Folia Geobotanica 39: 405–429, 2004

INFRASPECIFIC DIFFERENTIATION IN THE BALKAN DIPLOID *CARDAMINE ACRIS* (*BRASSICACEAE*): MOLECULAR AND MORPHOLOGICAL EVIDENCE

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Abstract: Molecular and morphological variation in the Balkan diploid *Cardamine acris* was studied by amplified fragment length polymorphism (AFLP) and multivariate morphometric analyses. Principal coordinate analysis and neighbour-joining analysis of the AFLP data showed three genetically differentiated groups of populations corresponding to their geographic origin. Following the results of molecular analysis three subspecies were recognized by a combination of morphological characters as shown by separate analyses of field-collected and cultivated plants. These subspecies are allopatric; *C. acris* subsp. *acris* occurs in most parts of the Balkan Peninsula, and newly described subsp. *pindicola* and subsp. *vardousiae* are endemic to the Northern Pindhos Mts. in Northwestern Greece, and Central Greece, respectively. The differentiation into geographically isolated subspecies is most probably caused by long-term isolation of populations in different mountain ranges of the Balkan Peninsula, possibly initiated during the cold periods of the Pleistocene.

Keywords: AFLP, Biogeography, Multivariate morphometrics, Subspecies, Taxonomy

INTRODUCTION

The genus *Cardamine* (*Brassicaceae*) is represented by ca. 27 species and subspecies in the Balkan Peninsula (JALAS & SUOMINEN 1994). The taxonomy of the *C. pratensis* group (MARHOLD & KIT TAN 1999, MARHOLD & ANČEV 1999, LIHOVÁ & MARHOLD 2003) and the *C. amara* group (MARHOLD et al. 1996, MARHOLD 1998) has been studied in detail in this region. So far, two taxa related to these groups, *C. acris* GRISEB. (sometimes treated as subspecies of *C. raphanifolia* POURR.) and *C. barbaraeoides* HALÁCSY (treated also as subspecies of *C. raphanifolia* or *C. amara* L.), have not been evaluated using a biosystematic approach, and their taxonomic treatments are inconsistent in various Floras. Recently, we have shown that *C. barbaraeoides* is a clearly distinguishable tetraploid confined to a small area in Northwestern Greece (PERNÝ et al., unpubl.data). That study also indicated that Greek populations of *C. acris* exhibit a broad range of variation in several morphological characters, motivating us to perform a detailed evaluation of the infraspecific variation in *C. acris* in its entire distribution area.

In this study, we examine the molecular and morphological variation among populations of *C. acris* from different regions of the Balkan Peninsula, to which we consider it endemic. Calabrian populations, previously hypothesized as related to *C. raphanifolia* subsp. *acris* (GRISEB.) O.E. SCHULZ (PIGNATTI 1982, JONES & AKEROYD 1993) were shown to represent a distinct hexaploid species, *C. silana* PERNÝ et MARHOLD (MARHOLD et al. 2003, PERNÝ et al. 2004). It was suggested that the Balkan *C. acris* is one of the parental species of allopolyploid *C. silana* (PERNÝ et al. 2004). Furthermore, plants from the Central Apennine Peninsula that were previously regarded as conspecific or closely related to *C. acris* (PIGNATTI 1982, JONES & AKEROYD 1993, JALAS & SUOMINEN 1994), were shown to belong to *C. amporitana* SENNEN et PAU, a well circumscribed tetraploid within the *C. amara* group (LIHOVÁ et al. 2004a,b). Finally, reports of *C. acris* from the Caucasus and Turkey, based on plants originally described as *C. seidlitziana* ALBOV (CULLEN 1965, KHATRI 1988), probably represent a taxon well-differentiated from *C. acris* (J. KUČERA et al., in prep.).

The original description of *C. acris* was based on plants from Mt. Kobelica in Macedonia (at present Former Yugoslav Republic of Macedonia, F.Y.R.O.M.), Mt. Kopaonik in Southern Serbia, and Mt. Nidgé (=Voras) on the border of F.Y.R.O.M. and Northwestern Greece (GRISEBACH 1843). In the years that followed, the species was also reported from other mountain areas in Serbia (PANČIĆ 1874), Bulgaria (VELENOVSKÝ 1886), and Greece (HALÁCSY 1901). GRISEBACH (1843) described *C. acris* as morphologically intermediate between *C. pratensis* L. and *C. latifolia* VAHL (= *C. raphanifolia* POURR.). In the monograph of the genus (SCHULZ 1903), and later in some Floras (CULLEN 1965, ASSENOV 1970, STRID 1986, JONES & AKEROYD 1993), it was treated as *C. raphanifolia* subsp. *acris*. STRID (1986) reported that the Balkan populations differ from the Iberian *C. raphanifolia* subsp. *raphanifolia* only in quantitative characters. Most authors dealing with the Balkan flora, however, have continued to use the original species level treatment (e.g. MAIRE & PETITMENGIN 1908, ADAMOVIĆ 1911, HAYEK 1927, RECHINGER 1943, STOJANOV et al. 1966, TRINAJSTIĆ 1976, KIT TAN 2002). Diploid chromosome numbers (2n=16) were reported for *C. acris* plants originating from Bulgaria (ANČEV et al. 1997).

Differentiation and speciation via hybridization and polyploidization are common in *Cardamine*, especially in the polyploid *C. amara*, *C. pratensis* and *C. raphanifolia* complexes (e.g. NEUFFER & JAHNCKE 1997, URBANSKA et al. 1997, MARHOLD 1999, MARHOLD et al. 2002a,b, 2004, LIHOVÁ et al. 2003, 2004a,b, PERNÝ et al. 2004, and unpubl. data). There are also examples of differentiation among geographically isolated diploid populations. For instance, four diploid subspecies of *C. amara* are recognized in Europe. They have allopatric or parapatric distributions, mostly restricted to different mountain ranges, and show various levels of morphological and molecular differentiation (MARHOLD 1992, 1995, 1998, LIHOVÁ et al. 2000, MARHOLD et al. 2002a, LIHOVÁ et al. 2004a,b).

Multivariate morphometric approaches have been successfully used to resolve complex infraspecific variation patterns and have led to the recognition of several infraspecific taxa in *Cardamine* (MARHOLD 1992, 1998, LIHOVÁ et al. 2000). Studies combining morphometric analyses with DNA-fingerprinting techniques, e.g. random amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP), are rather rare, but allow us to draw taxonomic conclusions with high confidence and bring insights into the evolutionary history of recently diverged taxa (HANSEN et al. 2000, LIHOVÁ et al. 2000, FJELLHEIM et al. 2001, ISHIDA et al. 2003, SAARELA et al. 2003).

MATERIAL AND METHODS

Material

Plants were sampled in the field on 21 localities (Fig. 1, Table 1) and conserved as herbarium specimens for morphometric analyses. Living plants were also collected from the same localities. To examine genetically determined morphological variation, plants were cultivated under similar environmental conditions in a common garden experiment at the Institute of Botany, Slovak Academy of Sciences, Bratislava, for one to three years. Herbarium specimens were taken from these plants for morphometric analysis. Most populations were, thus, represented by both field-collected specimens and specimens taken from cultivated plants (Table 1). One to three individuals per locality were checked for chromosome numbers (Table 1). Our sampling for AFLPs was designed to cover a wide range of total variation present across the geographic area of *C. acris*, rather than to assess intrapopulational variation. Therefore, samples (1 or 2 per population) originating from different regions were included (Table 1, Fig. 1).

Voucher specimens for all the analyses performed in this study are deposited in SAV. In addition, herbarium specimens of *C. acris* from herbaria B, BEO, BEOU, BM, BRNM, BRNU, C, JE, K, KRA, M, MA, PR, SO, SOM, W, WU, ZT were studied to assess geographic distribution. The geographical division of Greece into the eight regions adopted here (North East, North Central, Northern Pindhos, Southern Pindhos, East Central, Sterea Ellas, Peloponnisos, and Crete) follows STRID (1986). Note that the Northern Pindhos Mts. is a mountain range that covers only a part of the geographic area of Northern Pindhos.

Chromosome numbers

Chromosome numbers were counted in all populations used in morphometric and AFLP analyses from metaphase plates of meristematic cells from roots using a squash method as published in PERNÝ et al. (2004).

AFLP analysis

Total genomic DNA was extracted from silica gel-dried leaves following the CTAB protocol (DOYLE & DOYLE 1987) with minor modifications (see SCHÖNSWETTER et al. 2004). The AFLP procedure (VOS et al. 1995) followed the general protocol by Applied Biosystems (PE APPLIED BIOSYSTEMS 1996) with a few modifications (see SCHÖNSWETTER et al. 2004). PCR conditions for preselective and selective amplification followed LIHOVÁ et al. (2004a). On a basis of a primer test with 15 different primer combinations, three primer pairs were chosen for selective amplification: *Eco*RI-AAG-(HEX) and *Mse*I-CTG, *Eco*RI-ATC-(6FAM) and *Mse*I-CAG, *Eco*RI-AGC-(NED) and *Mse*I-CTG. The fluorescent labelled amplified fragments were run on an ABI 377 sequencer and analyzed with GeneScan® software (version 3.2, Applied Biosystems). Presence or absence of fragments ranging from 70 to 500 bp were scored for each sample (only distinct fragments were

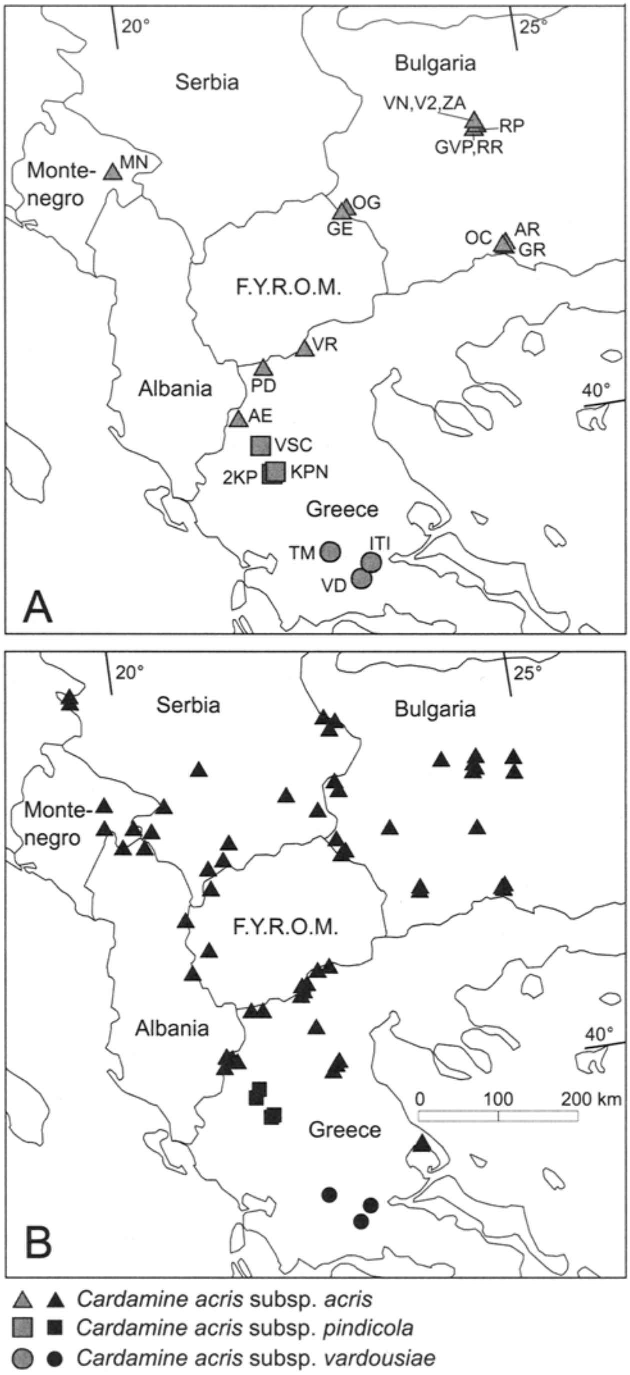

Fig. 1. A – Distribution of sample sites of *Cardamine acris* subsp. *acris*, subsp. *pindicola*, and subsp. *vardousiae*. For population codes see Table 1. B – Distribution of the three subspecies of *C. acris* based on herbarium specimens. F.Y.R.O.M. – Former Yugoslav Republic of Macedonia.

