Taxonomy of *Cardamine amara* (*Cruciferae*) in the Iberian Peninsula

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Summary

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Cardamine amara is represented in the Iberian Peninsula by two subspecies: the diploid (2*n* = 16) subsp. *pyrenaea*, occurring in the Eastern Pyrenees, and the tetraploid (*2n* = 32) subsp. *olotensis*, concentrated at the lower altitudes in Catalonia, N and NE of Barcelona. Morphometric analysis of populations of these taxa and four other subspecies of *C. amara*, which occur in other parts of Europe, supported their subspecific distinctness. RAPD (Random Amplified Polymorphic DNA) analysis of all six European subspecies of *C. amara* provided more evidence supporting this taxonomic treatment. Lectotypes were designated for the following names: *C. amara* var. *parviflora* Cadevall, *C. amara* subsp. *pyrenaea* Sennen, *C. amara* subsp. *siifolia* Sennen, and *C. amporitana* Sennen & Pau.

Keywords: *Cardamine*, chromosome numbers, Iberian Peninsula, morphometric analysis, RAPD, taxonomy.

Introduction

Cardamine amara L. is widespread in Europe extending to Asia. Recent studies of populations of *C. amara* in the Carpathians, Balkan Peninsula and the Alps (Marhold, 1992, 1995, 1999; Marhold & al., 1996) have shown considerable amount of karyological and morphological variation within this taxon. Four subspecies are currently recognised in Central Europe and the Balkan Peninsula: diploids (2*n* = 2*x* = 16): *C. amara* subsp. *amara*, widespread for most of the area of the species; *C. amara* subsp. *opicii* (J. Presl & C. Presl) Celak. from the Sudety Mts. and the Carpathians; *C. amara* subsp. *balcanica* Marhold et al., occurring in the mountains of SW Bulgaria and NE Greece; and tetraploid (2*n* = 4*x* = 32) *C. amara* subsp. *austriaca* Marhold from the Eastern Alps and neighbouring areas.

The occurrence of *C. amara* in the Iberian Peninsula is restricted to the Eastern Pyrenees (the westernmost localities are in Andorra) and to the lowland part of Catalonia N and NE of Barcelona (Rico, 1993). Populations from the lowland part of Catalonia were described as a separate taxon different from typical *C. amara* first by Sennen and Pau (Sennen, 1911) as *C. amporitana* Sennen & Pau, then by Cadevall y Diars (1915) as *C. amara* var. *parviflora* Cadevall, and finally by Bolòs (1952) as *C. amara* subsp. *olotensis* O. Bolòs. This taxon was mostly accepted, although Rico (1993) in *Flora Iberica* referred to it only in the note in the account of *C. amara*. L. E. Rico & J. J. Aldasoro (Rico, *in litt.*) determined a tetraploid chromosome number (2*n* = 32) for these plants from the Sierra del Montseny.

In 1929, Sennen described two subspecies of *Cardamine amara* from the Pyrenees, namely *C. amara* subsp. *pyrenaea* and *C. amara* subsp. *siifolia* (Sennen,

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1929). No other authors accepted these taxa. They were not mentioned, not even as synonyms in the recent edition of *Flora Iberica* (Rico, 1993), and populations of *C. amara* in the Pyrenees were treated in all local floras as belonging to the typical subspecies, *C. amara* subsp. *amara*. Only a few specimens from this area, deposited in BC, were identified by E. Vayreda y Vila at the end of the last century as "*C. amara* var. *opizii*".

For evaluation of the taxonomic position of Iberian populations of *C. amara*, morphometric methods, together with chromosome counts and study of the available Iberian herbarium material were used. As supplementary evidence, RAPD (Random Amplified Polymorphic DNA) analysis of all six European subspecies of *C. amara* was performed. RAPD is a PCR (Polymerase Chain Reaction) method using single randomly chosen ten-base primers and is based on the assumption that nuclear genomes contain loci where the primers can anneal to the opposite DNA strands at a distance that allows efficient PCR amplification of the DNA between them. Reviews concerning advantages and problems of this method were provided by, e.g., Bachmann (1997) and Parker & al. (1998).

Material and methods

Plant material. — Morphometric evaluation is based on population samples of 20–40 plants collected in the field and deposited in SAV. Iberian material consisted of three population samples of *C. amara* subsp. *olotensis*, altogether 101 plants, and eight population samples of *C. amara* from the Eastern Pyrenees, corresponding to both subsp. *pyrenaea* and subsp. *siifolia* as described by Sennen (1929). Altogether 238 plants were used (see Table 1 for localities). Extra-Iberian material used for morphometric analyses included population samples and data on morphology available from previous studies by the second author (Marhold, 1998, 1999), where also further details on this material can be found (including chromosome numbers): (1) ten population samples of *C. amara* L. subsp. *amara* ($2n = 16$) from the Czech Republic, Slovakia, Poland, Austria, Ukraine, and Romania (altogether 380 plants, populations no. 1, 13, 25, 33, 38, 46, 54, 55, 56, 58 cited by Marhold, 1998: 30–31); (2) ten population samples of *C. amara* subsp. *opicii* (J. Presl & C. Presl) Celak. from the Czech Republic, Slovakia, Poland, and Ukraine (altogether 378 plants, populations no. 4, 16, 20, 21, 22, 23, 28, 40, 50, 51 cited by Marhold, 1998: 31); and (3) ten populations of *C. amara* subsp. *austriaca* Marhold from the Czech Republic and Austria [altogether 368 plants from the following localities: Czech Republic: Horní Vltavice (840 m); Kvilda (1040 m); Austria: Ybbstaler Hütte (1360 m); Raxalpe (1400 m); Oberst Klinke Hütte (1480 m); Stuhleck (1196 m); Speiksee (1940 m); Schoberbach (2050 m); Fraganter Hütte (1780 m); and Sadnigbach (2130–2150 m); for details about localities see Marhold, 1999: 79–81]. Bulgarian populations of *C. amara* subsp. *balcanica* Marhold et al. were not included in these comparative studies as they are clearly morphologically different from the Spanish plants (cf. Marhold, 1998).

Table 1. List of populations of *Cardamine amara* subsp. *olotensis* and *Cardamine amara* subsp. *pyrenaea* from the Iberian Peninsula used for karyological, morphometric and RAPD analyses. x = used in the particular analysis, - = not used. SP = Spain, Catalonia; A = Andorra; *M* = Marhold; *V* = Vicens. Voucher specimens are deposited in SAV.

