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New Records on the Occurrence, Distribution and Chromosome Numbers of *Cardamine amara* and the *C. pratensis* Group (*Brassicaceae*) in Croatia

By

Jaromír KUČERA*) and Karol MARHOLD**)

With 2 Figures

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Summary

KUČERA J. & MARHOLD K. 2006. New records on the occurrence, distribution and chromosome numbers of *Cardamine amara* and the *C. pratensis* group (*Brassicaceae*) in Croatia. – *Phyton* (Horn, Austria) 46 (1) 99 – 111, 2 figures. – English with German summary.

New data on the distribution of *Cardamine amara* L. and the *C. pratensis* group in Croatia are presented. Till now only *C. pratensis* L. (s.str.) and *C. amara* subsp. *amara* were reported in floras and checklists for this area. Based on the study of herbarium specimens, five taxa, namely *C. amara* L. subsp. *amara*, *C. amara* subsp. *austriaca* MARHOLD, *C. matthioli* MORETTI, *C. majovskyi* MARHOLD & ZÁBORSKÝ, and *C. dentata* SCHULT., are confirmed for the area of Croatia. The occurrence of *C. majovskyi* and *C. amara* subsp. *austriaca* is affirmed also by chromosome number counts (both $2n = 32$). Although no specimens corresponding to *C. pratensis* s.str. were found, its occurrence in Croatia cannot be ruled out, as it occurs in neighbouring regions of Hungary and Slovenia.

* Dipl. Ing. J. KUČERA, Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 14, SK-845 23 Bratislava, Slovak Republic; e-mail: jaromir.kucera@savba.sk

** Assoc. Prof. Dr. K. MARHOLD, Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 14, SK-845 23 Bratislava, Slovak Republic & Department of Botany, Charles University, Benátská 2, CZ-128 01 Praha 2, Czech Republic; e-mail: karol.marhold@savba.sk

Zusammenfassung

KUČERA J. & MARHOLD K. 2006. Neue Angaben zu Vorkommen, Verbreitung und Chromosomenzahl von *Cardamine amara* und der *C. pratensis*-Gruppe in Kroatien. – Phytion (Horn, Austria) 46(1): 99 – 111, 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Neue Angaben zur Verbreitung von *Cardamine amara* L. und der *C. pratensis*-Gruppe in Kroatien werden hier präsentiert. Nur zwei Sippen, *C. pratensis* L. (s.str.) und *C. amara* subsp. *amara*, wurden bisher für dieses Gebiet angegeben. Beim Studium von Herbarmaterial konnten jedoch fünf Sippen in Kroatien festgestellt werden: *C. amara* subsp. *amara*, *C. amara* subsp. *austriaca* MARHOLD, *C. matthioli* MORETTI, *C. majovskyi* MARHOLD & ZÁBORSKÝ und *C. dentata* SCHULT. Das Vorkommen von *C. majovskyi* und *C. amara* subsp. *austriaca* konnte auch durch die Chromosomenzahlen ($2n = 32$) bestätigt werden. Obwohl keine Herbarbelege gefunden worden sind, die der Art *C. pratensis* s.str. zuzuordnen wären, kann ihr Vorkommen in Kroatien nicht ganz ausgeschlossen werden; diese Art wächst nämlich in angrenzenden Gebieten Ungarns und Sloweniens.

1. Introduction

According to NIKOLIĆ 1997, the genus *Cardamine* (*Brassicaceae*) is represented in Croatia by 17 species, including those sometimes treated within the separate genus *Dentaria*. Taxa from two taxonomically critical Eurasian assemblages, *Cardamine amara* L. and the *C. pratensis* group, have been recorded as well, but remained poorly explored in this area. Most authors reported them only as two species, *C. pratensis* L. and *C. amara* L., occurring in northwestern Croatia and without any notes on their infraspecific identity (NEILREICH 1868, SCHLOSSER & FARKAŠ-VUKOTI-NOVIĆ 1869, HAYEK 1925, DOMAC 1950 and TRINAJSTIĆ 1976). In the checklist of the Croatian vascular plants (NIKOLIĆ 1997, updated version available on-line on <http://hirc.botanic.hr/fcd/search.aspx>), they were listed as *C. amara* subsp. *amara* and *C. pratensis* subsp. *pratensis*.

