological characters. Central European samples: P30, 2*n* = 30; P38, 2*n* = 38; P44, PM44, 2*n* = 44; UC, *C. pratensis* "*ucranica* type" 2*n* = 16; RI, *C. rivularis* auct. non Schur, 2*n* = 16; labels are the same as in Marhold (1996). Iberian samples are numbered as in Table 1, with chromosome numbers indicated in parentheses, and marked by vertical bars.

AMOVA in *C. pratensis* s.str. without subdividing populations into groups showed moderate differentiation among populations, as 56.65% of total variation accounted for among-population variation. Partitioning of populations of *C. pratensis* s.str. into nine geographic regions revealed that 26.14% of the overall variation was explained by among-group variation, 31.35% by variation among populations, and 42.51% by that within populations (Table 6). When differentiating ploidy levels (seven groups), only 7.79% of the total variation was assigned to variation among cytotypes, while most of the variation was among (49.73%) and within populations (42.48%). AMOVA including only Iberian populations of *C. pratensis* s.str. subdivided geographically (six groups) gave higher value for the among-group variation (30.55%) than the analysis including also non-Iberian

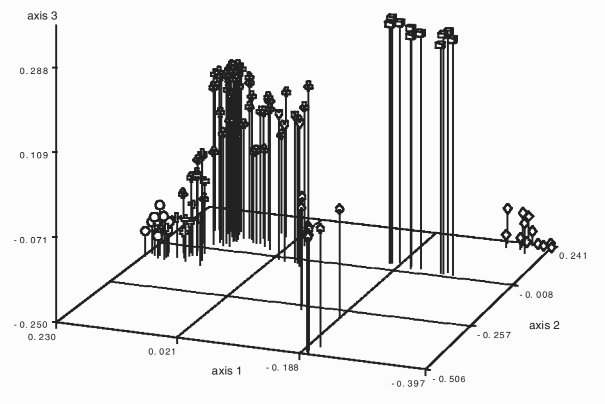

Fig. 10. Principal coordinate analysis of AFLP data of 114 samples of the *C. pratensis* group. Clubs, *C. pratensis* s.str.; diamonds, *C. crassifolia* (Eastern Pyrenees); cubes, *C. castellana* (central Iberian mountains); circles, *C. majovskii*; crosses, *C. matthioli*; hearts, *C. granulosa* (Piedmont); spades, samples from taxonomically uncertain populations from central Italy. The first three axes explain 13.52%, 11.14% and 7.88% of total variation.

samples. The Iberian cytotypes showed much higher differentiation (30.98 % vs. 7.79% with the inclusion of non-Iberian samples), but still, most variation was explained by among- and within-population differentiation. In accordance with neighbour-joining and PCoA results, only 12.51% of the total variation can be attributed to variation between Iberian and non-Iberian populations, suggesting that there is hardly any genetic differentiation between Iberian populations and those from other parts of Europe.

In the two-level AMOVA of populations of *C. crassifolia* from the Eastern Pyrenees and those from central Iberian mountains, strong differentiation among the populations was revealed (86.84%; Table 7). As can be seen from the three-level AMOVA, this is attributable mostly to the regional differentiation (E Pyrenees vs. central Iberian mountains Sierra de Gredos and Sierra de Albarracín; 62.77% of total variation), with a much lower value for among-population variation within a region (27.83%). When subdividing both Pyrenean and central Iberian populations into two geographic groups, even higher proportion of the variation accounted for among-region variation (77.53%). This is due to high genetic differentiation also between populations in Sierra de Gredos and Sierra de Albarracín (77.34%), as also shown in the neighbour-joining tree (Fig. 11).

Description of the new taxon. — Both morphological and molecular comparative analyses demonstrate that populations from central Iberian mountains, traditionally classified within *C. crassifolia*, are different from typical *C. crassifolia*, and should be regarded as a separate taxonomic entity. On a basis of this evidence, we describe here a new species, *Cardamine castellana* Lihová & Marhold. Morphological characters distinguishing this species from *C. crassifolia* and *C. pratensis* s.str. are summarized in Table 8.

Cardamine castellana Lihová & Marhold, **sp. nov.** – Holotype: Spain. prov. Ávila: Sierra de Gredos, Hoyos del Espino, near Hoyos del Collado, 1500 m, 10 May 2001, *J. Lihová s.n.* (SAV). Isotypes: SALA, BC, MA.

Descriptio: Herba perennis, (7–)13–23(–26) cm alta (ad infimum pedunculum). Rhizoma repens, obliquum vel erectum, plerumque incrassatum certe in parte superiore, tantum raro omnino tenue, interdum ferens plures floriferos caules (caespitosus), raro prostratos usque ascendentes stolones. Caulis ascendens usque erectus, simplex vel raro ramosus. Folia basalia glabra, pinnata, 1–4 jugata, rosulata vel congesta prope basin caulis; foliola terminalia late ovata usque subrotundata, integra vel in apice remote crenata, basi reniformia vel obtusa, majora foliolis lateralibus; foliola lateralia late elliptica, ovata usque subrotundata. Folia caulina (1–)2–6(–7), pinnatisecta, glabra, segmenta foliorum mediorum (4–)5–11(–13); segmentum terminale oblongum usque ellipticum vel oblanceolatum usque obovatum, integrum vel in apice remote crenatum, segmenta lateralia oblonga usque elliptica vel oblanceolata; numerus segmentorum inflorescentiam versus decrescens. Inflorescentia racemosa; petala saturata usque diluta rubelloviolacea, raro paene alba, anguste obovata, raro obovata, apice sinuata, (4.5–)5–8.3(–9) mm longa et (2–)2.3–3.6(–4.2) mm lata. Sepala elliptica usque anguste obovata, (2.4–)2.6–3.8(–4.2) mm longa. Staminum 6, tetradynama, antherae luteae, stamina filamenta longiora (3.1–)3.5–4.9(–5.6) mm longa, filamenta breviora (1.3–)1.9–3.1(–3.5) mm longa. Stigma conspicuum, dilatatum. Pedunculi patentes usque erecto-patentes, siliquae divergentes in eundem angulum ac pedunculi vel erectae. $2n = 2x = 16$.