Origin and collection data	Analysis		
	2*n*	morph.	RAPD (population code)
***Cardamine amara* subsp. *olotensis* O. Bolòs**			
SP: Olot, Paratges de la Deu, ca. 400 m, 10 May 1996, *M, Benedí & V s.n.*	32	x	L-66-OL2
SP: Olot, Parc Nou, ca. 400 m, 10 May 1996, *M, Benedí & V s.n.*	32	x	L-66-OL1
SP: Parc natural del Montseny, 1 km E of Santa Fe del Montseny, 1130 m, 11 May 1996, *M & Giráldez s.n.*	32	x	L-45-SFE
***Cardamine amara* subsp. *pyrenaea* Sennen**			
A: Grau Roig, close to Hostal Refugi de Cabana Roja, side tributary of Riu La Valira, 2095 m, 28 Aug 1996, *M & V s.n.*	16	-	S-89-CRJ
SP: Cerdanya, Martinet, Aransser, Estació d'esquí nòrdic d'Aransser, Prat Miró, 1950 m, 28 Aug 1996, *M & V s.n.*	16	-	-
SP: Cerdanya, near the road from Ribes de Freser to Puigcerdà, NW of the border of the regions of Ripollès and Cerdanya, ca. 0.75 km E of Casilla de Saltèguet, near the branch off the road to Baga de Saltèguet, 1725 m, 28 Aug 1996, *M & V s.n.*	16	-	-
SP: Near the mountain road from Tregura de Dalt (near Vilallonga de Ter) to Ribesaltes (near Ribes de Freser), near the rivulet Torrent de Meianell, 1985 m, 23 Aug 1996, *M & V s.n.*	16	-	-
SP: Ripollès, Queralbs, Coma de Freser, near the bridge over the Torrent de Freser, 1560 m, 7 Jul 1997, *M s.n.*	16	-	S-23-VAM
SP: Ripollès, Queralbs, Font de l'Home Mort, 1850-1970 m, 5 Jul 1997, *M s.n.*	16	x	S-14-HMO
SP: Ripollès, Queralbs, Núria, 2000 m, 26 Aug 1996, *M s.n.*	16	-	-
SP: Ripollès, Queralbs, Núria, below the junction of the valleys of Coma de Noucreus and Coma de Noufonts, 2130 m, 6 Jul 1997, *M s.n.*	16	x	-
SP: Ripollès, Queralbs, Núria, Coma d'Eina, 2065-2105 m, 8 Jul 1997, *M s.n.*	16	x	S-27-COE
SP: Ripollès, Queralbs, Núria, Coma de Noucreus, 2290-2310 m, 6 Jul 1997, *M s.n.*	16	x	-
SP: Ripollès, Queralbs, Núria, Coma de Noufonts, 2440 m, 25 Aug 1996, *M s.n.*	16	x	-
SP: Ripollès, Queralbs, Núria, Torrent de Finestrelles, 2140 m, 26 Aug 1996, *M s.n.*	16	-	-
SP: Ripollès, Queralbs, Planell de les Eugues, close to Refugi Manelic, near the rivulet el Freser, 1975 m, 24 Aug 1996, 7 Jul 1997, *M s.n.*	16	x	S-15-MN1
SP: Ripollès, Setcases, close to Refugi d'Ulldeter, NW of Pla dels Hospitalets, 2130 m, 23 Aug 1996, *M & V s.n.*	16	-	-
SP: Ripollès, Setcases, Pla dels Hospitalets, near the curve of the road, near the rivulet Clot de Coma Ermada, 1925 m, 23 Aug 1996, *M & V s.n.*	16	x	-
SP: Ripollès, source of the rivulet of Torrent de Fontalba, 2080 m, 8 Jul 1997, *M s.n.*	-	x	-

Table 2. List of extra-Iberian populations of *Cardamine amara* used for the RAPD analysis. Data on chromosome numbers with references are taken from previous papers, the others represent new reports (voucher specimens in SAV). *M* = Marhold.

Origin and collection data	2n	RAPD population code
Cardamine amara* subsp. *amara		
Austria: Lower Austria, Wechselgebiet, along the rivulet Leidingbach S of village Erlach, E of Leiding, 360 m, *Vitek s.n.*	16 (Marhold, 1999: 82)	A-61-WCH
Czech Republic: Trebonská pánev, Chlum u Trebone, nearby fishpond Podsedek, 430 m, *M s.n.*	16 (Marhold, 1999: 83)	A-12-RY1
Slovak Republic: Nízke Tatry Mts, close to the village of Gašparovo, 540 m, *M s.n.*	16	A-07-GAS
Slovak Republic: Slovenské rudohorie Mts, Drábsko, 980 m, *M s.n.*	16	A-83-DRB
Slovenia: Notranjsko, Loška dolina, river banks of Veliki Obrh, W of the village of Pudob, 570 m, *M s.n.*	16 (Marhold, 1999: 83)	A-20-PUB
Cardamine amara* subsp. *austriaca		
Austria: Salzburg, Mt. Hochkönig nearby the village Dienten, track from Erichhütte to Stegmoosalm, 1590 m, *Vitek s.n.*	32 (Marhold, 1999: 80)	T-60-HCH
Austria: North Tyrol, St. Anton am Arlberg, Steissbachtal, 1800 m, *Brandstätter, Chrtek & Mráz s.n.*	32 Marhold, 1999: 80)	T-78-STS
Slovenia: Štajerska, Pohorje, between Vuhred and Ribnica na Pohorju, 530 m, *M & Jogan s.n.*	32 (Marhold, 1999: 81)	T-50-ANT
Cardamine amara* subsp. *balcanica		
Bulgaria: Pirin Mts, Bansko, near rivulet Glazne, 1050 m, *M & Ancev s.n.*	not analysed	B-79-BAN
Bulgaria: Pirin Mts, Demjaniška poljana, 1620 m, *M & Ancev s.n.*	16 (Marhold & al., 1996: 203)	B-81-DMP
Bulgaria: Pirin Mts, near Demjanica chalet, 1850 m, *M & Ancev s.n.*	16 (Marhold & al., 1996: 203)	B-41-DM
Bulgaria: Rila Mts, between Borovec and Sitnjakovo, 1600 m, *M & Ancev s.n.*	16 (Marhold & al., 1996: 203)	B-80-SIT
Bulgaria: Vitoša Mts, Železnica, 1100–1150 m, *M s.n.*	16	B-74-ZLZ
Cardamine amara subsp. opicii		
Slovak Republic: Kremnické vrchy Mts, above the Kordícke sedlo Saddle, 1130 m, *M s.n.*	16 (Marhold, 1994: 29)	O-64-KRE
Slovak Republic: Slovenské rudohorie Mts, S of the village of Úhorná, between the mountains of Malá Pipitka and Pipitka, 875 m, *Mráz s.n.*	16	O-59-PIP
Slovak Republic: Slovenské rudohorie Mts, source of the rivulet Starovodský potok, S slopes of Mt. Hekerová, 1180 m, *Mráz s.n.*	16	O-75-HEK
Slovak Republic: Vysoké Tatry Mts, Malá Studená dolina Valley, 1595 m, *M s.n.*	16	O-03-MS1
Slovak Republic: Vysoké Tatry Mts, Malá Studená dolina Valley, 1630 m, *M s.n.*	16	O-49-MS2
Slovak Republic: Vysoké Tatry Mts, Velická dolina Valley, 1950 m, *M s.n.*	16 (Marhold, 1992: 134)	O-44-VEH
Slovak Republic: Západné Beskydy Mts, Mt. Babia hora, S slope, 1450 m, *Janovicová, Somogyi & Mráz s.n.*	16	O-22-BAB
Ukraine: Cornohora Mts, Mt. Hoverla, W slope, 1740 m, *Mráz s.n.*	16 (Marhold, 1994: 30)	O-99-HOV

For RAPD analyses, 29 individuals (each representing one population) of all six subspecies of *C. amara* were used. For localities and chromosome numbers see Tables 1, 2. Distribution of Iberian taxa: herbarium specimens from BC, BCC, BCF, BM, HGI, MA, MAF, and P were consulted.