analyzed) and transferred into a binary matrix using GenoGrapher (version 1.6.0, © Montana State University 1999; http://hordeum.msu.montana.edu/genographer/).

A principal coordinate analysis (PCoA; KRZANOWSKI 1990) based on Jaccard's coefficient was computed to recover non-hierarchical structure of AFLP data using SYN-TAX 2000 (PODANI 2001). A neighbour-joining tree based on the NEI & LI (1979) genetic distance was constructed using PAUP* (version 4.0b10, SWOFFORD 2003), with the following settings: Unweighted Least Squares was chosen as Objective Function; constraint branch lengths were set to be non-negative and branches of effectively zero length when searching was set to collapse. Group support was assessed by a bootstrap analysis with 5000 replications.

Morphometric analysis

Multivariate morphometrics was used to test the hypothesis that the groups of populations identified by AFLP analysis can be distinguished morphologically and taxonomically (for arguments in favour of this approach see HANSEN et al. 2000, FJELLHEIM et al. 2001).

Eighteen vegetative and five floral quantitative characters were measured on both field-collected and cultivated plants. Nine ratio characters were computed (Table 2). In addition, one binary floral character, colour of petals, was scored. To measure floral characters, one flower was randomly taken from each specimen. The floral parts were attached by adhesive tape to paper, dried and scanned on Microtek 9800XL scanner. The size was measured with Carnoy 2.0 (SCHOLS et al. 2002), and average value for each character was used in the analyses. Morphological characters included those traditionally used for delimitation of taxa related to *C. raphanifolia* as well as characters differentiating taxa within the related *C. amara* and *C. pratensis* groups.

Spearman correlation coefficients were computed to detect pairs of highly correlated characters. One character from a pair of highly correlated characters was excluded from further analyses.

Canonical discriminant analysis (CDA; KLECKA 1980, KRZANOWSKI 1990) was used as the hypothesis testing method, using groupings of populations resulting from AFLP analyses as groups and individual plants as units. CDA is an ordination method that maximizes differences between groups by weighting characters (KLECKA 1980, KRZANOWSKI 1990). Although several characters more or less deviated from the normal distribution, these deviations did not invalidate the presented CDA results. Multivariate normality is not required when CDA is used as an ordination procedure (KLECKA 1980, PIMENTEL 1981). To determine the characters that mostly contribute to the group separation, total canonical structure expressing correlation of characters with canonical axes was computed.

To obtain those characters that are not environmentally strongly influenced and thus are useful for identification, CDA was performed with field-collected plants and cultivated ones defined as two groups.

In the descriptions of taxa, 5% and 95% percentiles are given for character value ranges, with 1% and 99% percentiles in brackets. The SAS 8.2 package (SAS INSTITUTE 2000) was used for morphometric analyses.

Table 1. Populations of *Cardamine acris* used for the analyses: mitotic chromosome number (2n); number of individuals included in morphometric analyses (Morph-field – field-collected plants; Morph-cult – cultivated plants), and number of individuals included in the AFLP analysis. Countries: Bg – Bulgaria, Gr – Greece. Collectors: EG – Elka Georgieva, MA – Mincho E. Anchev, MP – Marián Perný, VK – Viktor Kučera.

Code	Origin and collection data	2n	Morph- -field	Morph- -cult	AFLP
Cardamine acris subsp. *acris*					
VN	Bg, Stara planina, Tetevenska planina, NW of Mt. Vezhen (2198 m), 1850 m, 21.VI.2000, MP & EG	16	25	6	1
V2	Bg, Stara planina, Tetevenska planina, NW of Mt. Vezhen, 1870 m, 21.VI.2000, MP & EG	16	9	7	-
ZA	Bg, Stara planina, Tetevenska planina, NW of Mt. Vezhen, Zavodna stream, 1500 m, 22.VI.2000, MP & EG	16	18	10	1
RP	Bg, Stara planina, Tetevenska planina, Ribarishki prokhod, 1650 m, 22.VI.2000, MP & EG	16	38	4	-
GVP	Bg, Stara planina, Tetevenska planina, Gorni vetrovity prokhod, 22.VI.2000, MP & EG	16	-	-	-
RR	Bg, Stara Planina, Tetevenska planina, Ravna Reka stream, ca. 3 km NEE of Veliovi Cherkvi Hill, ca. 1300 m a.s.l., 23.VI.2000, MP & EG	16	-	-	-
OG	Bg, Osogovska planina, above Osogovo chalet, 1600 m, 28.VI.2000, MP & MA	16	22	9	1
GE	Bg, Osogovska planina, W slopes from Gramadite (W of Trite Buky chalet) to the Ruen Hill, 1500–1600 m, 28.VI.2000, MP & MA	16	-	25	2
AR	Bg, Zapadni Rodopi, Pamporovo, Ardashla, 1450 m, 25.VI.2000, MP	16	30	24	2
OC	Bg, Zapadni Rodopi, Pamporovo, Orlitsa, 1600 m, 25.VI.2000, MP	16	-	-	1
GR	Bg, Zapadni Rodopi, Pamporovo, Golyamata Reka, 1490 m, 25.VI.2000, MP	16	-	-	1
MU	Bg, Zapadni Rodopi, Perelik range, spring fen by the path from village Mugla to Lednitsa chalet, 1300 m, 6.VII.2001, Hájek	16	-	-	-
SJ	Bg, Zapadni Rodopi, Smolyanski ezera area, 1400 m, 22.V.2002, MA & Polatschek	16	-	-	-
MN	Montenegro, Moikovach, Byelasitsa Mts., Mt. Zekova glava, 1900 m, 25.VII.2001, Šida et al.	16	-	-	1
1AE	Gr, Northern Pindhos, Ioannina/Kastoria prov., Gramos Mts., Aetomilitsa, SE slope of the valley ca. 5 km N of the village, 19.VI.2001, MP & VK	16	34	13	1
2AE	ca. 300 m from subpopulation 1AE, 19.VI.2001, MP & VK	16	-	8	1
3AE	ca. 300 m from subpopulation 2AE, 19.VI.2001, MP & VK	16	-	4	-
1VR	Gr, North Central, Pella prov., Voras Mts., Voras Ski Resort, stream along the road above the resort, 21.VI.2001, MP & VK	16	23	4	1
2VR	Gr, North Central, Pella prov., Voras Mts., Voras Ski Resort, spring near the resort, ca. 300 m from subpopulation 1VR, 21.VI.2001, MP & VK	16	-	3	-
3VR	Gr, North Central, Pella prov., Voras Mts., Voras Ski Resort, stream along the road below the resort, ca. 1km from subpopulation 2VR, 21.VI.2001, MP & VK	16	-	9	1
1PD	Gr, North Central, Florina prov., Varnous Mts., Pisoderi Ski Resort, ca. 2 km N of the ski resort, spring of stream below the unsurfaced road, 20.VI.2001, MP & VK	16	-	19	-
2PD	Gr, North Central, Florina prov., Varnous Mts., Pisoderi Ski Resort, close to the ski resort, wet places below a ski-lift, 20.VI.2001, MP & VK	16	-	2	1

Code	Origin and collection data	2n	Morph -field	Morph -cult	AFLP
3PD	Gr, North Central, Florina prov., Varnous Mts., Pisoderi Ski Resort, ca. 1 km NW of the ski resort, stream above the road, 20.VI.2001, MP & VK	16	-	2	1
4PD	Gr, North Central, Florina prov., Varnous Mts., Pisoderi, stream in the village above the road, 20.VI.2001, MP & VK	16	-	4	1
Cardamine acris subsp. *pindicola*					
1KPN	Gr, Northern Pindhos, Ioannina prov., Northern Pindhos Mts., Metsovo, Katara Pass, ca. 1.7 km S of Mt. Katara (1820 m), 18.VI.2001, MP & VK	16	-	4	1
2KPN	ca. 100 m from subpopulation 1KPN, 18.VI.2001, MP & VK	16	-	2	1
3KPN	ca. 100 m from subpopulation 2KPN, 18.VI.2001, MP & VK	16	-	9	1
2KP	Gr, Northern Pindhos, Ioannina prov., Northern Pindhos Mts., Metsovo, Katara Pass, ca. 2.5 km S of Mt. Katara, before crossroad to Haliki, 18.VI.2001, MP & VK; 29.V.2002, MP	16	22	3	1
VSC	Gr, Northern Pindhos, Grevena/Ioannina prov., Northern Pindhos Mts., Vasilitsa Ski Resort, little stream below road, 18.VI.2001, MP & VK	16	-	17	2
Cardamine acris subsp. *vardousiae*					
TM	Gr, Sterea Ellas, Evritania prov., Timfristos Mts., Karpenisi Ski Resort, stream ca. 2.5 km NW of the resort, 24.VI.2001, MP & VK	16	31	14	1
1VD	Gr, Sterea Ellas, Fokida prov., Vardousia Mts., ca. 6 km WSW of Athanasios Diakos, pass between the hills 2340 m and 2495 m, 23.VI.2001, MP & VK	16	19	5	2
2VD	stream ca. 500 m from subpopulation 1VD, 23.VI.2001, MP & VK	16	24	13	1
3VD	stream ca. 300 m from subpopulation 2VD, 23.VI.2001, MP & VK	16	-	5	1
1ITI	Gr, Sterea Ellas, Fthiotida prov., Iti Mts., ca. 10 km E of Kastania, near the crossroads close to brick spring, 24.VI.2001, MP & VK	16	-	13	1
2ITI	ca. 300 m from subpopulation 1ITI, 24.VI.2001, MP & VK	16	-	6	1

RESULTS

Chromosome numbers

All individuals of *C. acris* analyzed were diploid with 2n=2x=16 and all counts represent new reports (Table 1).