Cardamine amara L. is a species widespread in Europe and Asia, occurring on wet sites near streams and springs from lowlands up to the alpine belt. Based on extensive morphometric, karyological and molecular studies (MARHOLD 1992, MARHOLD & al. 1996, MARHOLD 1998, 1999, LIHOVÁ & al. 2000) five subspecies are currently recognised in Europe. The nominate subspecies *C. amara* subsp. *amara* is diploid ($2n = 16$) and it occurs throughout the European continent, with the exception of southernmost Europe and some, especially mountain areas. Three other diploid subspecies are geographically restricted to certain mountain ranges: *C. amara* subsp. *opicii* (J. PRESL & C. PRESL) ČELAK. from the Sudety Mts. and Carpathians, *C. amara* subsp. *balcanica* MARHOLD & al. from Bulgaria and Greece, and *C. amara* subsp. *pyrenaica* SENNEN from the Pyrenees. The only tetraploid subspecies, *C. amara* subsp. *austriaca* MARHOLD ($2n = 32$) is distributed in the Eastern Alps and neighbouring areas (MARHOLD 1992, 1999,

MARHOLD & al. 1996, LIHOVÁ & al. 2000). A closely related tetraploid species, *C. amporitana* SENNEN & PAU (previously known as *C. amara* subsp. *olotensis* O. BOLÒS; $2n = 32$) occurs in central Italy and Catalonia (Spain) (LIHOVÁ & al. 2000, 2004a). While *C. amporitana* can be distinguished from *C. amara* (apart from other characters) by the colour of anthers, the subspecies of *C. amara* differ mainly in quantitative characters such as number of stem leaves, number of leaflets of stem leaves, size of flower parts, branching of stem and width of stem base. Morphological differentiation between diploid *C. amara* subsp. *amara* and its putative autotetraploid derivative *C. amara* subsp. *austriaca* is less pronounced, but the ploidy level is well-correlated with the pollen grain size, and this character is often essential for reliable taxon identification in sympatric areas (MARHOLD 1999, LIHOVÁ & MARHOLD 2003a).

The *Cardamine pratensis* group, as traditionally recognized, is a polyploid complex widely distributed across Europe, and occurring also in Asia, northern Africa and northern North America. Ecologically, taxa of this group are found in wet sites, on meadows, pastures, in flood-plain forests, along streams and riverbanks from lowlands up to the alpine belt. They encompass taxa with ploidy levels from diploid to dodecaploid, including aneuploids and dysploids. Recent molecular studies (LIHOVÁ & al. 2004c, MARHOLD & al. 2004, LIHOVÁ & MARHOLD 2006) revealed that the separation of three species complexes, namely *C. amara*, *C. pratensis*, and *C. raphanifolia*, is not as strict as previously considered and that the parents of polyploid taxa at least in some cases originated from more than one of these species complexes. Nevertheless, for practical purposes it is still useful to keep the concept of the *C. pratensis* group. It includes several 'basal' diploid ($2n = 16$) species (FRANZKE & HURKA 2000, LIHOVÁ & al. 2003, 2004b) restricted in their distribution to relatively small areas in the Mediterranean: *C. crassifolia* POURR. (Eastern Pyrenees), *C. castellana* LIHOVÁ & MARHOLD (Iberian Peninsula), *C. apennina* LIHOVÁ & MARHOLD (central Italy), *C. rivularis* SCHUR (South Carpathians, Bulgaria) and *C. penzesii* ANČEV & MARHOLD (Black Sea coast in Bulgaria and Turkey). In addition, the group comprises several diploid and polyploid taxa forming the 'derived' group (FRANZKE & HURKA 2000), occurring entirely or mostly to the north of the Mediterranean: *C. pratensis* L. s. str. distributed in whole Europe with several cytotypes from diploid to heptaploid level (here also populations from the Alps, previously classified as *C. rivularis*, should be included, see MARHOLD 2000); *C. dentata* SCHULT. with octoploid to dodecaploid populations occurring mainly in northern Europe reaching to the south as far as to the Danube river; octoploid to decaploid *C. nymantii* GAND. from northernmost Europe; diploid *C. matthioli* MORETTI, distributed in central and southern part of Europe, and its putative autotetraploid derivative *C. majovskyi* MARHOLD & ZÁBORSKÝ, currently known from the eastern part of Pannonia, Carpathians, Austria and Slovenia. The

species of the *C. pratensis* group differ both in qualitative (morphology of rhizomes and basal parts of stem, position of hairs on the youngest rosette leaves, position of segments of stem leaves, presence/absence of stalks on the leaflets of the uppermost stem leaves, colour of anthers and petals) as well as in quantitative characters (size of flower parts, size of pollen grains, number of stem leaves, number of leaflets/segments on stem leaves). Diploid *C. matthioli* and tetraploid *C. majovskyi* differ mainly in the size of flower parts. For their identification the size of pollen grains is also very useful, especially if the plants on herbarium specimens are poorly preserved (MARHOLD 1996, LIHOVÁ & MARHOLD 2003b).