Morphometric analyses. — In order to reveal morphological relations of both Pyrenean and lowland Catalonian populations with other taxa of *C. amara* from different parts of Europe, basic statistics, canonical discriminant analysis, and parametric and k-nearest-neighbour non-parametric classificatory discriminant analyses of morphological characters were performed (Klecka, 1980; SAS Institute, 1990b). The non-parametric classificatory discriminant analysis was used, because the distribution of most characters deviated from normal. In both discriminant analyses individual plants were used as objects and different subspecies (delimited by their chromosome numbers and the area of occurrence) as groups. The following nine characters were measured or scored for each plant: width of stem (WS), mm; number of stem leaves (NL); maximum number of leaflets of leaves in the upper 4/5 of stem (NLL); degree of congestion of leaves beneath the inflorescence, expressed by the number of leaves reaching the base of the uppermost stem leaf (NLR); number of flowers (including buds) in the main inflorescence (NF); length of petals (LP), mm; width of petals (WP), mm; length of sepals (LS), mm; maximum length of filaments (LF), mm. Fresh floral parts were attached to adhesive tape and dried; other characters were measured on herbarium specimens. The character "branching of stem" used in previous studies (Marhold, 1992, 1998) was not included here, as it was invariable within the populations from the Pyrenees (no plant had a branched stem). It was, however, scored for all plants and it is considered in the discussion. All the above-mentioned analyses were performed using the SAS statistical package (SAS Institute, 1990a,b).

Chromosome numbers. — These were counted from mitotic plates in root-tips of plants taken from the localities (see Tables 1, 2) and cultivated at the Institute of Botany of the Slovak Academy of Sciences, Bratislava, Slovakia. The root tips were pre-treated with 0.002 M aqueous solution of hydroxyquinoline for 3 hrs, then fixed for 10 min to 24 hrs in a freshly prepared mixture of ethanol and acetic acid (3 : 1), hydrolysed for 5 min in a mixture of concentrated hydrochloric acid and ethanol (1 : 1), washed in water, and then stained with acetic or propionic orcein. Temporary slides were made by the squash method. Voucher specimens are deposited in SAV.

DNA-extraction. — DNA was extracted following the method of Doyle & Doyle (1987) modified for microcentrifuge tubes, from 50 mg frozen leaf material from the plants, taken from the field and cultivated in the greenhouse of the University of Osnabrück under uniform conditions.

RAPD. — PCR reactions were carried out in 50 µl volumes with 200µM each dNTP, 2 mM $MgCl_2$, 10 pmol primer (OPERON Technologies, Alameda, California, U.S.A.), 50 ng template DNA, 1× PCR reaction buffer (Gene Craft, Biotechnologies Transfer, Münster, Germany), and 0.75 U polymerase (Gene Craft). The PCR programme was: 44 cycles at 94°C/30s (first cycle 2 min), 36°C/30s with a ramping phase of 0.4°C/s, 72°C/1 min (last cycle 4 min). We used the thermocycler

Trio-Thermoblock (Biometra). Products were separated on 1.5% agarose gels in TBE buffer together with molecular weight ladder, stained with ethidium bromide and photodocumented on an UV bench. Out of 60 primers screened for the genus *Cardamine* (see Neuffer & Jahncke, 1997), 11 primers provided informative markers, namely B07, B11, B15, B20, H03, H05, H16, H19, R02, R13, and R14, (for the sequences see Neuffer & Jahncke, 1998). A neighbour-joining distance analysis (Saitou & Nei, 1987) including the bootstrap option (TREECON program package, Van de Peer & de Wachter, 1994) and principal coordinates analysis using Jaccard's coefficient (SYN-TAX 5.1 program package, Podani, 1997) were used for evaluation of data.

Results

Chromosome numbers. — The analysis of chromosome numbers of the plants from the Iberian Peninsula (see Table 1) confirmed the unpublished record by E. Rico & J.J. Aldasoro from the Parc natural del Montseny (Rico, *in litt.*) that plants described as *C. amara* subsp. *olotensis* are invariably tetraploid, with $2n = 32$. Plants analysed within the present study also included those from the *locus classicus* in the town of Olot (Parc Nou). The analysis of plants collected in the Eastern Pyrenees, in Spain and Andorra, revealed that these plants are diploid with $2n = 16$, like *C. amara* subsp. *amara*, *C. amara* subsp. *opicii* and *C. amara* subsp. *balcanica*.

Morphometric analysis. — The canonical discriminant analysis of individual plants of *C. amara* subsp. *amara*, *C. amara* subsp. *pyrenaea*, and *C. amara* subsp. *olotensis* showed that *C. amara* subsp. *pyrenaea* differs from *C. amara* subsp. *amara* with respect to the number of stem leaves, number of leaflets and congestion of leaves below the inflorescence (see Fig. 1 and Table 3). In addition to these characters, branching of stem can be used for the identification of these taxa. While *C. amara* subsp. *pyrenaea* has invariably simple, unbranched stems, *C. amara* subsp. *amara* can have either simple or branched stems.

The main character differentiating *C. amara* subsp. *olotensis* from all other subspecies of *C. amara* is the colour of the anthers, being yellow in subsp. *olotensis* and violet in other five subspecies. According to the canonical discriminant analysis, the length of sepals differentiates *C. amara* subsp. *olotensis* and *C. amara* subsp. *amara* (separated along the second canonical axis on Fig. 1), and some other characters contribute to this division as well (see Table 3).

Table 3. Canonical discriminant analysis of *C. amara* (see Fig. 1). Correlation coefficients of morphological characters and canonical axes (CAN1, CAN2). Those exceeding the value 0.6 are marked in bold.