AFLP analysis

170 bands were scored, 13 of which were monomorphic, and 41 restricted to a single individual (Table 3). The highest number of bands (138) was observed in plants from Bulgaria, Montenegro and Northwestern Greece, in contrast to plants from the Northern Pindhos Mts. with only 75, and those from Central Greece with 79 bands. These differences might be, however, influenced by the different amount of studied plants. Average number of bands per individual ranged from 47.13 in plants from Northwestern Greece and Montenegro to 53.17 in plants from the Northern Pindhos Mts. The highest number of exclusive AFLP

Table 2. Characters included in morphometric analysis. [1] – character used only for ratio computation, [2] – the leaf closest to the midpoint of the leafy part of stem (LSL/2 distance).

Vegetative characters	
WIS	width of stem (mm)
LSL	height of stem to the base of the uppermost leaf (cm)
LSI[1]	height of stem to the base of the lowermost flower/fruit (cm)
NB	number of stem branches
NL	number of stem leaves
LC1	length of basal leaf (cm)
LC2[2]	length of middle stem leaf (cm)
LC3	length of the uppermost leaf (cm)
NFB	number of lateral leaflet pairs of basal leaf
LTB	length of terminal leaflet of basal leaf (cm)
WTB	width of terminal leaflet of basal leaf (cm)
LLB	length of first lateral leaflet of basal leaf (cm)
WLB	width of first lateral leaflet of basal leaf (cm)
NFS[2]	number lateral leaflet pairs of middle stem leafl
LTS[2]	length of terminal leaflet of middle stem leaf (cm)
WTS[2]	width of terminal leaflet of middle stem leaf (cm)
LLS[2]	length of first lateral leaflet of middle stem leaf (cm)
WLS[2]	width of first lateral leaflet of middle stem leaf (cm)
Flower characters	
LS	length of sepals (mm)
LP	length of petals (mm)
WP	width of petals (mm)
LFL	length of longer filaments (mm)
LFS	length of shorter filaments (mm)
Ratio characters	
LSL/LSI, NL/LSL, LC1/LSL, LC2/LSL, LC3/LSL, LC2/LC1, LC3/LC2, WP/LP, LFS/LFL	

bands was observed in plants from Bulgaria, Montenegro and Northwestern Greece (70). No diagnostic bands were present except the group from Northern Pindhos Mts. that had one diagnostic band (Table 3).

In the PCoA ordination diagram, three distinct groups of individuals were separated along the first two axes (Fig. 2). These groups corresponded to the geographic origin of the plants. Plants from Central Greece and the Northern Pindhos Mts. formed two groups separated along the first axis and the second axis. Plants from Bulgaria and Northwestern Greece (plus a single individual from Montenegro) were separated from these two groups along the first axis, and they were separated from each other along the third axis.

Also in the neighbour-joining tree, clustering was consistent with the geographic origin of plants (Fig. 3). The Northern Pindhos Mts. and Central Greece clusters received high bootstrap support (96% and 89%, respectively), and a cluster of plants from Bulgaria, Northwestern Greece and Montenegro was supported moderately (72%). Within the latter, two subclusters were formed, corresponding to individuals from Bulgaria and Northwestern Greece (including one sample from Montenegro), but without high bootstrap support (< 50%).

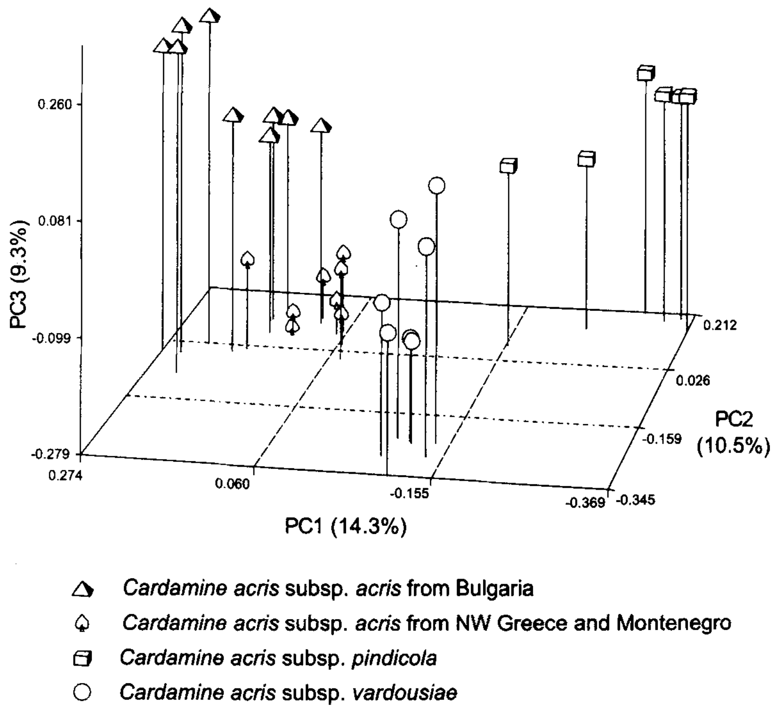

Fig. 2. Principal coordinate analysis of AFLP phenotypes of *Cardamine acris* based on Jaccard's coefficient.

Morphometric analysis

Character LSI was removed from further analyses due to the high correlation (Spearman correlation coefficient = 0.99) with character LSL.

Two separate canonical discriminant analyses (CDA) were caried out, based on individual plants and the groups based on the AFLP analysis and geographical criteria. The first was based on field-collected plants (Fig. 4) and the second one on the plants from the common garden experiment (Fig. 5). They both showed clear morphological differentiation among groups. There was only marginal overlap of individual specimens in separate CDAs of field-collected plants and cultivated plants. Correlation of ten characters with the first canonical axis in CDA of field-collected plants exceeded the arbitrary level of 0.6, in particular that of LSI/LSL and LC3/LSL (Table 4A). In the analysis of cultivated plants, only five characters (out of these ten) expressed correlation higher than 0.6 with the first axis, in particular the number of stem branches and LSL/LSI (Table 4B). In both analyses several characters contributed to the separation of objects along the second axis, but none of them has correlation with this axis higher than 0.6.

CDA based on 31 morphological characters with field-collected and cultivated plants defined as two groups, revealed a shift between these groups. Fourteen characters contributed mostly to the shift as seen from their correlations with canonical axis (exceeding arbitrary chosen value of 0.4, see Table 4C). Thus, they were assumed to be seriously influenced by the

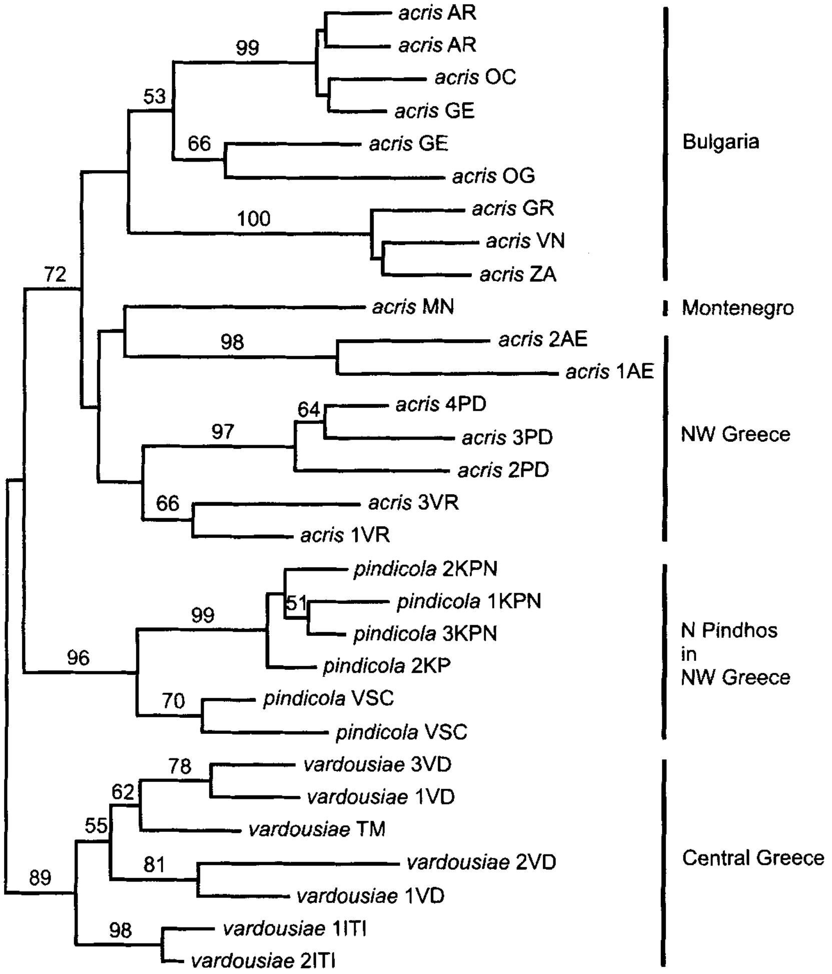

Fig. 3. Neighbour-joining analysis of AFLP data of *Cardamine acris* based on NEI & LI (1979) genetic distance. Bootstrap values above 50% are shown. For population codes see Table 1.

environmental conditions, and were not used for constructing an identification key. Especially those characters expressing the size of leaves differed strongly in their ranges between field-collected and cultivated plants (e.g., length of leaves, Fig. 5c,d; length and width of their terminal leaflet, Fig. 5e, g; or ratio LC1/LSL, Fig. 5j).

Populations from the Northern Pindhos Mts. had only white petals. Two out of three populations from Central Greece (TM and ITI, see Table 1) had pink petals, while within the population from Mt. Vardousia (VD, see Table 1) both white- and pink-flowered individuals were found; all plants of the subpopulation 2VD had white petals, those in the subpopulation 3VD had pink petals, and both white- and pink-flowered plants (in more-less equal proportion) grew in the subpopulation 1VD. Two plants originating from Stara Planina in

Table 3. Number of AFLP bands scored in the subspecies of *Cardamine acris*. BT (bands total) – total number of bands present in a given taxon (or in a group of populations); BA (bands average) – mean number ± standard deviation of bands present per individual; EB (exclusive bands) – bands present in a given taxon/group, but absent in other taxa/groups; DB (diagnostic bands) – bands present in all samples of a given taxon/group, but absent in other taxa/groups.