Description: Perennial herb, (7–)13–23(–26) cm tall (up to the lowest flower/fruit peduncle). Rhizome creeping, oblique or erect, mostly thickened at least in the upper part, only seldom entirely thin, sometimes with multiple stems (caespitose), and occasionally with prostrate to ascending thin stolons. Stem ascending to erect, simple or rarely branched above. Basal leaves glabrous, pinnate, with 1–4 pairs of leaflets, either forming a basal rosette or only congested near the stem base; terminal leaflet broadly ovate to roundish, entire or remotely crenate at apex, reniform or obtuse at base, larger than lateral ones, lateral leaflets broadly elliptic, ovate to roundish. Stem leaves (1–)2–6(–7), pinnatisect, glabrous, middle stem leaves with (4–)5–11(–13) segments; terminal segment oblong to elliptic or oblanceolate to obovate, entire or remotely crenate at apex, lateral ones oblong to elliptic or oblanceolate; number of segments gradually

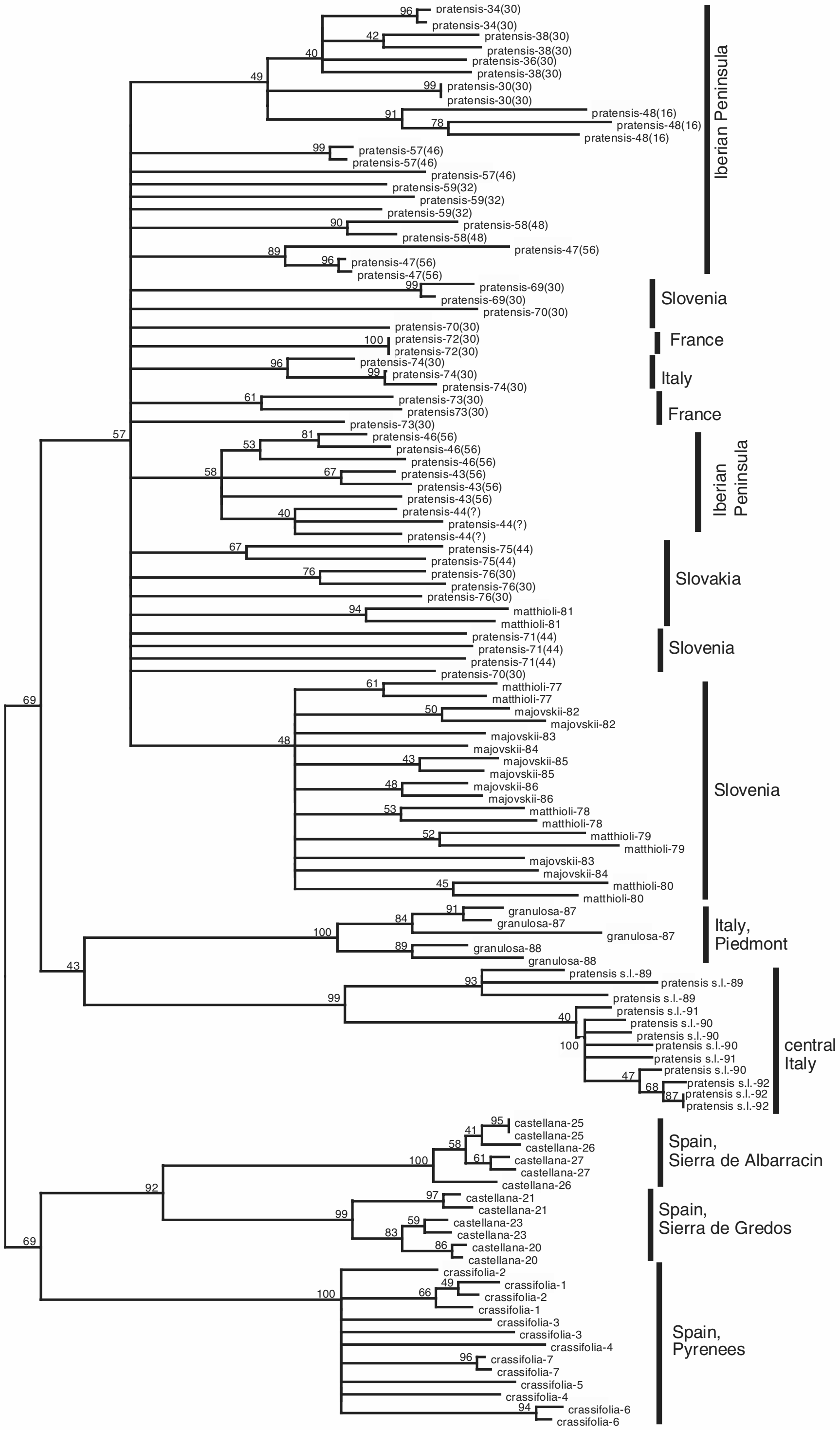

Fig. 11. Neighbour-joining tree of AFLP data of 114 samples of the *C. pratensis* group, including *C. pratensis* s.str. (with chromosome numbers indicated in parentheses), *C. matthioli*, *C. majovskii*, *C. crassifolia* (samples from the Eastern Pyrenees), *C. castellana* (samples from Sierra de Gredos and Sierra de Albarracín), *C. granulosa* (Piedmont) and individuals from taxonomically uncertain populations from central Italy. For numbers and origin of samples see Table 1. Values above branches are bootstraps using 2,000 replicates. Branches with less than 40% bootstrap are shown as unresolved.

diminishing towards the inflorescence. Inflorescence racemose; petals deep to pale reddish-violet, seldom almost white, narrowly obovate, sinuate at the tip, (4.5–)5–8.3(–9) mm long and (2–)2.3–3.6(–4.2) mm wide. Sepals elliptic to narrowly obovate, (2.4–)2.6–3.8(–4.2) mm long. Stamens 6, tetradynamous, anthers yellow, filaments of longer stamens (3.1–)3.5–4.9(–5.6) mm long, filaments of shorter stamens (1.3–)1.9–3.1(–3.5) mm long. Stigma conspicuous, enlarged. Peduncles patent to erect-patent, siliquae divergent from infructescence-rachis at the same angle as peduncles or erect. $2n = 2x = 16$.