CAN1	CAN2	Morphological characters
-0.0246	0.5801	width of stem (WS)
-0.3539	-0.3046	length of filaments (LF)
-0.0717	**0.7387**	length of sepals (LS)
0.5854	0.2908	width of petals (WP)
0.4841	0.3199	length of petals (LP)
0.2941	0.4807	number of flowers (NF)
-0.8127	-0.0216	number of leaflets (NLL)
-0.7523	0.4901	number of leaves (NL)
-0.7145	0.4913	congestion of leaves (NLR)

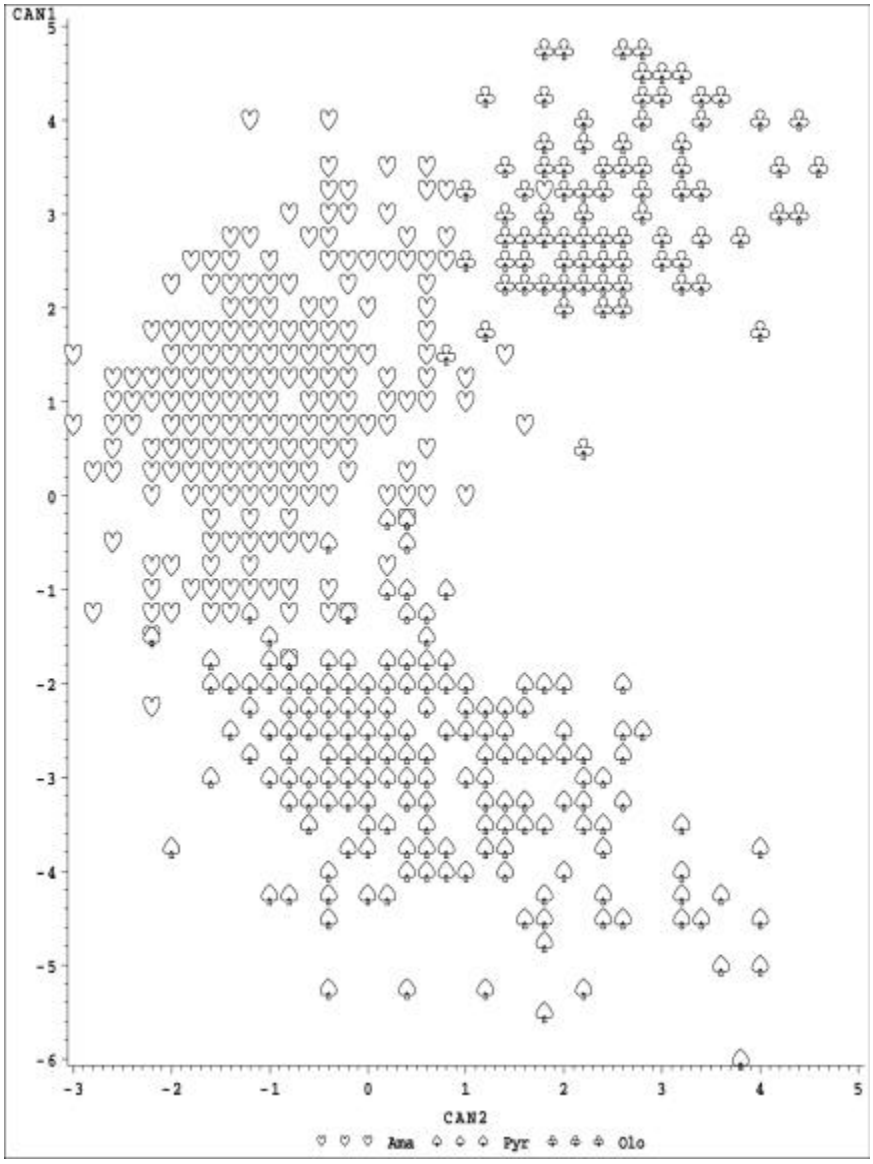

Fig. 1. Canonical discriminant analysis based on nine morphological characters of individuals of *C. amara* subsp. *amara* (heart, n = 380), *C. amara* subsp. *pyrenaea* (spade, n = 238), and *C. amara* subsp. *olotensis* (club, n = 101).

Table 4. Results of the classificatory discriminant analysis of *C. amara* subsp. *amara*, *C. amara* subsp. *austriaca*, *C. amara* subsp. *olotensis*, *C. amara* subsp. *opicii* and *C. amara* subsp. *pyrenaea*, based on morphological characters. Individual plants as OTUs and subspecies as groups. P = parametric analysis, N = non-parametric analysis.

Actual group		Predicted group membership (number of observations and percentage classified into groups)					
		amara	*austriaca*	*olotensis*	*opicii*	*pyrenaea*	Total
amara	P	**349**	20	3	1	7	380
	P	**91.84**	5.26	0.79	0.26	1.84	100.00%
	N	**344**	25	6	0	5	380
	N	**90.53**	6.58	1.58	0.00	1.32	100.00%
austriaca	P	51	**302**	1	6	8	368
	P	13.86	**82.07**	0.27	1.63	2.17	100.00%
	N	32	**327**	2	0	7	368
	N	8.70	**88.86**	0.54	0.00	1.90	100.00%
olotensis	P	2	0	**99**	0	0	101
	P	1.98	0.00	**98.02**	0.00	0.00	100.00%
	N	1	0	**100**	0	0	101
	N	0.99	0.00	**99.01**	0.00	0.00	100.00%
opicii	P	1	9	0	**326**	42	378
	P	0.26	2.38	0.00	**86.24**	11.11	100.00%
	N	5	7	0	**340**	26	378
	N	1.32	1.85	0.00	**89.95**	6.88	100.00%
pyrenaea	P	1	11	0	19	**207**	238
	P	0.42	4.62	0.00	7.98	**86.97**	100.00%
	N	3	7	0	13	**215**	238
	N	1.26	2.94	0.00	5.46	**90.34**	100.00%

There were only slight differences between the results of parametric and k-nearest-neighbour non-parametric classificatory discriminant analysis (Table 4). Morphological characters included in the analysis separated these taxa quite well. A higher amount of wrongly determined plants appeared between *C. amara* subsp. *amara* and *C. amara* subsp. *austriaca*, as well as between *C. amara* subsp. *pyrenaea* and *C. amara* subsp. *opicii*. In the first of these cases, the size of pollen grains, which reliably separates subsp. *amara* and subsp. *austriaca* (Marhold, 1999) was not included in the analysis (as data for other subspecies are not currently available). On the other hand, there are certain morphological similarities between *C. amara* subsp. *pyrenaea* and *C. amara* subsp. *opicii*. Tables 5 and 6 show basic statistics of the evaluated characters of *C. amara* subsp. *olotensis*, and subsp. *pyrenaea* in comparison with data on subsp. *amara*, published by Marhold (1998), based on data from 1457 plants. From these data it is clear that *C. amara* subsp. *pyrenaea* can be identified only by using a combination of several characters. Although there are quantitative morphological characters which, in their combination, separate subsp. *olotensis* from both subsp. *amara* and subsp. *pyrenaea*, one has to bear in mind that the character best separating subsp. *olotensis*, yellow colour of anthers, was not included in the morphometric analyses, because of its qualitative nature and unequivocal ability to separate this taxon.

RAPD analysis. — The interpretation of RAPD patterns resulted in 196 markers excluding bands found in only one individual. Each studied plant represented different composition of bands. In the neighbour-joining distance analysis only clus-

Table 5. Results of the exploratory data analysis of three subspecies of *Cardamine amara*. Data for subsp. *amara* are from Marhold (1998) in which details of localities can be found.