The aim of this paper is to evaluate Croatian material of the *C. pratensis* group and *C. amara* based on available herbarium specimens and our own field collections. Chromosome numbers were counted from several localities and the distribution of individual taxa in Croatia was discussed in the context of their distribution pattern in neighbouring countries.

2. Material and Methods

Seven populations of the *C. pratensis* group and *C. amara* were sampled in the studied area (Table 1). Plants collected in the field were transferred to the experimental field of the Institute of Botany of the Slovak Academy of Sciences in Bratislava. Chromosome numbers were determined from mitotic metaphases observed in the root tips of the cultivated plants. The root tips were pretreated with 0.002 M hydroxyquinoline for 5 hours, fixed in a freshly prepared mixture of concentrated ethanol and acetic acid (1 : 1), and finally hydrolyzed in a mixture of concentrated hydrochloric acid and ethanol (1 : 1) for 1–2 min. Squashes were made in a drop of 45% acetic acid under a cellophane square (MURÍN 1960), and stained in 10% solution of Giemsa stock solution in Sörensen phosphate buffer for 1 hour. Voucher specimens are deposited in SAV (herbarium acronyms follow HOLMGREN & al. 1990).

For acquiring distribution data, we studied herbarium specimens from the herbaria ZA and ZAHO. As for the distribution survey in Croatia, we followed the phytogeographical division of the country by NIKOLIĆ & al. 1998.

Ploidy levels of herbarium specimens were assessed by measuring the size of pollen grains obtained from flower buds. Pollen grains from one flower bud per specimen were mounted on a microscopic slide in acetocarmine jelly (KEARNS & INOUE 1993) and left overnight before measurements were done. Thirty pollen grains were measured per slide (i.e. per flower) and mean values were compared to those of pollen grains from plants with known chromosome numbers from Croatia and other regions (Austria, Slovakia – voucher specimens from the studies by MARHOLD & ZÁBORSKÝ 1986, MARHOLD 1994a, 1999).

3. Results and Discussion

3.1. *Cardamine amara* L.

3.1.1. Karyology and Taxa

In the three karyologically analysed populations of *C. amara* from Croatia only the tetraploid level ($2n = 32$) was determined, which corre-

sponds to *C. amara* subsp. *austriaca* (Table 1). The mean values of the size of pollen grains ranged here from 22.9 to 24.3 μm . Subsequent screening of the size of pollen grains from herbarium specimens of *C. amara* originating from Croatia revealed two size classes, namely 17.7–20.6 μm and 21.0–26.1 μm (for localities see below). These two classes of the size of pollen grains correspond well with the values, which we measured on the plants of *C. amara* subsp. *amara* and subsp. *austriaca* with known chromosome numbers ($2n = 16$ and 32 respectively) collected outside of Croatia, in Austria and Slovakia, as well as with the values published for these taxa from the area of neighbouring Slovenia (LIHOVÁ & MARHOLD 2003a).

Based on the above analyses we can confirm the occurrence of two infraspecific taxa of *C. amara* in Croatia, namely *C. a.* subsp. *amara*, which was already reported for this area (NIKOLIĆ 1997), and subsp. *austriaca* as a new taxon for the country. Both subspecies of *C. amara* are distributed in the northwestern part of Croatia in Zapadnopanonska makroregija (Western Pannonian Macroregion) (Fig. 1).

C. amara L. subsp. *amara* is distributed mainly in lowlands (or at lower altitudes) in the phytogeographical regions of Ravnjačko-Krška mezoregija (Slunj), Pokupska mezoregija (Karlovac) and Gornjeposavska mezoregija (Samobor).