Distribution and habitat: Spain. Sierra de Gúdar, Sierra de Albarracín, Sierra de Gredos, Sierra Segundera, Cordillera Cantabrica; wet meadows and pastures, stream banks, 1200–2000 m.

Key to the Iberian representatives of the *Cardamine pratensis* group

1a. Whole rhizome equally thin, as wide as stem . . . 2
1b. Whole or only upper part of rhizome thickened . . 3
2a. Rhizome horizontally creeping, stem ascending; basal leaves with 1–2 pairs of lateral leaflets; petals broadly obovate or obovate, 3.5–5.9 mm wide . . . . *C. crassifolia*
2b. Rhizome horizontally creeping to erect, stem ascending to erect; basal leaves with 1–4 pairs of leaflets; petals narrowly obovate, 2.3–3.6 mm wide . . . . *C. castellana*
3a. Middle stem leaves with 9–21 segments; petals broadly obovate or obovate, 9–15 mm long, 5–9 mm wide . . . . *C. pratensis* s.str.
3b. Middle stem leaves with 5–11 segments; petals narrowly obovate, 5–8 mm long, 2.3–3.6 mm wide . . . . *C. castellana*

Full list of studied specimens of the *Cardamine pratensis* group in the Iberian Peninsula is given in the internet version of Taxon.

DISCUSSION

Combined karyological, morphological and molecular studies suggest that three taxa of the *Cardamine pratensis* group can be recognized in the Iberian Peninsula: *C. pratensis* s.str., *C. crassifolia*, and the herein described new species, *C. castellana*. Whereas *C. pratensis* s.str. is composed of several cytotypes, ranging from diploid to heptaploid, the latter two Iberian endemic species are diploid. All three taxa show morphological differences in both qualitative and quantitative characters, and based on molecular AFLP data they are also genetically clearly differentiated.

***Cardamine pratensis* s.str., karyological and morphological diversity.** — *Cardamine pratensis* s.str. is a highly polymorphic polyploid species widespread in Europe. Although Lövkvist (1956) indicated that in maritime districts of Portugal, NW Spain and SW France, a diploid taxon, different from *C. pratensis* s.str., could be recognized, we cannot support his view. In both morphological and molecular evaluation, Iberian populations clearly fall within the variation of this species in other parts of Europe (Fig. 9, Table 6). Previously, only diploid and triploid chromosome numbers for this taxon were reported from the Iberian Peninsula (both from Portugal; Lövkvist, 1956; Queirós, 1973), but we revealed a much wider karyological variation (Table 1, Fig. 1). We recorded diploid populations in Portugal (Beira Litoral region), in the Spanish province Cantabria, and in the Central Pyrenees. Diploids of *C. pratensis* s.str. otherwise occur scattered across Europe; they have been found in Central Europe (e.g., Lövkvist, 1956; Urbanska-Worytkiewicz & Landolt, 1974; Marhold, 1994, 1996, 1999), in Belgium (Vyvey & Stieperaere, 1984), and France (Guinochet, 1946; Bernard, 1974; Vyvey & Stieperaere, 1984). In the Central Pyrenees (province Huesca) we found one diploid population (Table 1, population no. 61) morphologically somewhat deviating from other Iberian populations. Due to small floral organs and lower number of leaf segments in some individuals, in morphometric analyses it was placed close to *C. crassifolia* (Fig. 2). Most of the plants had, however, erect stems, short and thick rhizomes, and true basal rosettes with a high number of leaflets, all traits characteristic of *C. pratensis* s.str. Still, in a few cases tendency to ascending stems typical for *C. crassifolia* and rhizomes resembling *C. castellana* was observed. We have seen similar specimens also from other, adjacent localities (see Appendix, specimens marked by asterisks), and morphological peculiarity of plants from that area has been already noticed by Rico (1993). Bolòs & Vigo (1990) classified populations from the Central Pyrenees as *C. pratensis* subsp. *rivularis* (Schur) Nyman (≡ *C. rivularis* Schur), which in fact is a different taxon restricted to the mountains of Bulgaria and the Southern Carpathians, having violet anthers and appressed hairs on the rachis of basal leaves (vs. yellow anthers and patent hairs present here; Marhold, 1994a; Franzke & Hurka, 2000). Several authors, however, have misinterpreted this taxon, and reported it erroneously from higher altitudes in the Eastern Carpathians, Eastern Alps, Apennines, Central Massif, and also from the Pyrenees (Lövkvist, 1956; Urbanska-Worytkiewicz & Landolt, 1974; Landolt, 1984). As conspicuous morphological variability, broad ecological range of habitats and large plasticity exist in *C. pratensis* s.str., we assume that the central Pyrenean plants, although at marginal position, still fall within the variation of *C. pratensis* s.str., and should be classified within this species. However, past or more recent introgression from *C. crassifolia*, occuring more eastward, cannot be excluded. Detailed study is needed, including several populations from that area, and evaluating them also with molecular markers.