Character	Mean	Standard deviation	Percentiles	
			1% (5%)	99% (95%)
width of stem - WS (mm)				
amara	2.49	0.83	1 (1.5)	5 (4)
olotensis	3.94	1.50	1.5 (2)	9 (6.5)
pyrenaea	3.20	1.08	1 (2)	7 (5)
maximum length of filaments - LF (mm)				
amara	5.73	0.85	3.5 (4.3)	7.5 (7.1)
olotensis	4.99	0.44	4.2 (4.3)	6.1 (5.6)
pyrenaea	5.94	0.46	4.9 (5.2)	6.9 (6.6)
length of sepals - LS (mm)				
amara	3.56	0.29	2.9 (3.1)	4.1 (4)
olotensis	4.26	0.39	3.5 (3.6)	5.2 (4.9)
pyrenaea	3.94	0.45	3.1 (3.3)	5.2 (4.9)
width of petals - WP (mm)				
amara	4.60	0.57	3.5 (3.8)	6.4 (5.7)
olotensis	5.41	0.62	4.5 (4.5)	6.9 (6.6)
pyrenaea	4.14	0.54	3.1 (3.5)	5.2 (5.2)
length of petals - LP (mm)				
amara	8.32	0.97	6.1 (6.8)	10.2 (9.9)
olotensis	9.52	0.90	8 (8.3)	11.6 (11.5)
pyrenaea	7.82	0.82	6.1 (6.2)	9.4 (9)
number of flowers (including buds) in the main inflorescence - NF				
amara	15.63	5.75	5 (6)	29 (25)
olotensis	23.83	5.83	9 (13)	36 (34)
pyrenaea	15.61	5.08	5 (7)	27 (23)
maximum number of leaflets of the leaves in the upper 4/5 of stem - NLL				
amara	8.19	1.67	5 (6)	13 (11)
olotensis	6.46	1.09	4 (5)	9 (8)
pyrenaea	11.17	1.47	7 (9)	15 (13)
number of leaves - NL				
amara	8.45	3.35	3 (4)	17 (15)
olotensis	11.54	2.33	8 (9)	18 (17)
pyrenaea	21.37	6.82	10 (12)	42 (34)
degree of congestion of leaves beneath the inflorescence, expressed by the number of leaves reaching the base of the uppermost stem leaf - NLR				
amara	1.27	0.93	0 (0)	4 (3)
olotensis	2.13	0.89	1 (1)	4 (4)
pyrenaea	4.44	1.76	2 (2)	10 (7)

Table 6. Frequency of states of character BS (branching of stem) in three subspecies of *Cardamine amara*. Data for subsp. *amara* are from Marhold (1998), where details of localities can be found.

	Number of plants with	
	BS = 1 (stem branched)	BS = 0 (stem not branched)
amara	271	109
olotensis	87	14
pyrenaea	0	238

ters of *C. amara* subsp. *pyrenaea*, subsp. *olotensis*, subsp. *balcanica* and subsp. *opicii* were supported by bootstrap values higher than 50% (see Fig. 2). The remaining two subspecies (subsp. *amara* and subsp. *austriaca*) were supported by much lower bootstrap values, which indicate that they are much less differentiated

from each other. The first principal coordinates analysis showed subsp. *olotensis* in a clearly separated position from the rest of the material along the first axis (diagram not shown). In the second analysis (without subsp. *olotensis*, Fig. 3), subsp. *opicii*, subsp. *balcanica* and subsp. *pyrenaea* formed well-defined groupings, while subsp. *amara* and subsp. *austriaca* were separated only along the third axis, which is less important in comparison with the first two.

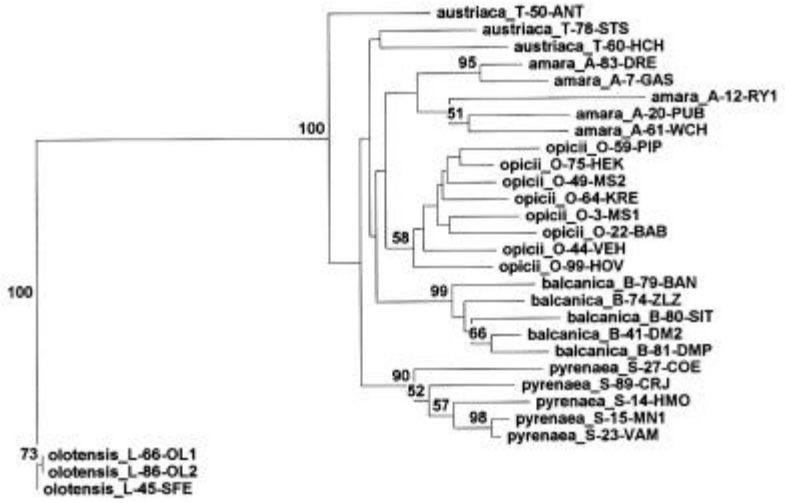

Fig. 2. Neighbour-joining distance analysis of RAPD data of the six subspecies of *Cardamine amara* (n = 29). Only bootstrap values higher than 50% are shown. For population abbreviations see Tables 1 and 2.

Discussion

This study of Iberian populations of *Cardamine amara* provides further data on variation of this species in its European area of distribution. This species is differentiated into three diploid subspecies confined to major mountain ranges, one diploid subspecies widespread in Europe, and two tetraploid subspecies, occupying the Eastern Alps and neighbouring areas, and the lowland part of Catalonia. From the morphological evaluation it is clear that these two tetraploid taxa originated independently. This view is supported also by the RAPD data presented here and data on isozymes (Marhold & al., in prep.).

The new morphological data presented here and the previously published data on *C. amara* from other parts of Europe (Marhold, 1992, 1998, 1999) show that its subspecies differ only in respect to quantitative characters. The exception is subsp. *olotensis*, which is differentiated by the qualitative character—colour of anthers. High mountain diploid subspecies (subsp. *pyrenaea*, subsp. *balcanica*, and subsp. *opicii*) tend to be more uniform and distinct with respect to their morphology, and

