Cardamine maritima group (Brassicaceae) in the amphi-Adriatic area: A hotspot of species diversity revealed by DNA sequences and morphological variation

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Abstract The western Balkans, characterized by high rates of endemism, are recognized as one of the most diverse and species-rich areas of Europe. The present study focuses on diploid speciation in a poorly explored ancestral group of the genus *Cardamine* that is distributed in the amphi-Adriatic territory. Morphological data and DNA sequences (plastid *trnL* intron and *trnL-trnF* spacer, ITS of nrDNA, and single-copy nuclear gene *CHS*) were used to address taxonomic, phylogenetic and evolutionary questions. Recognition of several previously described, but recently not accepted narrow endemics is supported (*C. fialae*, *C. rupestris*, *C. serbica*). *Cardamine maritima* s.str. is segregated into three distinct entities, which were given species rank. A new taxonomic concept of the group is proposed, including identification key, synonymy, typifications, *C. montenegrina* sp. nov. and *C. adriatica* nom. nov. Chloroplast data indicate radiation of the ancestral lineage into a series of distinct endemic species; lack of resolution at internal nodes and incongruence with nuclear phylogenies suggest that this radiation occurred rapidly. Nuclear data indicate more recent hybridization events. It is assumed that glacial-induced local-scale and altitudinal migrations along the Balkan coastline and in adjacent massifs caused contacts between previously isolated populations, resulting in occasional interspecific gene-flow. Trans-Adriatic contacts and gene exchange are also suggested.

Keywords *Cardamine*; chalcone synthase; morphometrics; nuclear ribosomal ITS; *trnL-trnF*; western Balkans

■ INTRODUCTION

The genus *Cardamine* L. (Brassicaceae) comprises around 200 species distributed on all continents except for Antarctica, and includes diploids to high polyploids. Around 50 species are currently recognized in Europe (Lihová & Marhold, 2006). Several species groups, especially taxonomically critical polyploid complexes, have received much attention in recent studies. As a result, new taxonomic concepts were established, and the Mediterranean was identified as an area with high species diversity that also harbours evolutionary basal diploid lineages (Lihová & al., 2000, 2003, 2004; Perný & al., 2005). The present study is focused on a poorly known European species group, which comprises annual to biennial populations, typically found on calcareous rocks or screes of the western Balkan and central Apennine Peninsula. In the *Flora Europaea* (Jones & Akeroyd, 1993) and the *Atlas Florae Europaeae* (Jalas & Suominen, 1994), only two species are recognized within this group: the western Balkan *C. maritima* Port. ex DC. and the Apennine *C. monteluccii* Brilli-Cat. & Gubellini. Another four taxa were described earlier from the area of the western Balkans, but all these names were later rendered to the synonymy of *C. maritima*: *C. fialae* Fritsch described from the vicinity of Klobuk village in Bosnia and Herzegovina (Fritsch, 1897), *C. maritima* var. *maglicensis* Rohlena from the Maglić Mts. in Serbia (Rohlena, 1906), *C. serbica* Pančić from the Tara Mts. in Serbia (Pančić, 1884), and *C. maritima* "proles" *rupestris* O.E. Schulz (Schulz, 1903) from the island Lošinj (Croatia) and the area of Stolac in Bosnia and Herzegovina (also named *C. rupestris* (O.E. Schulz) K. Malý; Malý, 1911).

Data on chromosome numbers have only been published for *C. monteluccii*, which was shown to be diploid with $2n = 16$ (Brilli-Cattarini & Gubellini, 1986). A recent phylogenetic reconstruction, comprising most European diploids of the genus, indicated that the above-mentioned putative taxa of the *C. maritima* group are monophyletic, constituting a basal lineage (Lihová & al., 2006). Two species with central and eastern Mediterranean distribution, *C. graeca* L. and *C. glauca* DC., and two alpine species, *C. alpina* Willd. and *C. resedifolia* L., were identified as their closest relatives. The lineage containing these taxa occupied a basal position also on the whole-genus phylogenetic tree (Carlsen & al., 2009). In our pilot study based on AFLP markers, we showed that *C. monteluccii* is a distinct species, and as many as five allopatric and genetically differentiated entities were indicated in the western Balkans (Kučera & al., 2008). They seemed to correspond to the following taxa: *C. fialae*, *C. serbica*, *C. rupestris*, and two allopatric entities within *C. maritima* sensu stricto (s.str.) (mainland Croatian vs. Montenegrin populations). *Cardamine maritima* var. *maglicensis* was suggested to be part of the variation of *C. serbica*.