C. amara subsp. *austriaca* MARHOLD seems to be frequent in Croatia mainly in highlands in the regions of Pokupska mezoregija (Ozalj, Karlovac, Rude) and Zagorska mezoregija (Krapina). As shown on Fig. 1, the area of *C. amara* subsp. *austriaca* in Croatia is an extension of its distribution in Slovenia (LIHOVÁ & MARHOLD 2003a). Inferring from this pattern, we can expect the occurrence of this subspecies also in the Croatian regions of Gornja Podravsko-Međimurska mezoregija, Ravnjačko-Krška mezoregija and in Podkapelska mezoregija. These data are in accordance with the overall distribution area of this tetraploid, which covers Eastern Alps and neighbouring regions.

As reported by MARHOLD 1999 and noted also by LIHOVÁ & MARHOLD 2003a, there are only minor morphological differences between *C. amara* subsp. *amara* and subsp. *austriaca*. They are displayed in the number of leaves and size of flower parts (see the identification key below). The most important difference, however, is in the size of pollen grains, which was confirmed also in the present study. This corresponds well with the assumed autopolyploid origin of tetraploid *C. amara* subsp. *austriaca* from diploid subsp. *amara* (LIHOVÁ & al. 2004a, LIHOVÁ & al. 2004c, MARHOLD 1999).

3.1.2. Identification Key to *Cardamine amara* in Croatia

- 1a. Width of stem base (1–) 1.5–3.5 (–4.5) mm, number of stem leaves (3–) 5–12 (–17), maximum number of leaflets of the leaves in the upper 4/5 of stem (5–) 7–11 (–13), number of flowers in the main inflorescence (3–) 7–22 (–28), length of

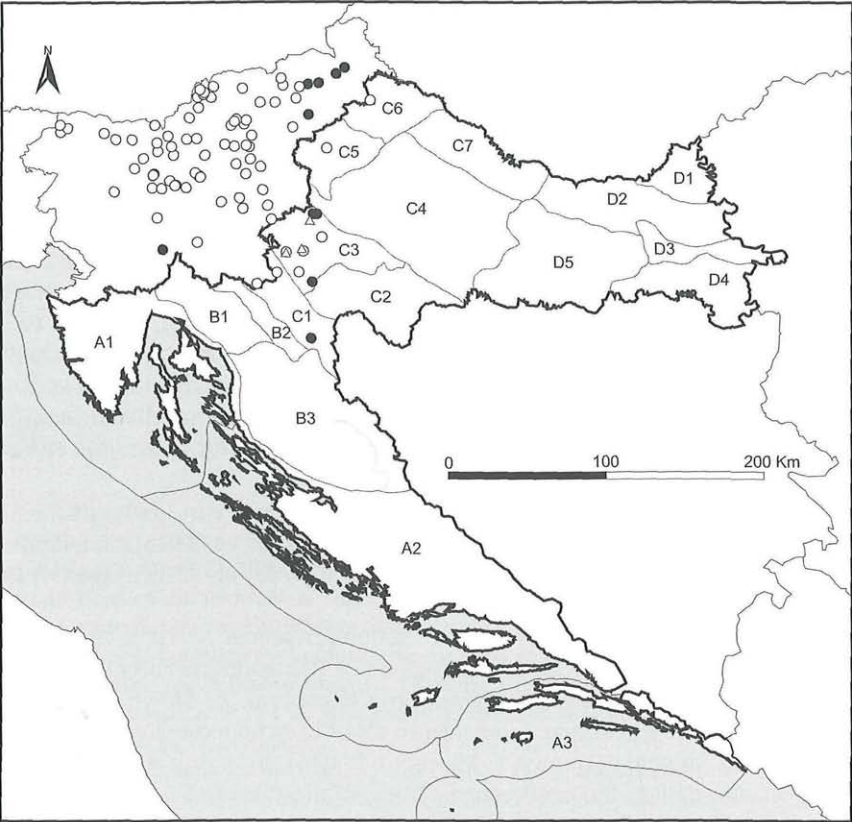


Fig. 1. Distribution map of *Cardamine amara* subsp. *amara* (solid symbols) and *C. amara* subsp. *austriaca* (empty symbols) in Croatia and Slovenia based on herbarium specimens and our own collections. Circles represent records based on herbarium specimens and triangles refer to the karyologically analysed Croatian populations (see Table 1). Distribution in Slovenia is based on data by LIHOVÁ & MARHOLD 2003a.

sepals (2.8–) 3.1–4.0 (–4.5) mm, length of filaments of the longer stamens (3.8–) 4.9–6.6 (–7.3) mm, average diameter of thirty pollen grains per plant individual (20.3–) 21.1–22.2 (–23.0) μm , $2n = 16$