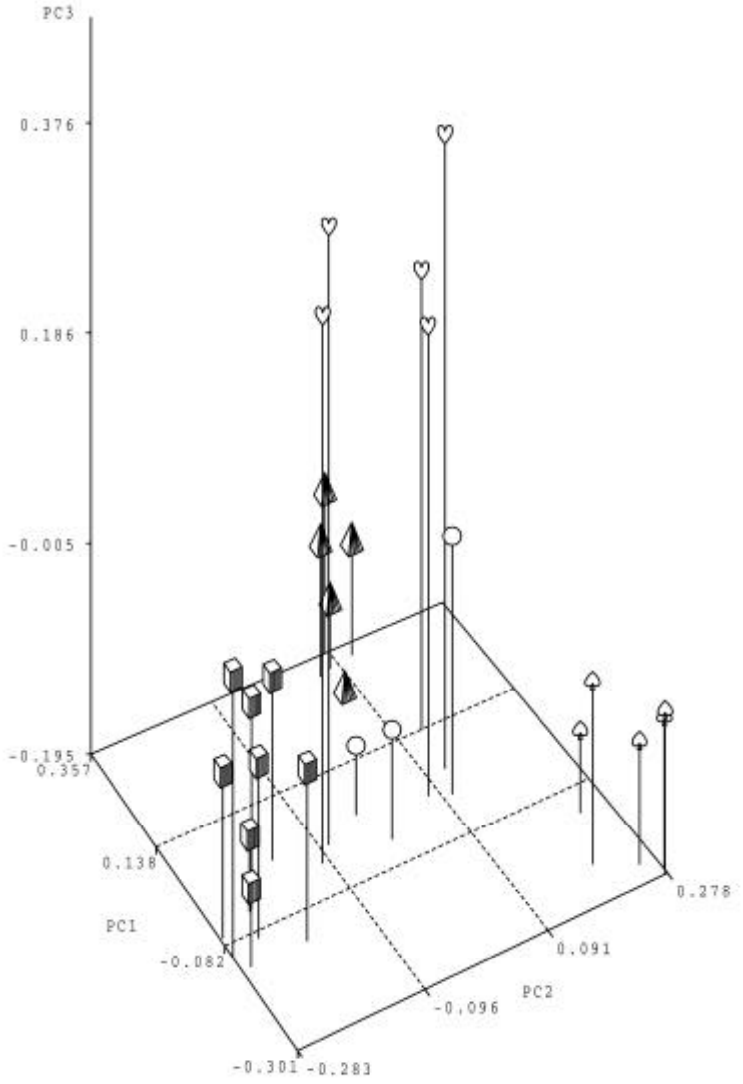

Fig. 3. Principal coordinates analysis of RAPD data of five subspecies of *Cardamine amara* (n = 26). Heart = subsp. *amara*, circle = subsp. *austriaca*, pyramid = subsp. *balcanica*, spade = subsp. *pyrenaea*, cube = subsp. *opicii*. First three axes represent 14.47, 13.48 and 9.55% of variation, respectively.

this is also supported by the RAPD data, at least for the first two taxa. *Cardamine amara* subsp. *austriaca*, most probably an autotetraploid derivative of subsp. *amara* (Marhold, 1999), is rather poorly separated in respect to both morphological (except for the size of pollen grains) and RAPD characters from the latter subspecies. Again, such an origin of subsp. *austriaca* is also supported by isozyme data (Marhold & al., in prep.).

Cardamine amara subsp. *olotensis* and to a large extent also subsp. *pyrenaea* occupy an area geographically isolated from the other subspecies (cf. Jalas & Suominen, 1994: 154, 155, subsp. *pyrenaea* in the Pyrenees mapped as subsp. *amara*) on the southwestern border of the distribution area. This isolated position might be the reason for the rather uniform morphology of these populations, and it is also reflected in their isozyme spectrum. Iberian subsp. *olotensis* and subsp. *pyrenaea* as well as Balkan subsp. *balcanica* possess alleles unknown in other subspecies of this species, while the other subspecies of *C. amara* differ only in respect to the allele frequencies (Marhold & al., in prep.). We might, therefore, speculate also about the relic nature of both Iberian and Balkan populations.

Bolòs (1952) and Rico (1993) argue that *C. amara* subsp. *olotensis* differs from the typical populations of *C. amara* with respect to morphological characters that make this subspecies more related to *C. raphanifolia* Pourr. In addition to the yellow colour of anthers, such characters include the shape of the stigma, shape of the terminal leaflet of lower stem leaves, glabrous stem leaves, basal leaves tendency to form a rosette, higher number of flowers in the inflorescence, and bigger size of the plants. For most of these characters, however, populations of subsp. *olotensis* fit into the overall variation of *C. amara*. The size of the plant of *C. amara* is rather variable and depends mostly on ecological conditions; plants growing in the shade tend to be taller and have larger leaves. The number of flowers, which Jones (1964) treated as an important differentiating character for the subspecies of *C. amara*, have no taxonomical value whatsoever (see Marhold, 1992; compare also data in Table 5). The terminal leaflet of the lower stem leaves tends to be, indeed, in subsp. *olotensis* larger (in comparison with lateral leaflets) and rotund, but this kind of variation is also found in subsp. *balcanica*. Stems and leaves of subsp. *olotensis* are usually glabrous, but occasionally plants with scattered hairs on stems and leaf margins can be found. In comparison with subsp. *amara* they are generally less hairy. The tendency of basal leaves to form rosettes is not as pronounced in subsp. *olotensis* as Bolòs (1952) claimed, and even the picture in the original publication of this subspecies is unrepresentative in this respect. Also with regard to stigma morphology subsp. *olotensis* is much more close to subsp. *amara* and other subspecies of *C. amara* than to *C. raphanifolia*. Nevertheless, one might question whether the rank of subspecies is appropriate for *C. amara* subsp. *olotensis*. For the time being, we prefer to keep this rank, which is also accepted by Jalas & Suominen (1994). However, after full evaluation of molecular data (including isozymes), and after study of populations from central Italy, probably closely related to subsp. *olotensis*, the rank of this taxon should be re-evaluated.

With respect to some morphological characters (number of leaves, number of leaflets of the stem leaves, congestion of leaves below inflorescence), there appears to be parallel evolution in the Pyrenees (subsp. *pyrenaea*) and also in the

Carpathians and Sudeten mountains (subsp. *opicii*). In addition to the characters included in the morphometric analysis, both subsp. *pyrenaea* and subsp. *opicii* have simple, unbranched stems (see Table 6). In other subspecies both simple and branched stems can be found. There is a significant morphological difference between subsp. *pyrenaea* and subsp. *opicii* in their indument, a character that was not included in the numerical analysis. While most of the plants of subsp. *pyrenaea* have stems and leaves glabrous or rarely with scattered hairs, the majority of populations of subsp. *opicii* contain both densely hairy and glabrous plants. Given the disparate geographic distribution of these two subspecies, a common origin for them is hard to explain. This hypothesis is not favoured by the RAPD data either.

The original materials of *C. amara* subsp. *siifolia*, deposited in BC (marked by ⊗ in the attached list of studied specimens), including the lectotype selected here, fully correspond to our concept of *C. amara* subsp. *pyrenaea*. From the point of view of nomenclature, both subspecific names have equal priority. However, we selected subsp. *pyrenaea* following Art. 11.5 of the *Code* (Greuter & al., 2000), because the classical locality of this name is known to us in detail.