Taxon/group (number of individuals)	BT	BA	EB	DB
subsp. *acris* (17)	138	50.82 ± 5.54	70	0
subsp. *acris* – Bulgaria (9)	105	54.11 ± 2.62	33	0
subsp. *acris* – Greece and Montenegro (8)	9	47.13 ± 5.72	25	0
subsp. *pindicola* (6)	75	53.17 ± 4.02	11	1
subsp. *vardousiae* (7)	79	50.43 ± 5.62	15	0
Total	170	51.20 ± 5.13	-	-

Bulgaria (RP, see Table 1) had white petals. All other individuals from Bulgaria, Northwestern Greece (except the Northern Pindhos Mts.), Albania and countries of former Yugoslavia represented by both specimens collected by us and those deposited in several herbaria (see Appendix) had petals of pink to reddish-violet colour.

DISCUSSION

We conclude that three subspecies of *C. acris* can be distinguished in the Balkan Peninsula: *C. acris* subsp. *acris*, and the here described subsp. *pindicola* and subsp. *vardousiae* (see Taxonomy). The genetic and morphological variation among the *C. acris* populations is mainly related to geographic distribution. The populations from Central Greece (subsp. *vardousiae*), and white flowering populations from the Northern Pindhos Mts. in Northwestern Greece (subsp. *pindicola*), represent two genetically well defined groups (PCoA ordination diagram and neighbour-joining tree of AFLP data, Figs. 2, 3). Their separate positions within *C. acris* were confirmed also in discriminant analyses of quantitative characters based on individual plants (Figs. 4, 5). Although there is a slight shift in the ordination diagram of the AFLP phenotypes of Bulgarian plants of *C. acris* subsp. *acris* in respect of those from Northwestern Greece and Montenegro (Fig. 2), neither of these groups received considerable bootstrap support in the neighbour-joining tree (Fig. 3). This suggests that despite high morphological and genetic variability among these populations, all belong to one subspecies. Our sampling does not cover the distribution area of *C. acris* subsp. *acris* completely. It was not possible to include population samples from Albania and from countries of the former Yugoslavia (except one sample from Montenegro used for AFLPs). Nevertheless, based on herbarium specimens studied from this area (see Appendix), we can conclude that no other morphologically distinct taxon can be distinguished within this geographic area.

A comparison between field-collected plants and those cultivated in the common garden experiment revealed shifts in most characters expressing organ sizes as well as in some ratio characters expressing shapes of organs (see Fig. 6, Table 3C). This was also observed in other plant groups (e.g., BRYSTING & ELVEN 2000, KAPLAN 2002). The ranges of values remained to a large extent the same or at least relative differences were retained for 17 out of 31

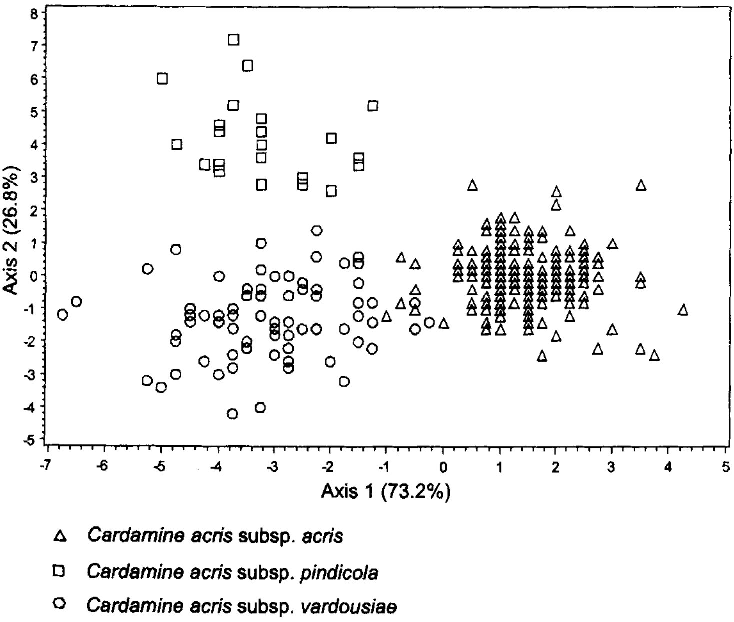

Fig. 4. Canonical discriminant analysis based on 31 morphological characters of field-collected individuals of *Cardamine acris*, with three subspecies as groups: subsp. *acris*, subsp. *pindicola*, and subsp. *vardousiae*.

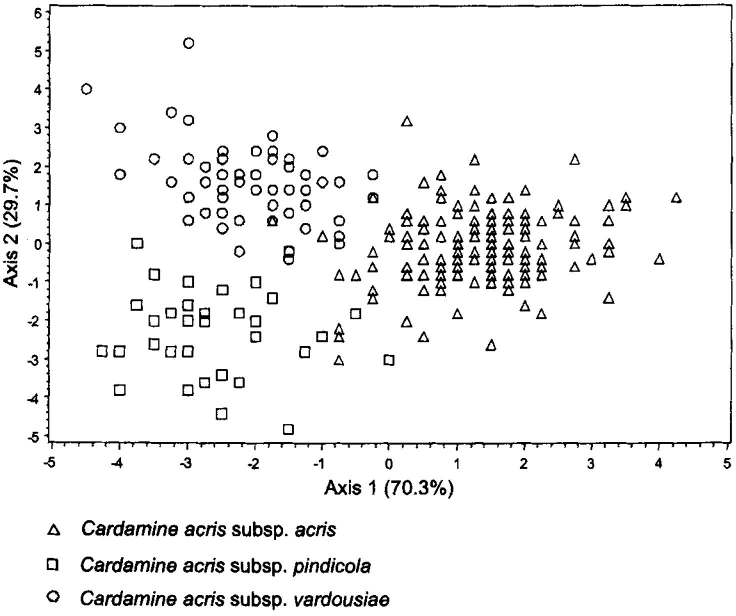

Fig. 5. Canonical discriminant analysis based on 31 morphological characters of cultivated individuals of *Cardamine acris*, with three subspecies as groups: subsp. *acris*, subsp. *pindicola*, and subsp. *vardousiae*.

Table 4. Canonical discriminant analysis based on 31 morphological characters and field-collected (A) and cultivated (B) individuals of *Cardamine acris*, with the three subspecies as groups: subsp. *acris*, subsp. *pindicola*, and subsp. *vardousiae*. CDA based on 31 morphological characters and individuals of *C. acris*, with field-collected and cultivated plants as groups (C). Total canonical structure expressing correlation of morphological characters with canonical axes (CAN1, CAN2) is presented; correlations exceeding the value 0.6 (A, B) or 0.4 (C) are marked in bold. For character abbreviations see Table 2.

Character	A		B		C
	CAN1	CAN2	CAN1	CAN2	CAN1
WIS	0.510	0.220	0.560	-0.310	-0.270
LSL	**0.710**	0.230	0.520	-0.480	-0.330
NB	**0.710**	0.100	**0.800**	-0.130	-0.050
NL	**0.660**	0.060	**0.640**	-0.280	0.330
LC1	0.370	0.140	0.530	-0.090	**-0.670**
LC2	0.380	0.300	0.120	-0.070	**-0.630**
LC3	-0.230	0.370	-0.410	-0.150	**-0.430**
NFB	**0.640**	-0.230	**0.670**	0.170	0.140
LTB	0.210	0.450	0.400	-0.210	**-0.420**
WTB	0.170	0.480	0.460	-0.050	**-0.410**
LLB	0.230	0.500	0.530	-0.060	**-0.420**
WLB	0.210	0.520	0.470	0.010	**-0.420**
NFS	0.450	-0.260	0.400	0.320	-0.380
LTS	0.210	0.410	-0.030	-0.310	**-0.480**
WTS	0.090	0.430	0.010	0.100	**-0.450**
LLS	0.360	0.410	0.290	-0.070	**-0.520**
WLS	0.080	0.520	0.160	0.200	**-0.490**
LS	**-0.710**	0.180	-0.440	-0.390	0.300
LP	**-0.620**	-0.030	**-0.640**	-0.140	0.390
WP	**-0.740**	0.080	-0.410	-0.080	0.330
LFL	-0.320	-0.130	-0.510	-0.350	0.370
LFS	-0.290	-0.230	-0.220	-0.360	0.400
LSL/LSI	**0.770**	0.110	**0.750**	-0.310	-0.010
NL/LSL	-0.420	-0.420	0.130	0.160	**0.550**
LC1/LSL	-0.520	-0.200	0.050	0.340	**-0.590**
LC2/LSL	-0.560	0.060	-0.430	0.460	**-0.560**
LC3/LSL	**-0.770**	-0.050	-0.590	0.280	-0.160
LC3/LC2	**-0.670**	-0.090	-0.450	-0.020	0.260
LC2/LC1	0.090	0.220	-0.450	0.050	0.160
WP/LP	-0.530	0.140	0.040	0.030	0.120
LFS/LFL	-0.070	-0.200	0.200	-0.190	0.230

characters. The comparison of characters of field-collected versus cultivated plants allowed us to obtain those characters that are not environmentally strongly influenced, and thus are useful for identifying and constructing an identifying key (see below).

Our study on the Balkan diploid *C. acris* provides an example of allopatric differentiation, where classification at the level of subspecies is most adequate. All subspecies of *C. acris* have more or less overlapping values of morphological characters (Fig. 5), and no diagnostic AFLP fragments are present in subsp. *acris* and subsp. *vardousiae*, and just one is in subsp. *pindicola* (Table 3). Moreover, neither morphological characters nor habitat preferences suggest adaptive differentiation. The rank of subspecies is appropriate if the separation of populations has been too short to result in distinct morphological divergence (e.g., MARHOLD 1998, LIHOVÁ et al. 2000, SÁEZ et al. 2002, GREGOR 2003) or when the differentiation is