The main goal of the study was to thoroughly revise the *C. maritima* group. We aimed (1) to establish a taxonomic concept considering both morphological and genetic differentiation among the populations and entities resolved; (2) to infer a molecular phylogenetic hypothesis for the relationships within the group and to its closest relatives; and (3) to explore and interpret the evolutionary and biogeographic history of the group.

MATERIALS AND METHODS

Taxon sampling. — The sampling focused on the area of the Apennine and western Balkan Peninsulas. We sampled multiple populations from each of the previously recognized taxa of the *Cardamine maritima* group, including type localities whenever possible (Appendix 1). All populations analyzed in the previous AFLP study (Kučera & al., 2008) were also examined. The plants were tentatively assigned to groups or species according to their morphology, geographic origin and the allopatric genetic entities suggested by AFLP data.

Morphometric analyses included only taxa from the *C. maritima* group; mostly 10–31 (rarely fewer) individuals were collected per population, with 35 populations and 816 specimens sampled in total. Molecular analyses were based on a broader taxon sampling; along with the *C. maritima* group, representative samples of its closest relatives were analyzed—*C. glauca* (S Apennine and Balkan Peninsulas), *C. pancicii* Hayek (endemic of Mt. Kopaonik, Serbia; Lakušić & al., 2006) *C. graeca* (Apennine and Balkan Peninsulas, Corsica), *C. resedifolia* (European Alpine System) and *C. alpina* (Pyrenees, Alps)—as well as two other morphologically similar species of so far uncertain phylogenetic position: *C. plumieri* Vill. (NW Apennine Peninsula, Corsica, Albania, Greece) and *C. carnosa* Waldst. & Kit. (W Balkan). Altogether, 56 populations were surveyed, with a sampling of 1–3 individuals per population (Appendix 1; Fig. 1).

Herbarium specimens were studied from: B, BEO, BEOU, BM, CLU, FI, GZU, K, KL, PR, PRC, SARA, SO, SOM, W, WU, Z, ZA and ZAHO.

Karyological analyses. — Chromosome numbers were recorded in 18 of the sampled populations. Chromosomes were counted from the root tips of germinating seeds. Seeds were collected in the field or from plants cultivated at the Institute of Botany in Bratislava. Root tips were pre-treated with 0.002 M hydroxyquinoline for 5 h, fixed in a freshly prepared mixture of concentrated ethanol and acetic acid (1 : 1), hydrolyzed in a mixture of concentrated hydrochloric acid and ethanol (1 : 1) for 1–2 min and rinsed in water. Squashes were made in a drop of 45% acetic acid under a cellophane square (Murín, 1960), and stained in a 10% Giemsa stock solution in Sörensen phosphate buffer for 1 h.

DNA extraction, amplification and sequencing. — Total genomic DNA was isolated from silica gel–dried leaves using the CTAB (2× cetyl trimethyl ammonium bromide) method (Doyle & Doyle, 1987) with minor modifications. Four genomic regions were surveyed: intron of *trnL* (cpDNA), *trnL-trnF* intergenic spacer (cpDNA), ITS region (ITS1–5.8S–ITS2) of nuclear ribosomal DNA (nrDNA), and single-copy nuclear gene *chalcone synthase* (*CHS*; major part of exon 2). PCRs were performed using universal primers: primers c, d, e, f for *trnL* and *trnL-trnF* of cpDNA (Taberlet & al., 1991), P1A, P4 for ITS (Francisco-Ortega & al., 1999), and primers CHS-FOR1 and CHSR1 for the *CHS* gene (Koch & al., 2000; Lihová & al., 2006). The PCR mix contained 10× reaction buffer that included $MgCl_2$ at 1.4 mM, 0.2 mM of each dNTP, 0.2 µM of each primer, and 0.6 U of *Taq* polymerase (Fermentas, St. Leon-Rot, Germany) in a total reaction volume of 40 µl. Amplifications were run in Mastercycler® ep gradient S (Eppendorf, Hamburg, Germany) using the following cycle profiles; cpDNA regions: 94°C (5 min), 35 cycles with 94°C (1 min), 54°C (1 min) and 72°C (45 s), and final extension at 72°C (10 min); ITS region: 94°C (5 min), 38 cycles with 94°C (30 s), 54°C (30 s) and 72°C (1 min), and final extension at 72°C (10 min); *CHS* gene: 94°C (5 min), 35 cycles with 94°C (30 s), 54°C (30 s) and 72°C (2 min), and final extension at 72°C (7 min). PCR products were purified using a Spinprep PCR clean-up kit (Calbiochem, Darmstadt, Germany) according to the protocol