Hypotetraploid populations ($2n = 30$) of *C. pratensis* s.str., which we found to prevail in the Atlantic region of Galicia and Portugal, represent the most widespread cytotype in Europe (Guinochet, 1946; Hussein, 1955; Lövkvist, 1956; Vyvey & Stieperaere, 1984; Marhold, 1994a, b). Because of two longer chromosomes in the karyotype, Lawrence (1931) assumed that this cytotype originated by chromosome fusion. As a result, 7- and 8-chromosome sets can then meet in the genome of polyploid plants, and contribute to the karyological complexity of the species (Lövkvist, 1956). Normal tetraploids with 32 chromosomes are less frequent in Europe (e.g., Vyvey & Stieperaere, 1984; Lippert & Heubl, 1988; Marhold, 1999), and in the Iberian Peninsula they seem to be concentrated in the Basque country and Central Pyrenees (Fig. 1). Populations with 40 chromosomes, here found in the province of Navarra, have been reported so far solely from France (central Jura; Guinochet, 1946). Interesting is the chromosome number $2n = 46$, which we confirmed for several plants in two localities in the provinces Guipuzcoa and Navarra. There are no other reports for such populations in Europe (only a single plant determined from France by Lövkvist, 1956), but these two populations are morphologically and geographically close to the normal hexaploid ones ($2n = 48$), thus they may have originated in them. Higher polyploids with 48 and 56 chromosomes, which we found in several localities in central and northern Spain, occur mainly in northern Europe (England, Finland, Denmark, Sweden; Hussein, 1955; Lövkvist, 1956; Dale & Elkington, 1974). Performing crossing experiments, Lövkvist (1956) revealed that *C. pratensis* s.str. is an allogamous species, and that plants of different ploidy levels (except for diploids) are partially to fully interfertile. Especially at higher ploidy levels they often cross and produce viable offspring with various aneuploid chromosome numbers. Weak reproductive barriers and existence of gene flow among individual cytotypes, together with two basic chromosome numbers ($x = 7, 8$), seem to be the reason for the karyological complexity of the species in Europe.

Based on our morphometric evaluation, we can confirm large morphological variation for this species in the Iberian Peninsula, already noticed by Montserrat-Recorder (1967) and Rico (1993), and known from other parts of Europe. Several attempts have been made to split this heterogeneous species and to recognize several separate taxa (e.g., *C. nemorosa*, *C. rivularis* auct. non Schur, *C. udicola*, *C. pratensis* subsp. *picra*, *C. pratensis* subsp. *major*; de Langhe & D'hose, 1976; Urbanska-Worytkiewicz & Landolt, 1974; Tomšovic, 1986). Resulting from previous studies (Landolt, 1984; Franzke & Hurka, 2000) and our observations, these cannot be regarded more than ecotypes or plasticity responses, without clear morphological and molecular differentiation. Morphometric comparison of different cytotypes of *C. pratensis* s.str. provided very similar results to those obtained by Marhold (1996). Although shifts in some characters at the populational level were found among the cytotypes, these, due to large overlap in the ranges, blurred at the level of individual plants. Several molecular markers used by Franzke & Hurka (2000) failed to resolve relationships and origin of different cytotypes of *C. pratensis* s.str. Neither the highly resolving AFLP-fingerprinting applied here has brought much more resolution, and thus, supported its current taxonomic treatment as a single species. Genetic affinity among geographically close populations of the same ploidy level was revealed, most likely reflecting current extensive gene flow over short distances. As suggested by genetic partitioning shown by AMOVA (Table 6), gene flow is restricted by both different ploidy levels and large geographic distances. Very low among-cytotype differentiation on the European scale, in contrast to higher differentiation among geographic regions, also indicates that the polyploids in *C. pratensis* s. str. might not have a single origin, but could have evolved independently and polytopically. Alternatively, such pattern could be attributed to gene flow across ploidy levels within regions. To support either of these hypotheses, however, much larger and detailed sampling across the whole distribution range would be needed.

Apart from *C. pratensis* s.str., populations resembling *C. dentata* have been reported from northern Spain (provinces Soria, Burgos, Navarra; Montserrat-Recorder, 1967; Rico, 1993). *Cardamine dentata* is a high polyploid ($2n = 7x$–$12x$) species distributed in central and northern Europe (Lövkvist, 1956). Although often misinterpreted by some authors (see Marhold, 1994a), morphologically it is well characterized by having all leaves pinnate with distinctly stalked and deciduous leaflets, and large white flowers (Marhold, 1994a). As we have not seen any herbarium specimens or plants in the field which would correspond to this taxon, and bearing in mind also its distribution, we find its occurrence in Spain as highly improbable.

***Cardamine crassifolia*, an endemic diploid species from the Eastern Pyrenees. —** From our herbarium and field studies, *C. crassifolia* should be regarded as a narrow endemic confined to the Eastern Pyrenees. The westernmost locality documented seems to be in Andorra, since westward from that, in the Central and Western Pyrenees, only diploid to pentaploid populations of *C. pratensis* s.str. occur (Table 1, Fig. 1, Appendix). Further records for *C. crassifolia* have been reported from various central Iberian mountains (Mateo Sanz & al., 1994); these, however, refer to the herein described new species *C. castellana* (Table 1, Fig. 1, Appendix). A similar distributional pattern as in *C. crassifolia* occurs also in another Iberian taxon, namely *C. amara* subsp. *pyrenaea* Sennen (Lihová & al., 2000). These two taxa, both diploids, have similar ecological requirements and co-occur in some localities. Most recently, even a sterile hybrid between them has been documented from two sites (Marhold & al., 2002).

A relic position of *C. crassifolia* within the *C. pratensis* group has already been proposed by Lövkvist (1956) on the basis of its distinct morphology and distribution. Recent molecular study combining several markers (Franzke & Hurka, 2000), as well as our AFLP data favour this view, as they reveal *C. crassifolia* as a genetically well-differentiated taxon, possibly representing one of the basal lineages of the *C. pratensis* group. The evolutionary history of this polyploid complex, however, might be even more complicated than presented by Franzke & Hurka (2000). Our preliminary molecular studies on polyploid *C. raphanifolia* (sensu *Flora Europaea*) show that it stands very close to the *C. pratensis* group (Lihová & al., submitted a). It seems that neither the *C. raphanifolia* nor *C. pratensis* groups, as currently understood, are monophyletic, and their evolution and diversification have involved taxa from both groups.