..... *C. amara* subsp. *amara*

- 1b. Width of stem base (1–) 1.5–4.0 (–6.5) mm, number of stem leaves (4–) 6–14 (–18), maximum number of leaflets of the leaves in the upper 4/5 of stem (6–) 7–13 (–15), number of flowers in the main inflorescence (4–) 7–26 (–35), length of sepals (3.1–) 3.6–4.7 (–5.2) mm, length of filaments of the longer stamens (4.9–) 5.7–7.3 (–7.8) mm, average diameter of thirty pollen grains per plant individual (23.0–) 23.5–25.1 (–25.7) μm , $2n = 32$

..... *C. amara* subsp. *austriaca*

Note: Previously published results of morphometric analyses (MARHOLD 1999) showed only minor morphological differences between *C. amara* subsp. *amara* and *C. amara* subsp. *austriaca*. Therefore, determination of chromosome number or measurements of the size of pollen grains is inevitable for the precise identification of single plants. Calibration based on measurements of plants with known ploidy level is necessary to interpret the pollen sizes properly.

3.1.3. Studied Herbarium Specimens from the Herbaria ZA and ZAHO

Cardamine amara L. subsp. *amara*

Zapadnopanonska makroregija: 1. Ravnjačko-Krška mezoregija: Flora Croatica, in locis udis silvarum ad Slunj, 25.4.1890, L. ROSSI no. 6447 (ZA). – 3. Pokupska mezoregija: Flora Croatica, Vukmanić prope Karlovac, 23.5.1904, L. ROSSI no. 15.357 (ZA). – 4. Gornjeposavska mezoregija: Stražnik, Samobor, 1878, ROSSI[?]. (ZA). – Flora Croatica, In fossis ad Domaslovec prope Samobor, 1.5.1878, L. ROSSI no. 325 (ZA). – Flora Croatica, in fossis Samobor, 4.1878, L. ROSSI no. 324 (ZA).

Cardamine amara subsp. *austriaca* MARHOLD

Zapadnopanonska makroregija: 3. Pokupska mezoregija: Flora Croatica, ad Ozalj, 11.5.1907, L. ROSSI no. 15.350 (ZA). – Flora croatica, okolina Karlovaca, Draganić, šuma Jelas, 17.4.1934, I. HORVAT (ZAHO). – Flora Croatica, okolina Karlovaca, Draganić, u šumi *Quercus robur*, 17.4.1934, I. HORVAT (ZAHO). – Flora Croatica, Karlovac, ad Ljažansko Polje, 15.4.1906, L. ROSSI no. 25.427 (ZA). – Herbarium Z. Udbinac, uz Brebernica na valo vlažnom mjestu u Zdenčini, 14.4.1957, Z. UDBINAC (ZA). – 5. Zagorska mezoregija: Krapina, Dolac, 25.3.1916, s. coll. (ZA).

Herbarium specimens without detailed localization:

Flora Croatica, in fontibus, ad rivulos, in acis uliginosis, Jos. Calas. SCHLOSSER no. 4309 (ZA).

Localities for the three populations investigated karyologically see Table 1.

3.2. *Cardamine pratensis* Group

3.2.1. Karyology and Taxa

In all six karyologically investigated populations of the *C. pratensis* group (Table 1), solely the tetraploid chromosome number of $2n = 32$ was found. This is in accordance with their morphology, which corresponds to the tetraploid *C. majovskyi* (size of flower parts, position of hairs on youngest rosette leaves, position of segments of stem leaves). The size of pollen grains of the plants from these tetraploid populations was $28.3\text{--}30.8\text{ }\mu\text{m}$. This is in agreement with the values published for *C. majovskyi* by LIHOVÁ & MARHOLD 2003b from neighbouring Slovenia. On the other

Table 1. Origin of the sampled populations used for karyological studies.