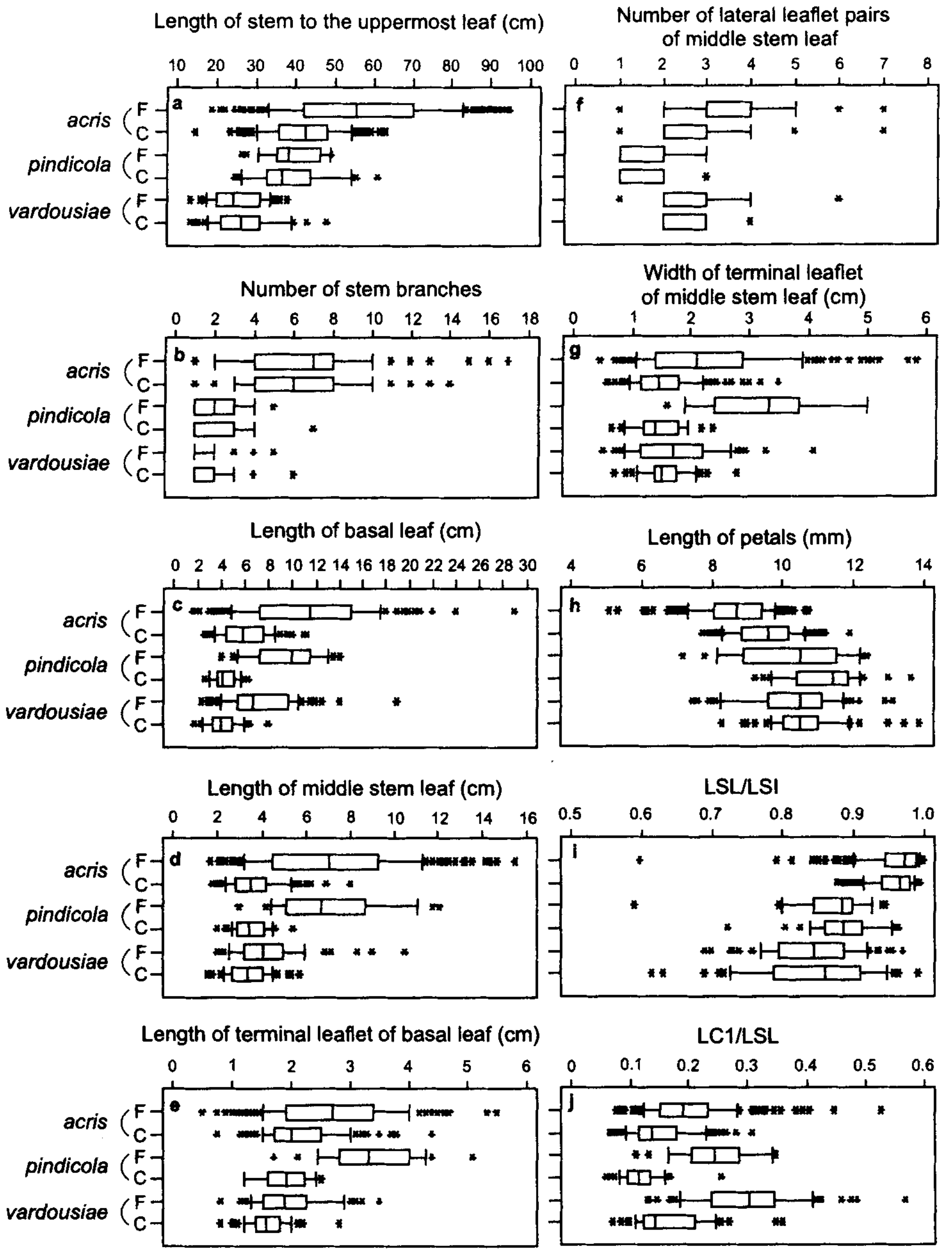

Fig. 6. Variation in selected morphological characters among the three subspecies, and compared between field-collected (F) and cultivated (C) plants. *Cardamine acris* subsp. *acris* – plants collected in the field (n = 199), cultivated plants (n = 153); subsp. *pindicola* – plants collected in the field (n = 22), cultivated plants (n = 35); subsp. *vardousiae* – plants collected in the field (n = 74), cultivated plants (n = 56). Rectangles define 25 and 75 percentiles; vertical lines show median; whiskers define 10 and 90 percentiles; asterisks show extreme values.

counteracted by occasional gene flow or introgression (e.g., MARHOLD 1992, KRAUSS 1996, HODÁLOVÁ et al. 2002). Both might be the case in *C. acris*.

The differentiation among three subspecies of *C. acris* is most probably caused by isolation in different mountain ranges in the Balkan Peninsula during the Pleistocene glaciations. Such isolations might have been related to shifts of altitudinal belts of vegetation (WILLIS 1994, FERRIS et al. 1999). *C. acris* is nowadays mainly found in montane and subalpine habitats between 1000 and 2100 m a. s. l. It needs a humid environment and it is reasonable to assume that it had a more compact distribution during the wetter periods of the Pleistocene. The predominant climate during cold stages, however, was drier than at present (FRENZEL et al. 1992). Steppes and forest-steppes of boreal type dominated the Balkan Peninsula. Thus, also during cold periods, the area of *C. acris* might have been fragmented. The more pronounced divergence between subsp. *pindicola* and subsp. *vardousiae* than between the Northwestern Greek and Bulgarian populations of subsp. *acris* might result from earlier isolation in southern latitudes due to dessication or other environmental factors.

Patterns of distribution similar to *C. acris* are found in several species or species complexes in *Brassicaceae* (JALAS & SUOMINEN 1994, JALAS et al. 1996, STRID & KIT TAN 2002). For example, *Aubrieta gracilis* BOISS. from the mountains of the southern half of the Balkan Peninsula differentiated in three subspecies: subsp. *scardica* (WETTST.) PITHOS occurs throughout most of the range of the species, while subsp. *glabrescens* (TURILL) AKEROYD is endemic to the North Pindhos Mts. in Northwestern Greece and susbsp. *gracilis* is endemic to Central Greece (AKEROYD & BALL 1993). Or, from *Draba lasiocarpa* ROCHEL s.l. with wide distribution (the Balkans, Southern and Eastern Carpathians, Eastern Alps), two narrow Greek endemics (treated either as species or subspecies) are distinguished: *D. laconica* STEVAN. et KIT TAN (Southern Peloponesos, Mt. Taygetos) and *D. lasiocarpa* subsp. *dolichostyla* (O. E. SCHULTZ) BUTTLER (Central Greece and Albania) (WALTERS 1993, STRID & KIT TAN 2002). *Aethionema saxatile* (L.) R. BR. differentiated into four subspecies in the Balkan Peninsula, with subsp. *athoum* (GRISEB.) HAYEK and subsp. *oreophilum* I.A. ANDERSON being endemic to southern half of the Balkan Peninsula, and subsp. *graecum* (BOISS. et SPRUNER) HAYEK endemic to Central Greece and Northeastern Peloponnisos (ANDERSON et al. 1983, JALAS et al. 1996, STRID & KIT TAN 2002). Other examples of Northern Pindhos or Central Greece endemics include, e.g., *Erysimum parnassi* (BOISS. et HELDR.) HAUSSKN. (JALAS & SUOMINEN 1994), or *Bornmuellera baldacii* subsp. *rechingeri* GREUTER and *B. tymphaea* (HAUSSKN.) HAUSSKN. (JALAS et al. 1996). Similar patterns of differentiation are found also in other families, e.g. the Southern Balkan endemic *Silene parnassica* BOISS. et SPRUNER (*Caryophyllaceae*) differentiated in five subspecies: subsp. *pindicola* (HAUSSKN.) GREUTER is restricted to Northern Pindhos and SE Albania, and another three subspecies are narrow endemics of various Southern Balkan mountains geographically isolated from subsp. *parnassica* (GREUTER 1997). Among three Balkan subspecies of *Silene pusilla* WALDST. et KIT., subsp. *tymphyaea* GREUTER is a narrow endemic of Northern Pindhos Mts. (GREUTER 1997). Further, many cases of geographic differentiation in Balkan flora are connected with polyploidization (for examples from *Brassicaceae* see, e.g., ANCHEV & TOMŠOVIC 1999, YURUKOVA-GRANCHAROVA et al. 2004, PERNÝ et al., unpubl. data).

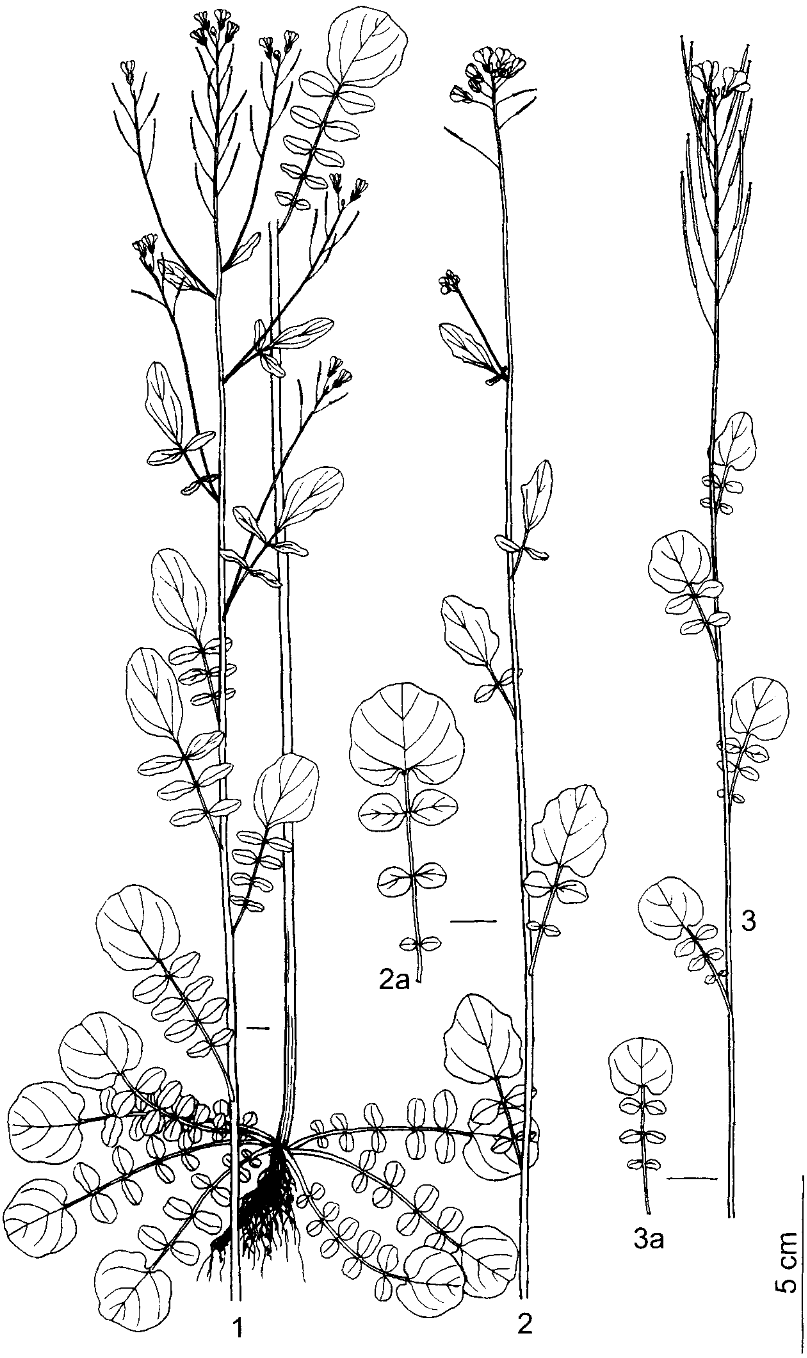

Fig. 7. Habitus of *Cardamine acris* subsp. *acris* (1), subsp. *pindicola* (2; 2a – basal rosette leaf) and subsp. *vardousiae* (3; 3a – basal rosette leaf). Leaf closest to the midpoint of leafy part of stem is indicated by arrow (drawing by Zlata Komárová).