The name *C. crassifolia* was first published by Pourret in 1788. A detailed description of this taxon was provided by Sennen, under the name *C. nuriae* Sennen (Sennen, 1917), and later *C. pratensis* subsp. *nuriae* (Sennen) Sennen (Sennen, 1929). Following Sennen's treatment, some authors have preferred subspecific level for this taxon, and this status was adopted in *Flora Europaea* (Jones & Akeroyd, 1993) as *C. pratensis* subsp. *crassifolia* (Pourr.) P. Fourn., as well as in *Flora Iberica* (Rico, 1993) under *C. pratensis* subsp. *nuriae*, having priority against the former name (for full nomenclatural account see Marhold & al., 2002). However, on the basis of clear molecular and morphological differentiation of this taxon, we prefer to keep it at the level of species, as also treated by previous authors studying the group in more detail (Lövkvist, 1956; Urbanska-Worytkiewicz & Landolt, 1974; Landolt, 1984; Franzke & Hurka, 2000).

***Cardamine castellana*, the newly described Iberian species. —** Populations occurring at higher altitudes in the central Iberian mountains (Sierra de Gúdar, Sierra de Albarracín, Sierra de Gredos, Sierra Segundera, and also Cordillera Cantabrica) have been included within *C. crassifolia* (Rico, 1993; Mateo Sanz & al., 1994). However, detailed morphological and molecular analyses reveal that these populations differ from *C. crassifolia* from the Eastern Pyrenees, and therefore deserve formal taxonomic recognition. This new taxon, *C. castellana*, shows interesting patterns of morphological variation. In most quantitative characters it resembles more *C. crassifolia*, whereas when examining morphology of rhizome and basal parts of the stem, it exhibits mixed characteristics of *C. crassifolia* and *C. pratensis* s.str. (Fig. 6). In the ITS sequence tree (Franzke & Hurka, 2000, under *C. crassifolia*) it appeared closer to *C. crassifolia* than to *C. pratensis* s.str., as did also in the neighbour-joining tree of AFLP data (Fig. 11). From a taxonomical point of view, the critical question is whether to adopt a specific or subspecific rank for this taxon. Both the genetic differentiation between *C. crassifolia* and *C. castellana* (revealed by the high between-taxon differentiation in AMOVA and documented also by several exclusive AFLP fragments in each of these two taxa, as opposed to the few they share) and differences in morphology show that they represent two well defined and distinct diploid taxa. To keep balanced and consistent taxonomic treatment of the *C. pratensis* group through the whole distributional range, and considering also the fact that it, although always treated and accepted as a group, might not be monophyletic (Lihová et al., submitted a), we find the species rank for *C. castellana* appropriate. Strong genetic differentiation revealed between populations from Sierra de Gredos and Sierra de Albarracín most probably indicates long-term isolation and absence of gene flow between these two mountain ranges, but without any morphological differentiation.

Although morphological and molecular data currently available do not provide sufficient resolution for inferring the origin and evolutionary history of *C. castellana*, two hypotheses might be suggested. The first is that *C. crassifolia* and *C. castellana* represent two closely related lineages, which have diverged from a common ancestor. They might have undergone morphological and genetic diversification due to their long geographic isolation and adaptation to different abiotic conditions. Alternatively, intermediacy in morphology may indicate hybrid origin of *C. castellana* between diploid *C. pratensis* s.str. and *C. crassifolia*. More insights into the genomic constitution of *C. castellana* and its close relatives are obviously needed.

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Appendix. List of studied specimens of the *Cardamine pratensis* group in the Iberian Peninsula. Localities are given in a shortened way. A list including full information as given on herbarium labels is available on request from the corresponding author. *morphologically problematic specimens; for details see discussion.