Taxon/locality	Chromosome number
<i>C. majovskyi</i> MARHOLD & ZÁBORSKÝ	
Croatia, Gornjeposavska mezoregija, Lonjsko polje lowlands, Kutina village, near highway, 45°28'07"N, 16°44'40"E, 100 m a.s.l., 19.4.2005, coll. KUČERA J. & KOLNÍK M.	2n = 32
Croatia, Pokupska mezoregija, Tušilovič village near Karlovac, 45°23'50"N, 15°36'51"E, 125 m a.s.l., 28.4.2005, coll. KUČERA J. & KOLNÍK M.	2n = 32
Croatia, Pokupska mezoregija, Ribnik village, 45°34'04"N, 15°21'09"E, 150 m a.s.l., 28.4.2005, coll. KUČERA J. & KOLNÍK M.	2n = 32
Croatia, Pokupska mezoregija, near Ozalj village, 45°36'37"N, 15°27'47"E, 155 m a.s.l., 28.4.2005, coll. KUČERA J. & KOLNÍK M.	2n = 32
Croatia, Gornjeposavska mezoregija, Braslovie village near Samobor, 45°45'02"N, 15°39'25"E, 440 m a.s.l., 28.4.2005, coll. KUČERA J. & KOLNÍK M.	2n = 32
Croatia, Pokupska mezoregija, Vrh village, 45°37'08"N, 15°35'55"E, 130 m a.s.l., 28.4.2005, coll. KUČERA J. & KOLNÍK M.	2n = 32
<i>C. amara</i> subsp. <i>austriaca</i> MARHOLD	
Croatia, Pokupska mezoregija, Ozalj village, 45°36'37"N, 15°27'47"E, 160 m a.s.l., 28.4.2005, coll. KUČERA J. & KOLNÍK M.	2n = 32
Croatia, Pokupska mezoregija, Vrh village, 45°37'08"N, 15°35'55"E, 130 m a.s.l., 28.4.2005, coll. KUČERA J. & KOLNÍK M.	2n = 32
Croatia, Pokupska mezoregija, Rude village near Samobor, 45°46'19"N, 15°41'08"E, 240 m a.s.l., 28.4.2005, coll. KUČERA J. & KOLNÍK M.	2n = 32

hand, no plants with patent hairs on rosette leaves (characteristic for *C. pratensis* s. str.) were found.

From the studied herbarium specimens deposited in ZA and ZAHO, one collection, namely that from the region of Gornjeposavska mezoregija (forests near Lipovljani) corresponded to *C. dentata*. The characteristic petiolate leaflets of all, even of uppermost stem leaves left no doubts about the taxonomic identity of these plants. The closest known localities of this taxon are in Hungary (Fig. 2; see also MARHOLD 1994b), thus this record shows the southern limit of the distribution area of *C. dentata*. While in northern Europe this taxon is more widespread with broader ecological amplitude (occupying broad range of wetland habitats), in southern parts of its distribution area it is more restricted to flood-plain forests along large rivers (see MARHOLD 1994b). In accordance with this pattern, the only