The southern part of the Balkan Peninsula was an important refugium during Pleistocene glaciations and many species have recolonized Europe from populations that survived there (HEWITT 1999). Moreover, we can conclude that mountains in this region, especially in Central and Northwestern Greece, have been important areas for differentiation of many Balkan endemics. This might be caused by isolation of plant populations in these mountains during Pleistocene climatic changes, but molecular phylogeographic studies of the Balkan flora are needed to clarify this hypothesis.

Key to subspecies of *Cardamine acris*

1. Petals white 2
- Petals pale pink to reddish-violet, exceptionally white 3
2. Number of leaflets of the rosette leaves 5–9, number of leaflets of the middle stem leaf 3–7; length of stem between the base and uppermost leaf 26–55 cm; endemic to the Northern Pindhos Mts. in Northwestern Greece .... *C. acris* subsp. *pindicola*
- Number of leaflets of the rosette leaves 7–11, number of leaflets of the middle stem leaf 5–9; length of stem between the base and uppermost leaf 16–38 cm; endemic to Central Greece .... *C. acris* subsp. *vardousiae*
3. Stem unbranched or with up to 2 branches; length of stem between the base and uppermost leaf 16–38 cm; maximum width of stem 1.3–4.0 mm; petals 8.1–12.2 mm long; endemic to Central Greece .... *C. acris* subsp. *vardousiae*
- Stem with 1–11 branches, rarely unbranched; length of the stem between the base and uppermost leaf 29–84 cm; maximum width of stem 2.0–7.8 mm; petals 7.2–10.7 mm long; widespread in the Balkan Peninsula, absent in Central Greece and in the Northern Pindhos Mts. in Northwestern Greece .... *C. acris* subsp. *acris*

TAXONOMY

Cardamine acris GRISEB., Spic. Fl. Rumel. 1: 253, 254 (1843).

≡ *Cardamine raphanifolia* subsp. *acris* (GRISEB.) O.E. SCHULZ, Bot. Jahrb. Syst. 33: 512 (1903).

Ind. loc.: "In regione alpina Macedoniae et Serbiae australis: socialis in herbosis humidis et ad rivulos m. Nidgé 4400′–5500′ (substr. marmor.), gregariae ad fontem m. Kobelitza juxta Mandram Weizensem alt. 4200′ (substr. micasch.)!; in rivulo m. Kopaunik 5700' alt. (Friedr.)."

Lectotypus (STRID 1986: 257): Ad fontem gregarie m. Kobelitza juxta Mandramt [sic!] (GRISEBACH, no. 943, GOET).

Description: Perennial herb, (25–)30–90(–102) cm tall; rhizome erect to ascending, rarely prostrate; stem erect, simple or with up to 11(–14) branches, glabrous and pruinose, (1.3–)1.8–7.0(–10.0) mm wide. Leaves forming a basal rosette. Young rosette leaves hairy, rarely glabrous, later becoming glabrous on the blade, but remaining hairy on margin, rarely completely glabrous, pinnate, (2.4–)3.1–17.0(–21.0) cm long, with (2–)3–7(–8) pairs of lateral leaflets, their terminal leaflet much larger than the lateral ones, orbicular to broadly circular, (0.9–)1.2–4.0(–4.7) cm long, (0.9–)1.2–4.2(–5.2) cm wide, lateral leaflets sessile, ovate or elliptic to orbicular, first lateral leaflets (0.3–)0.4–1.9(–2.5) cm long, (0.3–)0.4–1.8(–2.2) cm wide. Stem leaves 4–14(–17), mostly in the upper half of the stem, pinnate or pinnatisect, similar to the rosette leaves, hairy on their margin; middle stem leaves (1.8–)2.3–11.1(–14.3) cm long, with 1–5(–6) pairs of sessile lateral leaflets or segments; terminal leaflet or segment elliptic or obovate to almost orbicular, (0.8–)1.1–4.1(–5.3) cm long, (0.6–)0.9–4.0(–5.1) cm wide, lateral leaflets or segments elliptic, first lateral leaflets (0.3–)0.5–2.6(–3.1) cm long, (0.2–)0.3–1.4(–2.2) cm wide. Inflorescence racemose, peduncles glabrous. Sepals ovate with membranous margins, (2.6–)2.8–4.5(–4.8) mm long, (1.3–)1.4–2.4(–2.7) mm wide. Petals white or pale pink to reddish-violet, obovate, (6.1–)7.5–11.8(–13.0) mm long and (2.8–)3.6–7.1(–7.5) mm wide, with short claw, apex truncate to emarginate, glabrous. Stamens 6, tetradynamous, shorter filaments (1.7–)2.2–4.8(–5.1) mm long, longer filaments (3.0–)4.0–6.1(–6.6) mm long; anthers yellow. Stigma wider than style. 2n=2x=16.

Distribution and habitat: Balkan Peninsula; stream banks in wet meadows and pastures, streams in *Fagus* forests, 1000–2100 m.

Cardamine acris subsp. *acris*

- *Cardamine amara* subsp. *barbaraeoides* auct. non (HALÁCSY) MAIRE et PETITM., p.p.: KIT TAN (2002)

Description: Herb (29–)35–94(–102) cm tall. Stem (1.3–)2.0–7.8(–10.0) mm wide, with 1–11(–14) branches, rarely simple. Rosette leaves hairy, later becoming glabrous on blade, but hairy on margin, rarely completely glabrous, (2.6–)3.5–18.0(–21.0) cm long, with 3–7(–8) pairs of lateral leaflets, their terminal leaflet (0.9–)1.3–4.0(–4.7) cm long, (1.0–)1.3–4.3(–5.2) cm wide, first pair of lateral leaflets (0.3–)0.5–2.0(–2.5) cm long, (0.3–)0.5–1.8(–2.2) cm wide. Stem leaves (5–)6–14(–17); middle stem leaves (1.8–)2.3–11.5(–14.3) cm long, with (1–)2–5(–6) pairs of lateral leaflets or segments; terminal leaflet or segment (0.8–)1.1–4.3(–5.3) cm long, (0.6–)0.9–4.0(–5.1) cm wide, lateral leaflets or segments (0.3–)0.5–2.6(–3.1) cm long, (0.2–)0.3–1.4(–2.2) cm wide. Sepals (2.6–)2.8–4.2(–4.5) mm long, (1.3–)1.4–2.3(–2.5) mm wide. Petals pale pink to reddish-violet (only very exceptionally white), (6.1–)7.3–10.7(–11.2) mm long and (2.8–)3.5–6.4(–7.0) mm wide. Shorter filaments (1.7–)2.2–4.8(–5.1) mm long, longer filaments (3.0–)4.0–6.1(–6.6) mm long.

Cardamine acris subsp. *pindicola* PERNÝ & MARHOLD, subsp. nov.

- *Cardamine raphanifolia* subsp. *barbaraeoides* auct. non (HALÁCSY) STRID, p.p.: STRID (1986)
- *Cardamine amara* subsp. *barbaraeoides* auct. non (HALÁCSY) MAIRE & PETITM., p.p.: KIT TAN (2002)
- *Cardamine pindicola* HAUSSKNECHT, nom. inval.

Diagnosis: A *Cardamine acris* subsp. *acris* petalis albis, ramulis caulibus minoribus (1–4 nec 2–12) et foliolis foliorum rosularium minoribus (5–9 nec 7–15) differt. A *C. acris* subsp. *vardousiae* foliolis foliorum rosularium minoribus (5–9 nec 7–11) differt.

Holotypus: Greece, Ioannina, North Pindhos Mts., Metsovo, Katara Pass, ca. 2.5 km S of Mt. Katara (1820 m), before crossroad to Haliki, 29.V.2002, PERNÝ (SAV)

Description: Herb (35–)41–71(–77) cm tall. Stem (1.8–)2.0–5.0(–7.0) mm wide, simple or with up to 3(–6) branches. Rosette leaves hairy, later becoming glabrous on blade, but hairy on margin, rarely completely glabrous, (2.7–)3.0–13.0(–14.1) cm long, with (1–)2–4(–5) pairs of lateral leaflets, their terminal leaflet 1.2–4.3(–5.1) cm long, (0.9–)1.1–4.6(–5.8) cm wide, first lateral leaflets (0.2–)0.3–2.3(–2.9) cm long, (0.2–)0.3–2.1(–2.3) cm wide. Stem leaves 4–11(–14); middle stem leaves (2.0–)2.5–11.1(–12.1) cm long, with 1–3 pairs of lateral leaflets; terminal leaflet (1.1–)1.3–4.6(–5.3) cm long, (0.7–)0.8–5.0 cm wide, first lateral leaflets (0.4–)0.5–2.7(–3.0) cm long, 0.2–2.3(–2.7) cm wide. Sepals (3.1–)3.5–4.8(–5.1) mm long, (1.6–)1.7–2.9(–3.0) mm wide. Petals white, (7.2–)8.2–12.4(–13.7) mm long and (3.8–)4.0–7.6(–8.0) mm wide. Shorter filaments (2.2–)2.4–4.7(–5.8) mm long, longer filaments (4.2–)4.6–7.1(–7.6) mm long.

Cardamine acris subsp. *vardousiae* PERNÝ & MARHOLD, subsp. nov.

- *C. raphanifolia* subsp. *barbaraeoides* auct. non HALÁCSY, p.p.: STRID (1986)
- *Cardamine amara* subsp. *barbaraeoides* auct. non (HALÁCSY) MAIRE & PETITM., p.p.: KIT TAN (2002)

Diagnosis: A *Cardamine acris* subsp. *acris* ramulis caulibus minoribus (1–3 nec 2–12), caulibus angustioribus (1.3–4.0 nec 2.0–7.8 mm) et brevioribus (16–38 nec 29–84 cm) differt. A *Cardamine acris* subsp. *pindicola* foliolis foliorum rosularium pluribus (7–11 nec 5–9) et caulibus longioribus (26–55 nec 16–38 cm) differt.

Holotypus: Greece, Fokida, Vardousia Mts., ca. 6 km WSW of Athanasios Diakos, pass between the hills 2340 m and 2495 m, 23.VI.2001, PERNÝ & V. KUČERA (SAV)

Description: Herb (25–)26–48(–53) cm tall. Stem (1.0–)1.3–4.0(–5.0) mm wide, simple or with up to 2(–4) branches. Young rosette leaves hairy, rarely glabrous, later becoming glabrous, rarely loosely hairy on their margin, (2.3–)2.5–10.8(–14.0) cm long, with (2–)3–5(–6) pairs of lateral leaflets, their terminal leaflet (0.8–)1.2–2.9(–3.2) cm long, (1.0–)1.2–3.2(–3.6) cm wide, first lateral leaflets (0.3–)0.4–1.3(–1.5) cm long, (0.3–)0.4–1.3(–1.5) cm wide. Stem leaves 4–9(–11); middle stem leaves (1.8–)2.0–5.9(–9.0) cm long, with (1–)2–4 pairs of lateral leaflets; terminal leaflet (0.8)1.1–2.7(–3.1) cm long, (0.7–)0.8–2.7(–3.3) cm wide, first lateral leaflets 0.4–1.3(–1.7) cm long, 0.2–1.0(–1.5) cm wide. Sepals (2.8–)3.1–4.4(–5.0) mm long, (1.5–)1.6–2.2(–2.4) mm wide. Petals white or pale pink to reddish-violet, (7.6–)8.1–12.2(–13.5) mm long and (4.1–)4.6–7.3(–7.5) mm wide. Shorter filaments (2.1–)2.3–4.3(–5.2) mm long, longer filaments (4.1–)4.4–6.6(–7.4) mm long.