***Cardamine pratensis* L. s.str.**: **Spain. prov. Álava:** Villareal de Alava, Elosu, 1985, *Alejandre s.n.* (MA 339703); Arcabuztaiz, Beluntza, 1985, *Alejandre s.n.* (MA 339704); Álava, Miñaur-Landeta, 1983, *Morante & Uribe-Echebarria s.n.* (MA 523411); **prov. Ávila**: Del monte de Siones, *Clemente s.n.* (MA 47511); Larra, Roncal Alto, 1967, *P. Montserrat s.n.* (ZT); **prov. Asturias**: Mon, San Martín de Oscos, 1994, *Aedo, Aldasoro & Muñoz s.n.* (MA 539474); Oviedo, Beas, Puerto de la Espina, 1976, *Galiano* & al. *s.n.* (MA 201370); Entre Aviles y el Cabo de Peñas, 1973, *Díaz & Navarro s.n.* (MACB 3630); Cudillero, Cabo Vidio, 1992, *C. Aedo s.n.* (MA 624188); Valle de Belmonte en Asturias, *Rivas Goday s.n.* (MAF 83337); San Claudio, 1981, *Aedo s.n.* (MA 622525); Soto de Ribera, Concejo de Ribera de Arriba, 1984, *Aliseda s.n.* (MA 416050); Valle de Belmonte, 1970, *Rivas Goday s.n.* (MAF 83337); Villaviciosa, 1952, *Guinea s.n.* (RNG); Villaviciosa, mt. Calvera, 1952, *Guinea s.n.* (RNG); **prov. Burgos**: Cordillera Cantábrica, N of Puerto del Escudo, 1980, *Miles, Phillipson, Sinnreich & Williams s.n.* (RNG); Burgos, Puerto del Escudo, *Fernández Diez s.n.* (MA 204696); Cañon del rio Ubierna, 1984, *Galán & Martin s.n.* (MACB 36315, MA 465122); Burgos, Pantano del Ebro, 1969, *P. Montserrat s.n.* (JACA 314669, ZT); Burgos, Puerto del Escudo, 1976, *Amich & Fernández Diez s.n.* (SALA 8522); **prov. Cantabria**: Laredo, 1984, *Herrera s.n.* (BIO 0030); Merón, San Vicente de la Barquera, 1984, *Aedo s.n.* (MA 622547); Ramales de la Victoria, 1995, *Carrasco s.n.* (MA 556225); Cabezón de la Sal - San Vicente de la Barquera, 1994, *Pulgar s.n.* (SANT 29853); Ojambre, El Tejo, 1980, *Amich & Sánchez s.n.* (SALA 23479); Villacantid, 1989, *Valle* (SALAF 82384); Puerto de Palombera, 1926, *Font Quer s.n.* (BC 111461); **prov. A Coruña**: Carnota, A Curra - Panchés, 1995, *Louzán s.n.* (BIO 23027, MA 565153, SANT 32502); Carnota, A Curra, 1995, *Louzán s.n.* (MA 581174, SANT 35957); Dumbria, Ezaro, 1994, *Louzán s.n.* (SANT 35956); Valle del rio Tambre, 1966, *Rivas Goday* (MA 47529, MAF 17055); Oleiros, 1979, *Fernández Diez s.n.* (MA 224241, SALA 19427, BC 630678); Santiago, 1944, *Figueroa Agea s.n.* (SANT 01410); Ribeira, Parque Natural "Complexo Dunar de Corrudedo e lagoas de Carregal y Vixán", 1993, *Pulgar s.n.* (SANT 38711); Muxia, entre Frixe e Nemiña, 1994, *Louzán s.n.* (SANT 40435); Monfero, entas Fragas de Eume, 1992, *Amigo & Nornan s.n.* (SANT 28101); Carnota, Monfero, Taboada, Fraga de Caaveiro, 1994, *Soñora Gómez s.n.* (SANT 37623); Ortigueira Mera, Barranco Vilariño, 1994, *Soñora Gómez s.n.* (SANT 37602); Fistera, Rostiro, 1994, *Louzán s.n.* (SANT 40606); **prov. Guipuzcoa**: Alkiza, 1989, *Biurrun s.n.* (BIO 26646); San Sebastián, 1967, *Merxmüller & Oberwinkler s.n.* (M); Oleabzu, 1895, *Gandoger s.n.* (Flora Hispanica exsiccata, No. 29) (MA 47528); Hernani, 1895, *Gandoger s.n.* (Flora Hispanica exsiccata, No. 41) (MA 47527, COI); Pasajes, 1895, *Gandoger s.n.* (Flora Hispanica exsiccata, No. 37) (MA 47515, COI); Azkoitia, Asunción Epelde, 1992 (SANT 27016); Vergara am Puerto des Carga, 1967, *Scholz & Hiepko 1046* (B); Monte del Castillo de la Nola en S. Sebastián, 1875, *Mansferrer s.n.* (BC 03106); **prov. Huesca**: *Cotiella, 1969, *Fernández Casas s.n.* (MA 410447, 409900); *Liri, Solana del Gallinero, 1992, *Fernández & Sesé s.n.* (JACA 331492); Benasque, Peña Blanca, 1994, *P. Montserrat & Bevington s.n.* (JACA 96494); *Castanesa, Orillas del Lago de Bacibé, *P. Montserrat & Dussaussois s.n.* (JACA 162382); *San Juan de Plan, San Mamés-Fene Mayor, 1981, *P. Montserrat* & al. *s.n.* (JACA R115267); *Cerler, Empriu, 1989, *P. Montserrat s.n.* (JACA 146089); **prov. León**: Ponferrada, Montes de Valdueza, 1979, *Temprano 287ET* (MA 314934); Tal des Rio Esla nördl. Cistierna, 1967, *Scholz & Hiepko 955* (B); **prov. Lérida**: *Muntanya de Llacs, pr. Boí, 1944, *Bolòs & Font Quer s.n.* (BC 94601); Vall d' Aran, Lés, 1954, *Bolòs s.n.* (BC 140163); **prov. Lugo**: Quiroga, 1981 (SANT 15195); Monforte, 1908, *Gandoger s.n.* (COI); **prov. Madrid**: Miraflores de la Sierra, 1976, *Fernández Diez s.n.* (SALA 8627); Sierra Guadarrama, Pto. de los Cotos, 1971, *Merxmüller & Gleisner s.n.* (M); Sierra de Guadarrama, inter Puerto de Cotos & al Paular, 1976, *Fernández Casas*