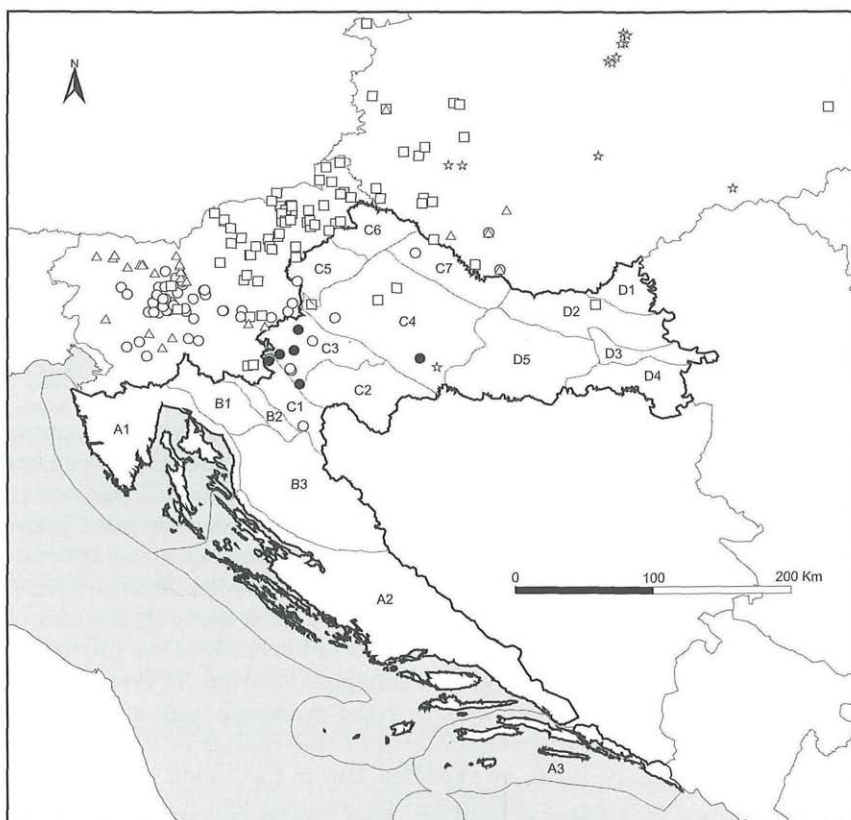


Fig. 2. Distribution map of *Cardamine pratensis* (triangles), *C. matthioli* (squares), *C. majovskyi* (circles) and *C. dentata* (stars) in Croatia, Slovenia and Hungary based on herbarium survey and our own collections. Empty symbols represent records based on herbarium specimens and solid symbols refer to the karyologically analysed Croatian populations (see Table 1). Distribution in Slovenia is based on data by LIHOVÁ & MARHOLD 2003b, that in Hungary on data by MARHOLD 1994a, 1994b.

Croatian locality known so far is in a flood-plain forest, and it is likely that more localities can be found along the river Sava in Croatia.

The remaining specimens studied from ZA and ZAHO corresponded by the gross morphology to *C. majovskyi* and *C. matthioli*. *C. matthioli* and its putative autotetraploid derivative *C. majovskyi* differ mainly in the size of flower parts and in the size of pollen grains (see MARHOLD 1994b and the identification key below). Because of the poor state of flowers on some specimens, we relied in the precise identification on the latter character. Screening of the size of pollen grains from these herbarium specimens originating from Croatia revealed two size classes, namely

20.7–27.2 μm and 27.7–30.4 μm (for localities see below). Based on these measurements, we can conclude that both diploid *C. matthioli* and tetraploid *C. majovskyi* occur in Croatia (for localities see below). Similar values of the size of pollen grains were published for these taxa also from the area of neighbouring Slovenia (LIHOVÁ & MARHOLD 2003b).

From Croatia only *C. pratensis* s.str. (= *C. pratensis* subsp. *pratensis*) was reported before (DOMAC 1950, TRINAJSTIĆ 1976, NIKOLIĆ 1997). However, the occurrence of *C. pratensis* s.str. could not be confirmed for the time being in Croatia, but taking into account the distribution pattern of this taxon in the neighbouring regions of Hungary and Slovenia (see Fig. 2, and MARHOLD 1994b, LIHOVÁ & MARHOLD 2003b) it might be found in the regions of Dravsko-dunavska nizina, Podravska mezoregija, or Pokupska mezoregija.

Based on morphological characters, karyological analyses, and results of measurements of the size of pollen grains we have confirmed three taxa of the *C. pratensis* group for this area, namely *C. matthioli*, *C. majovskyi* and *C. dentata* (Fig. 2). Distribution of all three taxa is concentrated to the northern part of Croatia, to the regions of Zapadnopanonska and Istočno-panonska makroregija (Western and Eastern Pannonian Macroregions). Their absence in other parts of Croatia is caused most likely by the lack of suitable habitats. *C. matthioli* is distributed in lowlands in the phytogeographical regions of Gornjeposavska mezoregija (Vrbovec, Sv. Ivan Žabno), Zagorska mezoregija (Pojatno) and Dravsko-dunavska nizina (Valpovo). Taking into account the distribution area of this species in neighbouring Slovenia and Hungary, it can be expected also in the region of Podravska mezoregija. *C. majovskyi* is distributed in lowlands and highlands in the regions of Ravnjačko-Krška mezoregija (Slunj), Pokupska mezoregija (Karlovac, Dubovac), Gornjeposavska mezoregija (Zagreb), Zagorska mezoregija (Brezje) and in Podravska mezoregija (Koprivnica). It can be expected also in the region of Dravsko-dunavska nizina, neighbouring with the area of its occurrence in Hungary.

3.2.2. Identification Key to the *Cardamine pratensis* Group in Croatia

- | | |
|---|---------------------|
| 1a. Cauline leaves pinnate, leaflets distinctly stalked | <i>C. dentata</i> |
| 1b. At least upper cauline leaves pinnatisect | 2 |
| 2a. At least some basal leaves hairy | 3 |
| 2b. Basal leaves glabrous or shrivelled at anthesis | 4 |
| 3a. Hairs on rachis of basal leaves patent | <i>C. pratensis</i> |
| 3b. Hairs on rachis of basal leaves appressed | 5 |
| 4a. Basal segments of mid-cauline leaves slightly ascending | <i>C. pratensis</i> |
| 4b. Basal segments of mid-cauline leaves slightly deflexed | 5 |