Acknowledgements: Thanks go to Viktor Kučera and other colleagues for help during our collection trips. We thank Lenka Mártonfiová (Košice) for useful discussion concerning the preparation of slides for chromosome number counts, Dmitar Lakušić (Beograd) for comments on distribution of Balkan taxa, Michael Barfuss (Vienna) for preparing polyacrylamide gels for AFLP analyses, Ivan Pišút (Bratislava) for Latin diagnoses, and Zlata Komárová (Bratislava) for drawings of plants. Peter Schols (Leuven) kindly provided program Carnoy 2.0. Special thanks go to Judita Lihová and Karol Marhold (Bratislava) for comments and corrections of the paper. The curators of herbaria B, BEO, BEO, BM, BRNM, BRNU, C, JE, K, KRA, M, MA, PR, SO, SOM, W, WU, ZT are acknowledged for allowing us to study herbarium specimens. The financial support was provided by project no. 3042 from the Grant Agency VEGA, Bratislava, Slovak Republic.

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Received 11 July 2004, revision received and accepted 13 October 2004
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APPENDIX

List of herbarium specimens studied

C. acris subsp. *acris*
Bulgaria: Stara Planina (Kl. Balkan) (s.d. G. ILIČ WU). Stara planina: Tetevenskiya balkan (VI.1898 S. GEORGIEV SO 28102). Zapadna Stara planina: do B"rza reka pod vr"h Midzhur, 1900 m (20.V.1993 V. VLADIMIROV SO 99236). Osogovska Planina (VIII.1887 J. VELENOVSKÝ BRNM; VIII. 1887 K. VANDAS PRC; 1.VII.1895 D. MICHAÎLOV SO 28100; 15.VII.1903 I.K. URUMOV SOM 32030). Balkan centr., infra cacumina Baba, 1400 m (2.V.1902 A.TOSHEV SOM 96435). Ruj Planina (16.VI.1895 D. MICHAÎLOV SO 28103); Mt. Ruj, 1400 m (24.VI.1933 B. ACHTAROFF SOM 32028). Etropolski Balkan (7.VI.1904 I.K. URUMOV SOM 32031). Karlovski Balkan (16.VII.1927 I.K. URUMOV SOM 32035). Trojanski Balkan (1895 I.K. URUMOV SOM 32020). Pirin, in subalpinis ad rivum Damjanitza (16.VII.1929 N. STOJANOFF, B. STEFANOFF & T. GEORGIEFF, Flora bulgarica exsiccata, K). Pirin: in pratis subalpinis ad rivam (VII.1931 V. KRIST PRC). Pirin: ad oppidum Bansko, vallis Damjanica (VII.1936 I. KLÁŠTERSKÝ & M. DEYL PRC). N Pirin, po polyana nad Gr"ncharska skala, 1000 m (28.V.1982 C. DENCHEV SO 93048). Rila australis, paludosis ad fontem Catal Cesme, sub cacuminem Carev Vrch, 2100 m (19.VI.1931 N. STOJANOFF & T. GEORGIEFF, Flora bulgarica exsicata, BRNU). Zapadna B"lgariya, Tr"nsko: K"rvavi kam"k,1600 m (29.V.1958 V. KITANOV SO 32660). Zapadni Rodopi, Pamporovo (V.1974 J. KOEVA SO 86725). **Albania:** Bertiscus, in valle rivuli Sočanska Bistrica, ad coram Kurdala, ca. 1600 m (18.VII.1933 RECHINGER fil. et SCHEFFER, Iter balcanicum 1933 No. 1080, K). Distrikt Kalis, Korab [Mts.], an quelligen Orten der Čafa Korabit, ca. 2200 m (1.VII.1918 I. DÖRFLER, Reisen in Nord-Albanien 1918 No. 755, WU). Distrikt Šala, an quelligen Orten der Fuša Nermajns bei Abata, ca. 1700 m (17.VI.1916, 19.VI.1916 I. DÖRFLER, Reisen in Nord-Albanien 1916 und 1918. No. 100 C (17.VI.1916); K, WU, BRNU (19.VI.1916)). Grammos Range, above Ersekë, 5500 ft. (27.VI.1933 A.H.G. ALSTON & N.Y. SANDWITH *1938* K). Montes Albaniae boreali-orientalis inter opp. Prizren et Debra jacentes, montes nivales Korab, supra pagum Radomir, ca. 1900 m (23.VII.1918 J.B. KÜMMERLE K). Shemeri (NE of Elbasan), 3000 ft. (22.V.1935 R.V. PENNINGTON, Flora of Albania No. 129, K). **F.Y.R.O.M.:** Debar, montibus Korab, prope karaul Skrtac, ca. 2300 m (15.VII.1937 F. WEBER PRC). Debar, montibus Korab: supra pag. Žirovnica, ca. 1900 m (16.VII.1937 F. WEBER BRNM). Korab [Mts.]: Mala vratna (19.VII.1939 I. RUDSKI BEO). Korab [Mts.]: Savrovo (VI.1935 V LINDTNER BEO). Ad Mavrova, ad fl. Radika reka, ad fin. Alban., 1250 m, (23.V.1918 J. BORNMÜLLER, Plantae Macedoniae No. 3343, JE). Golešnica Planina, Doln. Mandra-Begova (28.VI.1918 J. BORNMÜLLER, Plantae Macedoniae No. 3340; 26.VI.1918 J. BORNMÜLLER, Plantae Macedoniae No. 3347, JE). Mt. Kossov ad Zborsko (26.VI.1893 J. DÖRFLER, Iter turcicum secundum 1893 No. 29, JE, K, WU). Mt. Schelesna-Vrata prope Allchar (6.VII.1893 J. DÖRFLER, Iter turcicum secundum 1893 No. 30, JE, K, WU). Maurovi Hanovi, nad jezerem, ca. 1000 m (14.VI.1976 L. POKLUDA BRNM). N Macedonia, below Pepeljak, ca. 1800 m (10.VI.1935 H.P. THOMPSON, Flora of N Macedonia No. 693, K). Supra Štrbce, vpravo od Tetovskeho putu, ca. 1900 m (18.IX.1922 K. VANDAS PRC). Šar planina (21.VII.1989 V. STEVANOVIĆ & S. JOVANOVIĆ BEOU). Šar planina, Mt. Luboten [Ljubotin] (16.VI.1937 H.P. THOMPSON, Flora of N Macedonia No. 952, K; VII.1937 M. DEYL PRC; 1938 F. WEBER PRC). Šar Planina, Popova Šapka (5.VII.1959 J.D.A. STAINTON *7886* K; 1.VII.1982 F. ČERNOCH *39356*, Rem. Planta Peninsulae Balcanicae No. 11255, C, B 100127705; 1.VI.1971 N. DIKLIĆ BEO; 28.VII.1972 V. NIKOLIĆ et al. BEO). Šar Planina: zwischen der Popova Šapka und der Pašina Planina, ca. 2000 m (23.V.1968 M. BÄSSLER & I. QUASDORF, Flora von Jugoslawien Nr.718, B 100127679). Mt. Šar: Vernt-Karpa, ad 2300 m (1940 A. PICHLER BEO). Šar planina: Jezerska Čuka (28.VI.1980 S. JOVANOVIĆ et al. BEOU). Mt. Perister, prope Bitolj (Bitola), ad Golemo-jezero (21.VII.1934 V. LINDTNER BEO). Baba Planina, Mt. Perister, 1800–1820 m (6.VI.1976 P. FROST-OLSEN, Flora of Yugoslavia No. 308, C). Mt. Jablanica (18.VII.1921 s.coll. BEOU; VII.1939 P. ČERNJAVSKI BEO). Mt. Nidže (s.d., s.coll., BEOU). Mt. Bistra, supra pag. Sence (8.VII.1936 O. GREBENŠČIKOV BEO). **Montenegro:** Prokletije [Mts.]: Bor (12.VII.1934 F.S. COPELAND, Flora of Montenegro No. 52, K). Mt. Bertiscus [Prokletije Mts.]: in jugo Čakor prope Andrijevica, ca. 1700–1800 m (29.V.1958 K.H. RECHINGER, Iter Graecum IX. 1958 No. 19601, K). Prokletije [Mts.]: dolina Bjeluhe (15.VII.1973 V. NIKOLIĆ et al. BEO). Prokletije [Mts.]: Loganska Bistrica (15.VII.1933 P. ČERNJAVSKI et al. BEO). Prokletije [Mts.]: [Mt.] Glogice (16.VII.1933 P. ČERNJAVSKI et al. BEO). Prokletije [Mts.]: Čaf Bor – Sapica, 2000 m (8.VII.1994 D. LAKUŠIĆ & M. NIKETIĆ BEOU). Unter den Nordwestabhängen des Kom kučki, ca. 1800 m (5.VII.1916 E. JANCHEN WU). Kom (1909 K. MARIĆ BEOU). Mt. Koprivnik (12.VI.1928 N. KOŠANIN BEOU). Bjelasica [Mts.], Zekova glava, podnožje, 1700 m