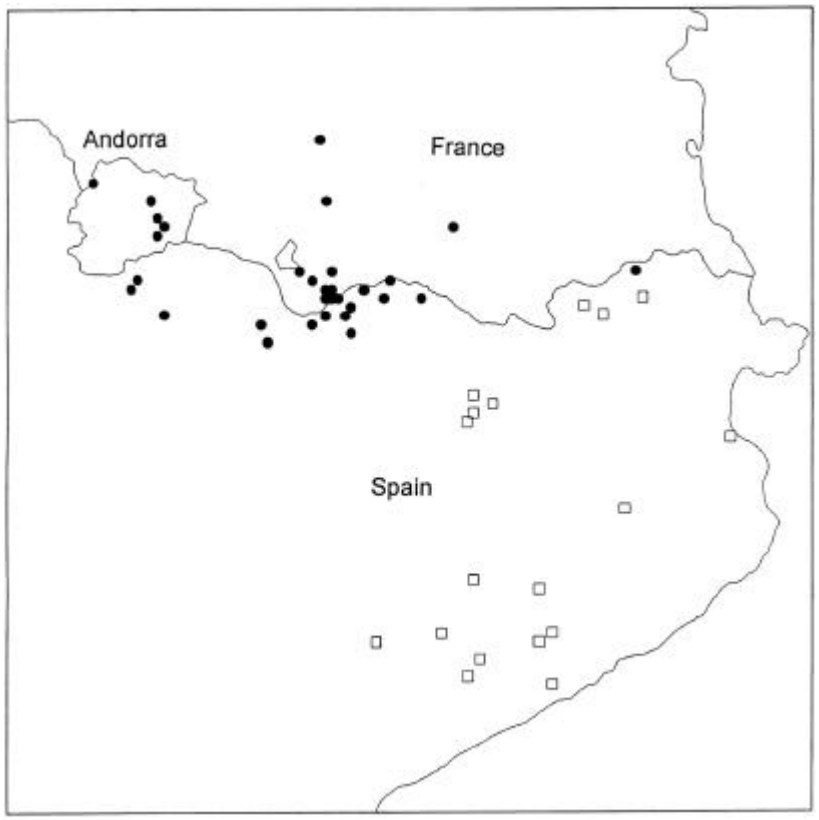

Fig. 4. Map of the distribution of *Cardamine amara* in the Iberian Peninsula based on herbarium specimens. Square = subsp. *olotensis*, circle = subsp. *pyrenaea*.

Taxonomic conspectus of *Cardamine amara* in the Iberian Peninsula

Cardamine amara L., Sp. Pl.: 656. 1753. Ind. loc.: "In Europae septentrionalis nemoribus". – Lectotype (Khatri, 1989: 92): LINN, no. 835.17!

Cardamine amara* subsp. *pyrenaea Sennen, Monde Pl. 30 (63/178): 6. 1929. Ind. loc.: "Nuria, bords des torrents, 2000 m et plus; Val de Llo, 1650 m; Val d'Eyne, 2150 m". – Lectotype (designated here): [Spain] Catalogne: Pyrénées à Nuria, torrents, 2100 m, 21 Jul 1914, *Sennen s.n.* (BC 827121!; isolectotype: BM 99973!).

= *Cardamine amara* subsp. *siifolia* Sennen, Monde Pl., Ser. 3, 30 (63/178): 6–7, 1929. Ind. loc.: "Le Canigou, le Cambredase, le Capcir: région alpine inférieure". – Lectotype (designated here): [France] Cerdagne au Cambredase [Cambras d'Azé], torrents S. Pierre, 1750 m, 22 Jul 1916, *Sennen s.n.* (BC 03122!).

Stem 10–35 cm tall, ascending, simple, (1–) 2–5 (–7) mm wide at base. Cauline leaves (10–) 12–34 (–42), congested below the inflorescence, with (7–) 9–13 (–15) leaflets. Lower cauline leaves not congested near the stem base. Leaflets of the lower and upper cauline leaves of the same shape, terminal leaflet not different from the lateral ones. Leaflets shortly petiolulate or sessile, entire or sinuate to crenate, narrowly to widely ovate. Inflorescence with (5–) 7–23 (–27) flowers. Sepals (3.1–) 3.3–4.9 (–5.2) mm long, petals (6.1–) 6.2–9 (–9.4) mm long and (3.1–) 3.5–5.2 (–5.2) mm wide. Filaments of longer (tetradynamous) stamens (4.9–) 5.2–6.6 (–6.9) mm long. Anthers blackish–violet before dehiscence. Stigma not conspicuous, as wide as the style.

Representative specimens (⊗ = original material of *C. amara* subsp. *siifolia*). **A n d o r r a .** Circo de Pessons, 31T CH80, 2000–2600 m, 21 Jul 1986, *Gómez, Luceño & Vargas 10113GL* (MA 321421); Grau Roig, river Valira, "Bordas de Envalira", 31T CH9212, 2050 m, 3 Jul 1992, *Nieto Feliner 3013GN & al.* (MA 516986); Pla Sorteny, Valle de Incles, near Rio a Junclar, 1900-2000 m, Aug 1949, *Losa & Montserrat s.n.* (BCF 35845); Planells d' Arcalís [CH72], 3 Jul 1980, *Soriano s.n.* (BCC 1468); Puerto de Envalira 31T CH9311, 2540 m, 31 Aug 1976, *Castroviejo & Valdés-Bermejo 1215EV* (MA 321414).

F r a n c e . Pyrenees Or. Cerdagne, au Cambredase [Cambras d'Azé], 1900 m, 3 Aug 1916, *Sennen s.n.* (BC 03146 ⊗; BC-Sennen 827116 ⊗); 1790 m, 22 Jul 1916, *Sennen s.n.* (BM 99974 ⊗); Le Cambredase [Cambras d'Azé], 1800 m, 9 Aug 1919, *Sennen s.n.* (BC-Sennen 827117 ⊗); Le Canigou [DH50], 13 Jul 1897, *Sennen s.n.* (BC-Sennen 827118 ⊗); Ruisseaux du Canigou, Jul 1872, *Cosson s.n.* (P); Capcir, V. de Galbe, F-66, Espoussouille, 1750 m, 9 Jun 1983, *Montserrat & Villar s.n.* (MA 257463); Courbitte, [près] d`Antist [near Bagnères-de-Bigorre], Jun, *Balle s.n.* (BC 133493); Matemale, 30 May 1960, *A. et O. de Bolòs s.n.* (BC 602600); Vallé dé la Ségre, 1847, *Franqueville s.n.* (P).

S p a i n . Catalonia, Pyrenees. Aranser, Sota Prat Miró, 1980 m, 17 Jul 1990, *Carrillo s.n.* (BCC); Val d' Eyne, 4 Aug 1924, *Barnades s.n.* (BC 604796, 603664); 4 Jul 1872, *Gadeceau s.n.* (BM 99970); 7 Jul 1849, *Grenier s.n.* (P); 24 Aug 1892, *Loret s.n.* (P); 2190 m, 26 Jul 1922, *Sennen s.n.* (BC-Sennen 827123); Val de Llo, 1690 m, 7 Aug 1923, *Sennen s.n.* (BC-Sennen 827122); Martinet, 1950, *Parcual s.n.*