- 5a. Petals 5.0–9.0 (–12.0) mm long and 2.5–5.5 (–6.0) mm wide, average diameter of thirty pollen grains per plant individual 20.7–27.2 μm , $2n = 16$ *C. matthioli*
- 5b. Petals 8.5–16.5 mm long and (5.0–) 5.5–12.0 mm wide, average diameter of thirty pollen grains per plant individual 27.7–30.4 μm , $2n = 32$ *C. majovskyi*

Note: Following the Art. 6.7 of the International Code of Botanical Nomenclature (McNEILL & al. 2006) the original spelling of the epithet '*majovskii*' should be corrected to '*majovskyi*'.

3.2.3. Studied Herbarium Specimens from the Herbaria ZA and ZAHO

Cardamine matthioli MORETTI

Zapadnopanonska makroregija: 4. Gornjeposavska mezoregija: u Lovrečini [Vrbovec], 10.4.1852, VUKOTINOVIĆ (ZA). – Flora Croatica, Sv. Ivan Žabno, južno od ceste prema Cirkveni, 29.3.1997, SPOMENKA CAR (ZA). – 5. Zagorska mezoregija: Flora croatica, Hrvatsko Zagorje, Pojatno, ispod Vel. Vrha, među joha, 15.4.1934, I. HORVAT (ZAHO). – Istočnopanonska makroregija: 2. Dravsko-dunavska nizina: Flora croatica, Podravska Hrvatska, okolina Valpova, u poplavnom području Drave kod Gata, 18.5.1939, I. HORVAT (ZAHO).

Herbarium specimens without detailed localization:

Auf Minsun in Croatien, IZABELLA SCHLOSSER (ZA). – Flora Croatica, In pratis uliginosis haud rara primo vere, Jos. Calas. SCHLOSSER no. 332 (ZA). – Flora Croatica, in pratis uliginosis hand rara primo vere, Jos. Calas. SCHLOSSER (ZA).

Cardamine majovskyi MARHOLD & ZÁBORSKÝ

Zapadnopanonska makroregija: 1. Ravnjačko-Krška mezoregija: Flora Croatica, In pratis humidis ad Slunj, 25.4.1890, L. ROSSI no. 6446 (ZA). – 3. Pokupska mezoregija: Flora Croatica, Karlovac, ad silvum Lužčić, 15.4.1919, L. ROSSI (ZA). – Flora Croatica, ad Karlovac, 29.3.1912, L. ROSSI no. 18.754 (ZA). – Flora Croatica, in humidis Ribnik ad Karlovac, 18.4.1883, L. ROSSI no. 321 (ZA). – Kroatien, Nasse Wiesen vor dem Lužčić wald b. Karlstadt, 8.4.1910, F. MORTON no. 18.755 (ZA). – Flora Croatica, in locis humidis ad Dubovac, 23.4.1887, L. ROSSI no. 321h (ZA). – 4. Gornjeposavska mezoregija: Okolo Zagreba, 4.1875, leg. ?? (ZA). – Auf Wiesen am Agram [Zagreb], 28.5.1881, WORMASTING (ZA). – Flora Croatica, trnje ad Zagreb, 27.3.1913, L. ROSSI no. 25.428 (ZA). – Am Agram [Zagreb] auf dem Wiesen, 9.4.1897, WORMASTING (ZA). – Auf feuchten Waldwiesen bei Agram [Zagreb], 4., leg. ?? (ZA). – Herbarium Zlata Udbinac, na livadi Cynosuretum eristati u Zdenčini, kod šume za Rabinom, 31.3.1957, Z. UDBINAC (ZA). – 5. Zagorska mezoregija: Flora croatica, Hrvatsko Zagorje, Dubravica, Brezje, 11.4.1919, I. HORVAT (ZAHO). – 7. Podravska mezoregija: Flora sjeverozapadne Bilogore, južno od Koprivnice, sjeverozapadni dio Kamenika, oko 160 m, 8.4.1988, SANJA MIKULIĆ (ZA).

Localities for the six populations investigated karyologically see Table 1.

Cardamine dentata SCHULT.

Zapadnopanonska makroregija: 4. Gornjeposavska mezoregija: Flora croatica, Lipovljani, fakultetska šuma, 11.5.1960, I. HORVAT (ZAHO).

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Autor(en)/Author(s): Kucera Jaromir, Marhold Karol

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