1113 (MA 394490); Sierra de Guadarrama, Cercedilla, 1912, *Vicioso & Beltrán s.n.* (MA 47503); Sierra de Guadarrama, Cercedilla, 1914, *Vicioso* (BC 03115, BC 03116, MA 47502); Somosierra, *Cutanda s.n.* (MA 47505); Cercedilla, 1968, *Irco Cadero s.n.* (MA 315521); Sierra Guadarrama, *Vicioso s.n.* (MA 47517); Guadarrama, *Lagasca s.n.* (MA 47504); Braojos (Madrid), 1918, *Vicioso s.n.* (MA 47501, BC 03111); El Escorial (Madrid), 1843, *Rodríguez s.n.* (MA 47506); Los Peñascales, 1942, *Guinea s.n.* (MA 321384); Paular - los Cotos, 1968, *P. Montserrat s.n.* (ZT); Puerto Los Cotos, 1968, *P. Montserrat s.n.* (JACA 226868); **prov. Navarra**: Pto. des Roncesvalles de Ibaneta, 1971, *Merxmüller & Gleisner s.n.* (M); Sierra Aralar, 1953, *Merxmüller & Wiedmann 874/53* (M); Idurrieta, cerca de Jaurrieta, 1994, *Nieto Feliner 3375GN* & al. (MA 545035); Baztán, Pto. Artesiega, barranco Olazas, 1980, *Aldezabal* & al. *s.n.* (MA 505704, BIO 7902); *Quintorreal, Collado de Sagardegui, 1982, *Belmonte s.n.* (MA 328131); Isaba, Túnel de Eraice, 1993, *P. Montserrat & Cernoch s.n.* (JACA 29093); Larra, Dolina, *P. Montserrat s.n.* (JACA 473367); Contrasario, Ochagavia, Irati, 1987, *Villar & G. Montserrat s.n.* (JACA 0116387); Ulzama, 1965, *P. Montserrat s.n.* (JACA 9165); Irurzun, río Araquil, 1970, *P. Montserrat s.n.* (JACA 71570); Huarte-Araquil, 1978, *P. Montserrat & Bascones s.n.* (JACA 30578); valle de Ulzana supra Pamplona, 1966, *P. Montserrat 16* (ZT); Urbasa, Segatos, 1956, *P. Montserrat s.n.* (ZT); Echarri-Aranaz, 1990, *Olano s.n.* (BIO 21463); **prov. Orense**: Verín. Rabal. Rio Tamega, 1987, *Rico & Giráldez s.n.* (MA 466936, MACB 32820, SALA 47079); Parada do Sil, entre Casuia e Baledo, 1996, Amigo, Ortiz, *Louzán 762 & Marisa* (SANT 034648); Allariz, 1981, Amich, Giráldez, *Rico & Sánchez s.n.* (SALA 32381); Villaviciosa, 1989, *Amor & Giráldez s.n.* (SALAF 85566); **prov. Pontevedra**: Villagarcía, 1972, *Valdés Bermejo s.n.* (MAF 82404); La Guardia, 1899, *Merino s.n.* (MA 47522); San Adrián, Vilaboa, 1970, *Castroviejo s.n.* (MA 196798); Bueu, Monte Ermelo, 1986, *García Martínez s.n.* (MA 332066); Mirador de Catoira, 1969, *Valdes s.n.* (MA 308739); El Grove, prope Pedras Negras, entre el Istmo de la Lanzada y El Grove, 1994, *Carrasco, Pajarón & Silva-Pardo s.n.* (MA 621680); entre Cuntis y Portela, 1982, *Valdés Bermejo & Silva s.n.* (MA 529820); rio Deza, 1982, *Horjales & Redondo s.n.* (SANT); **prov. Segovia**: Cantalejo, 1974, *Segura Zubizarreta s.n.* (M); Prádena, La Callejuela, 1983, *Romero s.n.* (SALA 37249); Cantalejo, Navacentella, 1983, *Romero s.n.* (SALA 37248); Frumales, en el Vado de la Vaca, río Cega, 1982, *Bayón s.n.* (MA 321379); Sierra de Guadarrama, Pinar de Valsaín, 1913, *Vicioso s.n.* (MA 47519); Valsain, *Cutanda s.n.* (MA 47508); Fuente el Olmo de Fuentidueña, *Romero s.n.* (MA 567636); entre Cotos y Rascafría, 1974, *López & Valdés Bermejo s.n.* (MA 315409); Puerto de Somosierra subiendo de Riaza, 1974, *Bellot & al s.n.* (MA 315500); La Granja, 1992, *García Adá & Lopéz s.n.* (MA 562790); Santo Domingo de Pirón, 1987, *Egido & García s.n.* (MA 562791); **prov. Soria**: San Leonardo, 1935, *Ceballos s.n.* (MA 47507); **prov. Teruel**: Bronchales, Sierra Alta, 1962, *Kjellqvist & Löve 511* (RNG); prope Teruel, *López Seoanez s.n.* (MA 47525); **prov. Valladolid**: Rábano, 1983, *Romero s.n.* (SALA 41872); **prov. Vizcaya**: Lekeitio, 1983, *G. Montserrat* & al. *s.n.* (BIO 1570); Mekoleta, Ochandiano, 1983, *Gomez s.n.* (BIO 1569); Bilbao, 1906, *Sennen s.n.* (BC-Sennen); **prov. Zamora**: Ribadelago, borde del Camino por Seoanez, 1987, *Roa s.n.* (MA 510717); Ribadelago, 1948, *Losa & P. Montserrat s.n.* (BC 114205); Olleros de Tera, Pradera Redonda, 1989, *García Río s.n.* (SALA 56243); **Portugal. Beira Litoral**: Eixo, Arrujo, 1965, *A. Fernandes & al. s.n.* (M, COI); Aveiro, Eixo, 1954, *J. & al. s.n.* (COI); Aveiro, Requeixo, Pateira de Fermentelos, 1978, *Marques s.n.* (COI); Ilhavo, zminta da Valenta, 1954, *J. Matos, A. Matos & Marques s.n.* (COI); Arredores de Montemór-o Volho, Fôja, 1911, *Carvalho s.n.* (P, COI); Mata de Fôja, 1942, *Lemos s.n.* (COI); Aveiro, Azurara, 1954, *J. Matos & al. s.n.* (COI, P); Vilarinho da Lousa, Soito, Águas de Albi, 1961, *A. Fernandes & al. s.n.* (P, COI); Arredores da Figueira da Foz, Villa Verde, 1890, *Moller s.n.* (P); Serra de Nogueira, aldeia de Nogueira, 1990, *Sequeira & Coelho 68* (MA 557314); pr. Águeda, ponte de Perraes, 1953, *Romariz & Mendes s.n.* (MA 268571); Aveiro, Águeda, em frente de Espinhel, Pateira de Fermentelos, 1978, *Marques s.n.* (COI); Aveiro, Águeda, Fermentelos, arredores do Porto da Minhoteira, 1977, *Marques & Pereira s.n.* (COI); Aveiro, Águeda, Fermentelos, entre Porto d' Asna, Sorgacal e Porto da Minhoteira, 1977, *Marques s.n.* (COI); Eirol, entre Aveiro e Águeda, 1965, *A. Fernandes & al. s.n.* (COI); Eixo, próximo de Eirol, 1969 *s.coll.* (COI); Ponte, da Azurva, 1965, *A. Fernandes, R. Fernandes & Paiva* (COI); Aguada de Baixo, 1965, *A. Fernandes, R. Fernandes & J. Matos s.n.* (COI); Águeda, Ribeira de Ferreiros, pr. Recardaes, 1958, *Santos s.n.* (COI); Rio Novo, 1892 (COI); Paul de Arzila, *Fernandes Costa s.n.* (COI); proximo do Paul d' Arzila, 1932, *de Lousa s.n.* (COI); Miranda do Côrvo, Gudinhela, 1943, *de Lousa s.n.* (COI); Serra da Louza, 1883, *Henriques s.n.* (COI); Arredes de Coimbra, 1880, *Ferreira s.n.* (COI); Alfaleros, 1929, *Carrisso & Mendonca s.n.* (COI); entre a Ponte de Varela e S. Yacinto, *s.coll.*, 1980 (COI); Coimbra, Arzila, 1988, *Cristina & Lopez s.n.* (COI); **Douro Litoral**: Porto, Matosinhos, 1889 (COI); Leca do Bailio, 1883, *Casimiro Barbosa s.n.* (COI); Porto, Ribeiro d' Avintes, 1888, *Leite s.n.* (COI); Vergas (próximo de Mira), 1970, *A. Fernandes & al. s.n.* (COI); Vergas Cabecinhas, Galsáz, 1973, *A. Fernandes & al. s.n.* (COI); Leca de Palmeira, Boa Nova, 1977, *Malato-Beliz 13231 & Guerra* (M); **Estremadura**: Serra de Cintra, 1840 (COI); **Minho**: Braga, Pico de Begahados, 1909, *Ferreira s.n.* (COI); Arredes de Braga-Monte do Crasto, 1883, *Lequeira s.n.* (COI); Serra de Geraz, 1918, *Felgueiros s.n.* (COI); entre Valenca e Sao Pédro da Torre, Veiga da Mira, 1946, *da Lilva s.n.* (COI); Vila Verde, Cabarelas, 1970, *A. Fernandes, R. Fernandes & J. Matos s.n.* (COI); Póvoa de Lanhoso, 1894, *Sampaio s.n.* (COI); **France**. Basses Pyrenees, Cambo les Bains, Arnaga - La Nive, 1972, *P. Montserrat s.n.* (ZT); Pyrenees Occidentales, Pau, 1991, *Nicol, Lys 64260 & Arudy* (ZT); Pyr. Atlantiques, Vers Taron, 1991, *Nicol, Lys & Arudy s.n.* (ZT); Pyrénées, Les Eaux Bonnes, *Rowland, Davis, Hyan* & al. *s.n.* (RNG); Pyrenees centrales, Bagnéres, de Luchon, Bourg d'Oueil, 1992, *Castroviejo 12050* & al. (MA 512382).