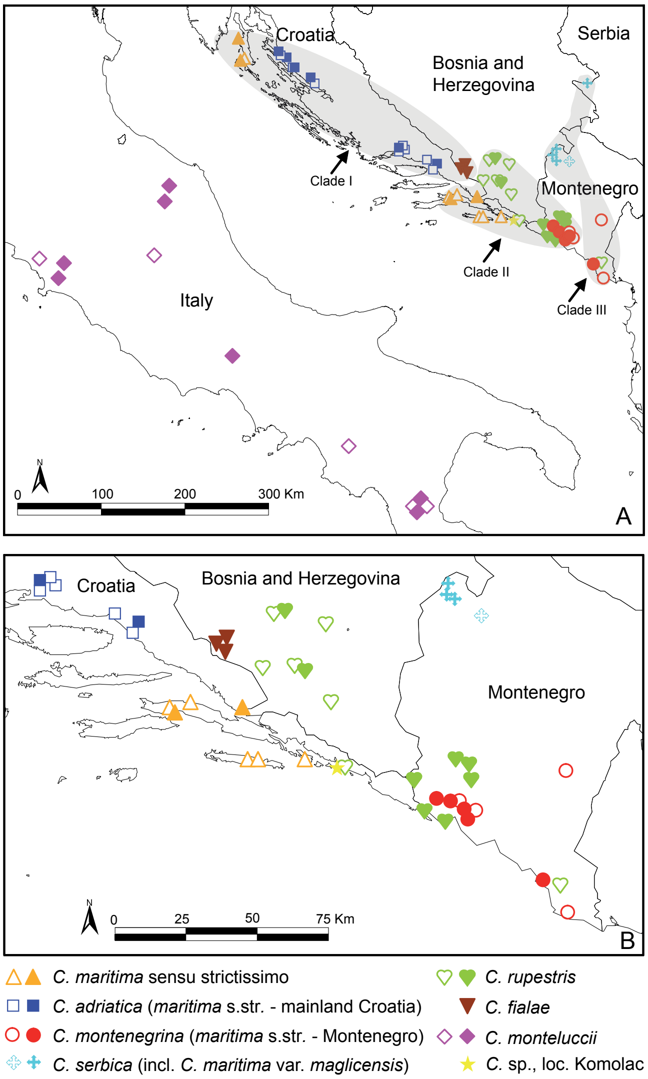

Fig. 1. Map of sample sites (filled symbols, see Appendix 1) and geographic distribution of the studied taxa of the *Cardamine maritima* group based on available herbarium records (open symbols, see Appendix 2 in the Electronic Supplement). **A,** overview of the study area; the three clades identified in the ITS phylogeny (Fig. 3) are shown in grey ovals; **B,** on the southern part of the studied Balkan area. *Cardamine maritima* sensu strictissimo refers to *C. maritima* s.str. in the sense of original description; *C. adriatica* and *C. montenegrina* refer to *C. maritima* s.str. from mainland Croatia and Montenegro, respectively.

of the manufacturer. Sequencing, using the original PCR primers, was performed at the BITCET Consortium at the Department of Molecular Biology, Comenius University, Bratislava (automated sequencer ABI PRISM 3130xl).

Due to the presence of multiple copies of nonfunctional *trnF*$^{(GAA)}$ pseudogenes reported from Brassicaceae (incl. *Cardamine*; see Koch & al., 2005), the 3′-region of the *trnL-trnF* intergenic spacer is characterized by structural mutations evolving at a high mutation rate, limiting its use for phylogenetic purposes. To avoid ambiguity in sequence alignment and indel handling, part of the 3′-region was eliminated from phylogenetic reconstructions. Electropherograms of ITS sequences were inspected for the presence of overlapping peaks, indicating the occurrence of substitutions between different ITS repeats in the same individual. Quantitative criteria to distinguish intraindividual ITS variation (i.e., intraindividual polymorphisms) from noise background followed recommendations by Fuertes Aguilar & Nieto Feliner (2003). IUPAC ambiguity codes were used for coding such polymorphic positions. The primers CHS-FOR1 and CHSR1 amplify almost the whole *CHS* gene, which includes two exons and one intron (Lihová & al., 2006: fig. 2). Sequencing of the amplified *CHS* gene was done with the reverse primer CHSRl only. Thus, a major part of exon 2 was obtained, aligned and used for phylogenetic reconstruction. In our previous study (Lihová & al., 2006) we showed that sequences of the *CHS* intron were unalignable among the closest relatives due to many short insertion/deletions, and exon 1 was considerably shorter (ca. 200 bp) than exon 2 (ca. 900 bp). Therefore, exon 2 was targeted in this study.