(23.VI.2002 G. TOMOVIĆ BEOU). **Serbia:** Kopaonik [Mts.] (16.VII.1938 I. RUDSKI BEO). Kopaonik [Mts.]: Milanov vrh (VII.1938 I. RUDSKI BEO). Kopaonik [Mts.]: Metode, oko gejzira (27.V.2001 V. MITROVIĆ & V. DORDEVIĆ BEOU). Kopaonik [Mts.]: Krst tresavice, 1700 m (3.VII.1998 D. LAKUŠIĆ BEOU). Kopaonik [Mts.]: Obronci Karamana, oko puta za Gobelju, 1700 m (8.VIII.1986 D. LAKUŠIĆ & M. NIKETIĆ BEOU). Kopaonik [Mts.]: Karamanski potok, 1800 m (12.VII.1988 S. STANIĆ BEOU). Kopaonik [Mts.]: Suvo Rudište, 1700 m, exp. N (5.VII.1991 D. LAKUŠIĆ BEOU). Kopaonik [Mts.]: Mala Reka, 900 m (6.VII.1989 D. LAKUŠIĆ BEOU). Kopaonik [Mts.]: Pajino Preslo, 1700 m (4.VII.1991 D. LAKUŠIĆ BEOU). Kopaonik [Mts.]: Oštre stene (Gobelja), 1 700 m (4.VI.1985 V. STEVANOVIĆ & M. NIKETIĆ BEOU). Kopaonik [Mts.]: Markove stene – Crni jelak (26.V.2001 D. LAKUŠIĆ & V. MITROVIĆ BEOU). Kosovo: Mokra Planina (16.VII.1932 I. RUDSKI BEO). Kosovo: Senovo, Jablanički petak, 1700 m (28.VI.1932 I. RUDSKI BEO). Kosovo, Prokletije, Pločica, 1800–2000 m (17.VII.1973 V. NIKOLIĆ et al. BEO). Kosovo, Šar planina, Stojkova kuća-Jezerska čuka, 1600–2400 m (23.VII.1980 V. NIKOLIĆ et al. BEO). Kosovo, Šar planina, prema Jažinačkom jezeru, ca. 2100 m (21.VII.1978 V. NIKOLIĆ et al. BEO). Kosovo, Šar planina, od Prevalca prema Bistri, 1600–2200 m (23.VII.1975 V. NIKOLIĆ & N. DIKLIĆ BEO). Šar planina: severne padine Bistre prema Prevalcu, 1600–1800 m (14.VII.1968 V. NIKOLIĆ & N. DIKLIĆ BEO). Mt. Ljubotin, Kepišar Lak (VI.1900 BIERBACH BEOU). Prokletije, Bjelički potok, 1700 m (3.VII. 1995 V. STEVANOVIĆ et al. BEOU). Kukavica [Mts.]: Preka Voda (18.VI.1960 V. NIKOLIĆ BEO). Vlasina, Jezero (13.–17.VI.1967 V. NIKOLIĆ & N. DIKLIĆ BEO). Mt. Ruj, Zvonce, NW, 1800 m (18.VI.1966 V. NIKOLIĆ & N. DIKLIĆ BEO). Mt. Ošljak, ad Bela Voda (7.VI.1923 SOŠKA BEOU). Prevalac (21.V.1922 N. KOŠANIN BEOU). Bistrica: Brezavce (VI.1924 s.coll. BEOU). Tara planina, Crveni potok (28.V.1994 V. STEVANOVIĆ et al. BEOU; 23.V.2001 V. STEVANOVIĆ & S. JOVANOVIĆ BEOU). Tara [Mts.]: Mitrovac, Rzav (20.VI.1978 V. NIKOLIĆ et al. BEO). Stara planina, Babin zub, podnožje, 1500 m (8.VI.1997 V. RANDJ et al. BEOU). Stara planina, Sveti Nikola, Orlova stena (Hajdučki kamen) (18.VI.1998 V. STEVANOVIĆ et al. BEOU). Mt. Pločica-Bogićevica: klisura Tropoje (18.VII.1933 P. ČERNJAVSKI et al. BEO). Mt. Ned inat: pod jezera (29.VII.1933 P. ČERNJAVSKI ET AL. BEO). **Greece:** Epirus, Nom. Ioanninon, Ep. Konitsis, Mt. Grammos, ravines SW of Souflikas, 5 km N of the village Aetomilitsa, 1800–1900 m (17.VII.1977 P. HARTVIG et al. *6983*, C). Nom. Florina, Dimos: Melitti, Mt. Kajmakcalan, 40°54'45" N, 21°48'25" E, 2100 m (1.VII.1999 A. SCHULER: Pflanzen aus Griechenland, Nr. 99/752, B 100127702). Macedonia, Mt. Kajmakčalan (Voras Oros), S part, NNW of Panagitsa, between Kalivia Giannakoula and the summit area, near Paleo Bladzi, 1850–1950 m (9.VII.1976 A. STRID et al. *12165*, C). Mt. Kajmakčalan, NE part, ca. 13 km along forest road NNW of Pevkoton (N of Aridea), near Platza, 1300 m (8.VII.1976 A. STRID et al. *12114*, C; 19.VI.1990 Herbarium WILLING No. 10.813, B 100127693). Nom. Florinis, Mt. Piperitsa, NNE of the summit, 1800–1850 m (11.VII.1981 STRID et al. *19046* C, B 100127681). Nom. Florinis, Mt. Varnous (Peristeri), 5 km S-SSE of the peak of Kortsa Toumba (peak 2334), near a spring Vrisi Kalo Nero, 1700–1800 m (26.VI.1981 STRID et al. *18125* C, B 100127682; 26.VI.1981 E. STAMATIADOU 22057 C). NW Macedonia, Pisoderion, ca. 4500 ft. (3.VI.1932 A.H.G. ALSTON & N.Y. SANDWITH *635* K). Nom. Pela, Dimos: Aridea, Mt. Voras, path to Kali Pediada (Dobro Pole), 41°01'01" N, 21°54'08" E, 1650 m (1.VII.1999 A. SCHULER: Pflanzen aus Griechenland, Nr. 99/752, B 100127702). Nom. Pellis, Mt. Tzena, along path from Notia to the summit area, 1800–2000 m (19.VII.1981 STRID et al. *19228* C, B 100127680). Nom. Kozanis, Pieria-Massiv, 1.9 km O Katafijio, NO-Hängen der Pieria, 40°15'30" N, 22°10' E, 1640–1710 m (5.VI.1990 Herbarium WILLING No. 9.333, B 100127685). Pieria-Massiv: N-Hängen der Tourla, 40°13'30" N, 22°7'30" E, 1280 m (4.VI.1990 Herbarium WILLING No. 9.285, B 100127689). Nom. Kozanis, Ep. Kozanis, 6.8 km OSO Servia, 40°10' N, 22°4'30" E, 1450 m (13.V.1988 Herbarium WILLING No. 3702, B 100127690). Nom. Imathia, Vermion Massiv, SW Ano Seli, 40°35' N, 21°55'30" E, 1450–1500 m (7.VI.1990 Herbarium WILLING No. 9.571, B 100127686), 1.0–1.8 km N Ano Seli, 1700 m, 40°35'30"–36'00" N, 21°56'0–30" E (9.VI.1992 Herbarium WILLING No. 17.513, B 100127699), NW Ano Seli, 1550–1600 m, 40°35'30" N, 21°55'30" E (7.VI.1990 Herbarium WILLING No. 9.577, B 100127688). Nom. Kastoria, Ep. Kastoria, Quellgebiet des Baroumas, an der Albanischen Grenze, 40°26' N, 20°47'30"–48' E, 1760 m (11./12.VI.1989 Herbarium WILLING No. 6279, B 100127694; 24.VI.1990 Herbarium WILLING No. 11.250a, B 100127687). Ep. Kastoria: SO-Hang des Flambouro, Eripa, 40°24'30" N, 20°51'30" E, 1330–1360 m (11.VI.1989 Herbarium WILLING No. 6151a, B 100127696, No. 6151b B 100127695, B 100127696). Ep. Kastoria: 1.0 kmNO Horio Grammo, 40°23'30" N, 20°50'30" E, 1340 m (11.VI.1989 Herbarium WILLING No. 6162, B 100127697). Nom. Magnisia, Ep. Volou, 0.9 km WSW Hania, 39°23'30" N, 23°2'30" E, 1100 m (18.V.1993 Herbarium WILLING No. 26.576, B 100127700). Ep. Volou: 3.0 SO Portaria, 39°22' N, 23°1'30" E, 880 m (17.V.1993 Herbarium WILLING No. 26.514, B 100127701).

C. acris subsp. *pindicola*
Greece: Distr. Konitsa, Mt. Smolika[s], supra Kerasovo (19.VII.1896 A. BALDACCI, Iter albanicum (epiroticum) quartum, K, WU). Mt. Smolikas: Skapdi Schopati, 7500 ft. (3.VII.1937 E. K. BALLS & W.B. COURLAY, Flora of Greece No. B3478, K). Mt. Smolikas: in declivibus borealibus supra pagum Kerasovo, 1800 m (9.VII.1958 K.H. RECHINGER, Iter Graecum IX., 1958, No. 21076, C, K). Mt. Smolikas: ad fontem "Thisino", 2000 m (9.VII.1958, K.H. RECHINGER, Iter graecum IX. No. 20823, B100000947). Distr. Konitsa: 10 km W of Samarina, 5 km S of the village Paraskevi, N-facing slopes, 1800–1850 m (15.VI.1976 P. HARTVIG et al. *5890* C). W Macedonia, nom. Grevenon, Ep. Grevenon: the valley between Mt. Gomara and Mt. Vasilitsa, ca. 7 km SW of Samarina, 1750 m (24.VI.1976 P. HARTVIG et al. *5275* C). Pindus Tymphaeus: in summo montis Zygos (Lakmon veter.) supra Metzovo, alt. 4500–5000' (VII.1885 C. HAUSSKNECHT, Iter Graecum 1885, JE). Distr. Ioannina, ad confines Thessaliae, Montes Pindus, in jugo Katara supra Metsovo, ca. 1600–1800 m (29.VII.1956 K.H. RECHINGER, Iter Graecum VIII., 1956, 18399, K). Katara [Pass], 4 km NO of Metsovo, ca. 1700 m (3.VII.1971 B. ALDÉN *1436* C). Trikalon, Kalambakas, E of Katara Pass, ca. 4 km E of the summit, 1400 m. Lat. 39°48′ N, Long. 21°12′ E (19.V.1985 A. STRID *24579* C).

C. acris subsp. *vardousiae*
Greece: Aetolia, Mt. Wardusia (16.V.1899 C. LEONIS, Flora Graeca, curavit I. DÖRFLER, No.378, B 100127683, BRNU). Nom. Fokidos, Ep. Parnassidos, Mt. Vardousia, 3.5–4 km W of Athanasios Diakos, 1900 m, 38°42′ N, 22°08′ E (11.VI.1985 L.-A. GUSTAVSSON *9279* C). Vardhousia-Massiv, Stavros, 1640–1750 m, 38°42′ N, 22°7′ E (31.V.1988 Herbarium WILLING No. 4770, B 00127698). Nom. Phthiotidos: Mt. Iti, Aufstieg von Neochorion zum Berg Petrotos, 1700 m (16.VI.1982 I. HAGEMANN et al. *208*, B 100127684, C). Mt. Iti: 5 km E of Neochorion, ca. 1900 m (23.VI.1986 L.-A. GUSTAVSSON *9835* C). Iti-Massiv, 3.5 km S Ipati, 38°50′ N, 22°14′ E, 1430 m (5.V.1991 Herbarium WILLING No. 14.443, B 100127691). Iti-Massiv: O Neohori, W Petrotos-Gipfel, Fuss des Petrotos, 38°47′0–30″ N, 21°13′-12′30″ E, 1370–1750 m (7.VI.1991 Herbarium WILLING No. 14.725a, B 100127692).