***Cardamine crassifolia* Pourr.**: **Spain**. Catalogne, Pyrénées à Núria, 1913, *Sennen s.n.* (BC-Sennen 805517); Gerona, Queralbs, vall de Núria, Nou Creu, 1993, *Monasterio* & al. *s.n.* (MA 528891); Catalogne, Pyrénées à Núria, 1914 (MA 47513, BC-Cadevall 816244); Nuria, come de les Mulleres, 1921, *Barnades s.n.* (BC 603665); Cerdanya, Pr. de La Llagona, 1960, *A. de Bolòs & E. de Bolòs s.n.* (BC 602596); In Cataloniae Pyrenaeis, Núria, Nou Fonts, 1943, *A. de Bolòs & E. de Bolòs s.n.* (BC 111454); Núria, Vall de les Mollera, 1958, *Vigo, Villar, Recabado* & al. *s.n.* (BC 601500); Núria, in Pyrenaeis, 1919, *Larriga s.n.* (BC 111460); In Pyrenaeis Cataloniae, Núria, 1880, *Mansferrer s.n.* (BC 03107); In Pyrenaeis Cataloniae, supra Meranges, 1950, *Capell* (BC 114241); **France**. Pyrénées Orbs., Val d'Eyne, 1919, *Sennen s.n.* (BC-Sennen); Cerdagne, Font Romeu, 1928, *Sennen s.n.* (BC-Sennen); Pyrénées Orbs., Le Capcir à Camporreils, 1919, *Sennen s.n.* (BC-Sennen); Cerdagne aux Bouillouses, 1916, *Sennen s.n.* (BC-Sennen); Cerdagne, Vallée d'Angoustrine, 1919, *Jude-Marie s.n.* (MA 47509); Cerdagne, Vallée d'Angoustrine, 1919, *Sennen s.n.* (BC-Sennen); Cerdagne, Val de Llo, 1923, *Sennen s.n.* (BC-Sennen); Espoussouille (Capcir, V. de Galve), 1983, *P. Montserrat & Villar* (MA 257466); Ariège, Etang de Llaurenti, 1984, *Landolt s.n.* (ZT); Pyrenees orient., Val de Galbe, P. del Pla de l'Ouriet, 1984, *Landolt s.n.* (ZT); Dept. Pyrénees Orientales, Lac de Bouillouses, Et. del Vivé, 1971, *Merxmüller & Zollitsch s.n.* (M); **Andorra**. Andorra, 1992, *Aedo* & al. *s.n.* (MA 523560).

***Cardamine castellana* Lihová & Marhold**: **Spain. prov. Asturias**: Puerto de Somiedo entre León y Asturias, 1970, *Rivas Goday & Izco s.n.* (MA 22424); **prov. Ávila**: Sierra de Gredos, Hoyos del Espino - Refugio del Rey 1971, *Merxmüller & Gleisner s.n.* (M); Piedrahita, 1983, *Valle & Iglesias s.n.* (SALAF 72684); Hoyo del Espino, 1983, *Ladero & Iglesias s.n.* (SALAF 65070); Puerto de la Peña Negra, 1982, *Rico s.n.* (SALA 26083); Pinar de Hoyocasero, 1987, *Giráldez & Rico s.n.* (SALA 56244); **prov. Teruel**: Orihuela del Tremedal, 1907, *Pau s.n.* (MA 47518); **prov. Zamora**: Porto, Valle del río Bibey, 1989, *Casaseca, Rico & Giráldez s.n.* (MA 466933); Porto, Sierra Segundera, Moncalvillo, 1993, *Aldasoro, Martínez Ortega, Pérez & Rico s.n.* (SALA 56245); San Martín de Castañeda, 1978, *Fernández Diez, Rico & Sánchez s.n.* (SALA 25150); Porto, Valle del río Bibey, 1989, *Casaseca & al. s.n.* (SALA 47050).