All sequences were submitted to GenBank (Appendix 1). The sequences were edited and aligned manually using BioEdit (v.7.0.4.1; Hall, 1999). The alignments are available in TreeBase (study number S2336).

Phylogenetic analyses. — Based on previous phylogenetic studies (Franzke & al., 1998; Bleeker & al., 2002) taxa from *Rorippa* Scop. and *Barbarea* W.T. Aiton were used as outgroups. Alignments of two chloroplast regions (*trnL*, *trnL-trnF*) were combined into a single cpDNA dataset. The datasets (*trnL* + *trnL-trnF*, ITS, *CHS*) were analyzed by Bayesian inference (Huelsenbeck & Ronquist, 2001) as implemented in MrBayes v.3.1.2. and the maximum likelihood method with a stochastic genetic algorithm-like approach used in GARLI program (v.0.96; Zwickl, 2006). Models of nucleotide substitutions appropriate for the individual datasets were identified by MODELTEST v.3.7 (Posada & Crandall, 1998) in conjunction with PAUP* v.4.0b10 (Swofford, 2001). The Akaike information criterion (AIC) and hierarchical likelihood ratio test (hLRT) were used to determine the model that best fits the dataset. In case of conflict a likelihood ratio test was applied (Huelsenbeck & Crandall, 1997).

The Bayesian analysis was run with the four chains for four million generations and with the following settings; the cpDNA dataset (TVM + G model): six substitution rates (nst = 6), gamma distribution (rates = gamma), and Temp = 0.05 (a value achieving efficient swaps between the chains); the ITS dataset (SYM + G model): six substitution rates, gamma distribution, fixing the equilibrium nucleotide frequencies to be equal (statefreqpr = fixed(equal)), and Temp = 0.06; the *CHS* dataset (SYM + I + G model): six substitution rates, gamma distribution assuming also invariable sites (rates = invgamma), fixing the nucleotide frequencies to be equal, and Temp = 0.05. The trees generated were summarized by computing a majority-rule consensus tree, excluding the trees of the burn-in phase. The percentage of trees recovering an individual node was indicated on the consensus tree by the node's posterior probability (PP). Four independent runs were conducted for each dataset, and the topologies and PP values of the consensus trees were compared.

Maximum likelihood analyses (GARLI) were run in multiple search replicates with different starting tree topologies (random and stepwise) and search parameters, giving confidence that the same topology and similar lnL scores are repeatedly found. Model specification settings corresponded to the models as determined above: 6-rate matrix, gamma distribution and equal state frequencies for ITS and *CHS* data; and 4-rate matrix (aabcad), gamma distribution and estimated state frequencies for the cpDNA dataset. Bootstrap support (BS) was calculated on the basis of 500 resamplings.

Morphometric analyses. — A total of 28 morphological characters (quantitative or binary) were recorded, and two ratios were derived (Table 1). Floral parts of one well-developed flower per plant were attached to paper by adhesive tape, dried, scanned on Microtek ScanMaker 9800XL, and measured with the Carnoy program (Schols & al., 2002). Fruit characters were recorded on a restricted set of 561 specimens, and analyzed separately from the vegetative and floral characters.

Both principal component analysis (PCA; Sneath & Sokal, 1973; Krzanowski, 1990) and discriminant analyses (canonical discriminant analysis [CDA], and non-parametric classificatory discriminant analysis; Klecka, 1980) were employed. A cross-validation procedure was used in the classificatory discriminant analysis, in which the classification criterion is based on $n - 1$ individuals and then applied for the individual left out. Discriminant analyses generally require multivariate normal distribution of the characters, but were shown to be considerably robust against deviations in this respect (Thorpe, 1976; Klecka, 1980).

Due to the high number of studied taxonomic entities (8) and the complexity of their morphological variation, herbarium specimens were divided into two parts based on the presence or absence of auricles at the leaf base. This trait is stable and well pronounced within the group. In addition, specimens of *C. maritima* s.str. (from Croatia and Montenegro, but excluding *C. fialae*, *C. monteluccii*, *C. rupestris* and *C. serbica*; see Fig. 1 and Results) were also analyzed separately to gain more insight into the differentiation among its allopatric genetic groupings. Thus, three datasets were subjected to PCA and CDA: (1) non-auriculate plants (417 individuals, 24 characters) of *C. maritima* s.str. from the type locality (Pelješac Peninsula) and the Croatian islands of Cres and Lošinj (= *C. maritima* sensu strictissimo), *C. monteluccii*, *C. rupestris*, and a taxonomically uncertain population from Komolac, southern Croatia; (2) auriculate plants (399 individuals, 20 chars.) of *C. maritima* s.str. from mainland Croatia and from Montenegro, *C. fialae*, and

Table 1. List of characters used in morphometric analyses.