(BC 608271); La Molina, *Vayreda s.n.* (BC-herb. Vayreda); Noufonts prés Nuria, 2100 m, 1 et 4 Aug 1913, *Sennen s.n.* (BM 99972); Nuria, 1900 m, 19 Jul 1922, *Cuatrecasas s.n.* (MAF 16969); 2100 m, 8 Aug 1924, *Hno. Gonzalo s.n.* (BC-Sennen 827120); 2300 m, 24 Aug 1949, *Font Quer s.n.* (BC 11 1795); Jul 1870, *Masferrer s.n.* (BC 03141); Nuria, *Vayreda s.n.* (BC-herb. Vayreda); inter Núria et Nou-Creus, 2200 m, Jul 1950, *Capell s.n.* (BC 114296); Núria, versus Noufonts, 1 Aug 1943, *A. et O. de Bolòs s.n.* (BC 111468); Núria, St. Gil, 9 Aug 1934, *Dauder Rodés s.n.* (BM 99971); Penticouse, Jul 1848, *Franqueville s.n.* (P); *Ant. Le Grand s.n.* (BC-Sennen 827119); Pérafeu, Jun 1873, *Vayreda s.n.* (BC-herb. Vayreda), May, *Vayreda s.n.* (BC-herb. Vayreda); Queralbs,Vall de Ribes–Font de l'Home Mort, 1850 m, 21 Jul 1968, *Vigo & Anglada s.n.* (BC 601547); Requeséns, 600–700 m, Apr 1886, *Trémols s.n.* (MA 47486); Vall de Ribes, Coma de Vaca, 2150 m, 30 Jun 1961, *Vigo s.n.* (BC 596472); Coma de Vaca, 20 Jul 1880, *Mansferrer s.n.* (BC 03262); Saltéguet, 1800 m, 12 Jul 1987, *Soriano s.n.* (BCC); Setcases, Montagne de Morens, Jun 1847, *Bourgeau, Pyrénées Espagnoles 414* (P); Val de Setcases, Rivera de Morens, 2000 m, 22 Jul 1921, *Cuatrecasas s.n.* (MAF 16968, 82638); Setcases, Pla dels Hospitalets, 1900 m, 17 Jul 1922, *Cuatrecasas s.n.* (BC 03135).

Cardamine amara* subsp. *olotensis O. Bolòs, Collect. Bot. (Barcelona) 3: 187–188, 1952. Ind. loc.: "Olot, Parc Nou". – Holotype: Olot, in pratis humidis Parc Nou, 1 May 1950, *A. de Bolòs s.n.* (BC 112809!).

= *Cardamine amporitana* Sennen & Pau, Bull. Acad. Int. Géogr. Bot. 20 (259): 104–105, 1911. Ind. loc.: "Espagne, Catalogne, ruisseau del Macho à Cabanas". – Lectotype (designated here following the suggestion by E. Rico, *in litt.*): Catalogne: Cabanas, ruisseau [printed label]; Cabanas, bords du ruisseau Gordo del Macho [written by hand], 4 Jun 1908, *Sennen, Plantes d'Espagne 530* (MA 47582!; isolectotypes: P!, BC 03152!, BC-Sennen 827115!, 805503!, MA 47581!). = *Cardamine amara* var. *parviflora* Cadevall, Fl. Catalunya 1: 136, 1915. Ind. loc.: "al Empordá; reguerols de Cabanes (Sen.!) ". – Lectotype (designated here): Catalogne: Cabanas, ruisseau [printed label]; Cabanas, bords du ruisseau Gordo del Macho [written by hand], 4 Jun 1908, *Sennen, Plantes d' Espagne 530* (MA 47582!; isolectotypes: P!, BC 03152!, BC-Sennen 827115!, 805503! MA 47581!, the same specimens as for *C. amporitana* Sennen & Pau).

Stem 20–65 cm tall, erect, branched above, seldom simple, (1–) 1.5–4.5 (–5) mm wide at base. Cauline leaves (8–) 9–17 (–18), not congested below the inflorescence, with (4–) 5–8 (–9) leaflets. Lower cauline leaves more or less congested near the stem base. Lower and upper cauline leaves with leaflets of slightly different shape. Lower ones with shortly petiolulate, entire leaflets, terminal leaflet suborbiculate or obovate, lateral ones obovate to elliptic. Upper leaves with shortly petiolulate or sessile, entire or sinuate to lobate leaflets, both terminal and lateral ones obovate, elliptic to oblong. Inflorescence with (9–) 13–34 (–36) flowers in the main inflorescence. Sepals (3.5–) 3.6–4.9 (–5.2) mm long. Petals (8–) 8.3–11.5 (–11.6) mm long and (4.5–) 4.5–6.6 (–6.9) mm wide. Filaments of longer (tetradynamous) stamens (4.2–) 4.3–5.6 (–6.1) mm long. Anthers yellow before dehiscence. Stigma slightly wider, sometimes as wide as the style.

Representative specimens. **S p a i n . Catalonia:** Agullana, *Vayreda s.n.* (BC-herb. Vayreda); Cantallops, 31T DG 99, 140 m, 22 Mar 1994, *Villar* & *Font s.n.* (HGI 6771); Celrà, 31T DG 85, 45 m, 5 Apr 1999, *Bisbe s.n.* (HGI 15893); [Martorell de la Selva DG72] Empalme, 1 May 1936, *Font Quer s.n.* (BC 634443); Gerona, Jun 1837, *R. de Bolòs s.n.* (BC 145091); Maçanet de la Selva, 31T DG 72, 60 m, 15 May 1987, *Vilar s.n.* (HGI 11476); Martorell de la Selva [DG72], 100 m, 5 May 1943, *A. de Bolòs s.n.* (BC 123856); 29 Apr 1934, *Font Quer s.n.* (BC 82062); [Sant Hilari DG53] Monsolí [Mansolí], Espinalbas [Espinelves], May, *Vayreda s.n.* (BC-herb. Vayreda); Montseny, l'Avencó, 400 m, 6 May 1948, *A. et O. de Bolòs s.n.* (BC 105965, 105966); Montseny, Gualba, 15 May 1915, *Font Quer s.n.* (BC 03138); Montseny, St. Celoni, 3 Jun 1917, *Garriga s.n.* (BC 03140); Montseny, Santa Fe, 1100 m, 21 Jul 1948, *O. de Bolòs s.n.* (BC 105964); 2 May 1948, *A. de Bolòs s.n.* (BC 105963); 3 Jun 1917, *Garriga s.n.* (BC 03139); Olot, May 1977, *A.C. Costa s.n.* (BC 614897); Apr, *Vayreda s.n.* (BC-herb. Vayreda, BC 03113); Olot, 400 m, Apr 1871, *Vayreda s.n.* (BC 03134); May 1871, *Vayreda s.n.* (BC 614895); 450 m, Apr, *Vayreda s.n.* (MA 47488); Olot, la Boixeda, la Canya [DG57], Apr, *A. de Bolòs s.n.* (BC 113061); 5 May 1943, *A. de Bolòs s.n.* (BC 123856); 18 Apr 1921, *Font Quer s.n.* (BC 111736); Olot, Castellfollit, Apr 1872, *Vayreda s.n.* (BC-herb. Vayreda); Olot, Font Moixina, Apr 1926, *A. de Bolòs s.n.* (BC 113057); Jun 1925, *A. de Bolòs s.n.* (BC 113072); 15 Apr 1976, *Ninot & al. s.n.* (BCC); Querós [DG54], 300 m, 1 May 1949, *Llensa s.n.* (BC 111926); Santa Coloma de Farners 31T DG 73, 200 m, 15 Apr 1986, *Vilar s.n.* (HGI 8708); Sant Joan les Fonts, 31T DG 57, 330 m, 20 May 1985, *Viñas s.n.* (HGI 11475); Tordera, 35 m, 22 Apr 1945, *Font Quer s.n.* (BC 101680); La Vajol, 31T DG 89, 540 m, 19 May 1996, *Font s.n.* (HGI 10147); Ventalló, 31T EG 06, 5 m, 31 Mar 1999, *Gesti s.n.* (HGI 15842).

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