Vegetative characters	
PAA[c]	Presence or absence of auricules on the base of stem leaves (1/0)
SLM[a,b]	Leaflet margin (1/0—coarsely crenate or serrate)
NLL[a,b,c]	Number of leaflets of the middle stem leaf
NL[a,b,c]	Number of stem leaves
NLI[a,b,c]	Number of lateral inflorescences on the main stem
HST[a,b,c]	Presence or absence of hairs on stem (1/0)
LTL,[a] WTL[a]	Length and width of the terminal leaflet of the middle stem leaf [cm]
NTTL[a,b]	Number of teeth/crenae on the terminal leaflet of the middle stem leaf
LLL,[a] WLL[a]	Length and width of the basalmost lateral leaflets of the middle stem leaf [cm]
NTLL[a,b,c]	Number of teeth/crenae on the most distal lateral leaflets of the middle stem leaf
NLS[a,c]	Number of lateral stems
LS[a,b,c]	Length of stem [cm]
WSB[a,b,c]	Width of stem base [mm]
Floral characters	
LBS,[a,b,c] WBS[a,b,c]	Maximum length and width of longer sepals [mm]
LSS,[a,b,c] WSS[a,b,c]	Maximum length and width of shorter sepals [mm]
LP,[a,b,c] WP[a,b,c]	Maximum length and width of petals [mm]
LSF,[a,b,c] LLF[a,b,c]	Maximum length of shorter and longer filaments [mm]
HS[b]	Presence or absence of hairs on the sepals (1/0)
Fruit characters	
LS, WS	Length and width of siliqua (excluding peduncle and style) [cm]
LST	Length of style [cm]
LPE	Length of peduncle [cm]
Ratio characters	
LTL/WTL[a,b,c]	
LLL/WLL[a,b]	

[a] Characters used for the dataset of non-auriculate populations (those lacking auricles at leaf base).
[b] Used for the dataset of auriculate populations (those with auricles at leaf base).
[c] Used for the dataset *Cardamine maritima* s.str.

C. serbica (incl. *C. maritima* var. *maglicensis*); (3) *C. maritima* s. str. (364 specimens, 18 chars.) from several Croatian islands, the Pelješac Peninsula, mainland Croatia, and Montenegro. All multivariate morphometric analyses were performed using SAS v.9.1.3. software (SAS Institute, 2007).

RESULTS

Karyological analyses. — Only the diploid chromosome number $2n = 16$ was found in the examined populations (Appendix 1). Thus, each of the studied taxa was confirmed to be diploid, in accordance with the previously published record on *Cardamine monteluccii* (Brilli-Cattarini & Gubellini, 1986).

Phylogenetic analyses. — *cpDNA dataset.* – The *trnL* and *trnL-trnF* alignments comprised 71 ingroup sequences; *trnL* alignment was 531 bp long (individual sequences spanned 465–500 bp) with eight indels (>2 bp) introduced; the trimmed 5′ part of *trnL-trnF* alignment was 362 bp long (sequences spanned 295–346 bp) with three indels (>2 bp) introduced. The combined cpDNA (*trnL* + *trnL-trnF*) alignment comprised 893 bp (sequences spanning 760–817 bp), of which 98 sites were variable, and 11 indels (>2 bp). Indels ≤2 bp referred to polyN or microsatellite stretches, and were not considered in phylogenetic inferences. Those >2 bp were recorded and indicated on a resulting phylogenetic tree.

The Bayesian majority-rule consensus tree had the same topology across the four replicate runs (Fig. 2). Only minor differences in PP values for some nodes were observed. The maximum likelihood tree (score lnL = –2073.52) showed one difference in comparison with the Bayesian consensus tree, which concerned a clade with low resolution at its internal branches (depicted in Fig 2). Two main clades were inferred (with PP = 1.00, BS = 99%, and PP = 0.95, BS = 71%, respectively). One clade included *Cardamine alpina*, *C. glauca*, *C. pancicii*, and *C. resedifolia*, which were resolved into two sister subclades. The other main clade comprised all remaining taxa; the resolution at its internal nodes was low (displaying

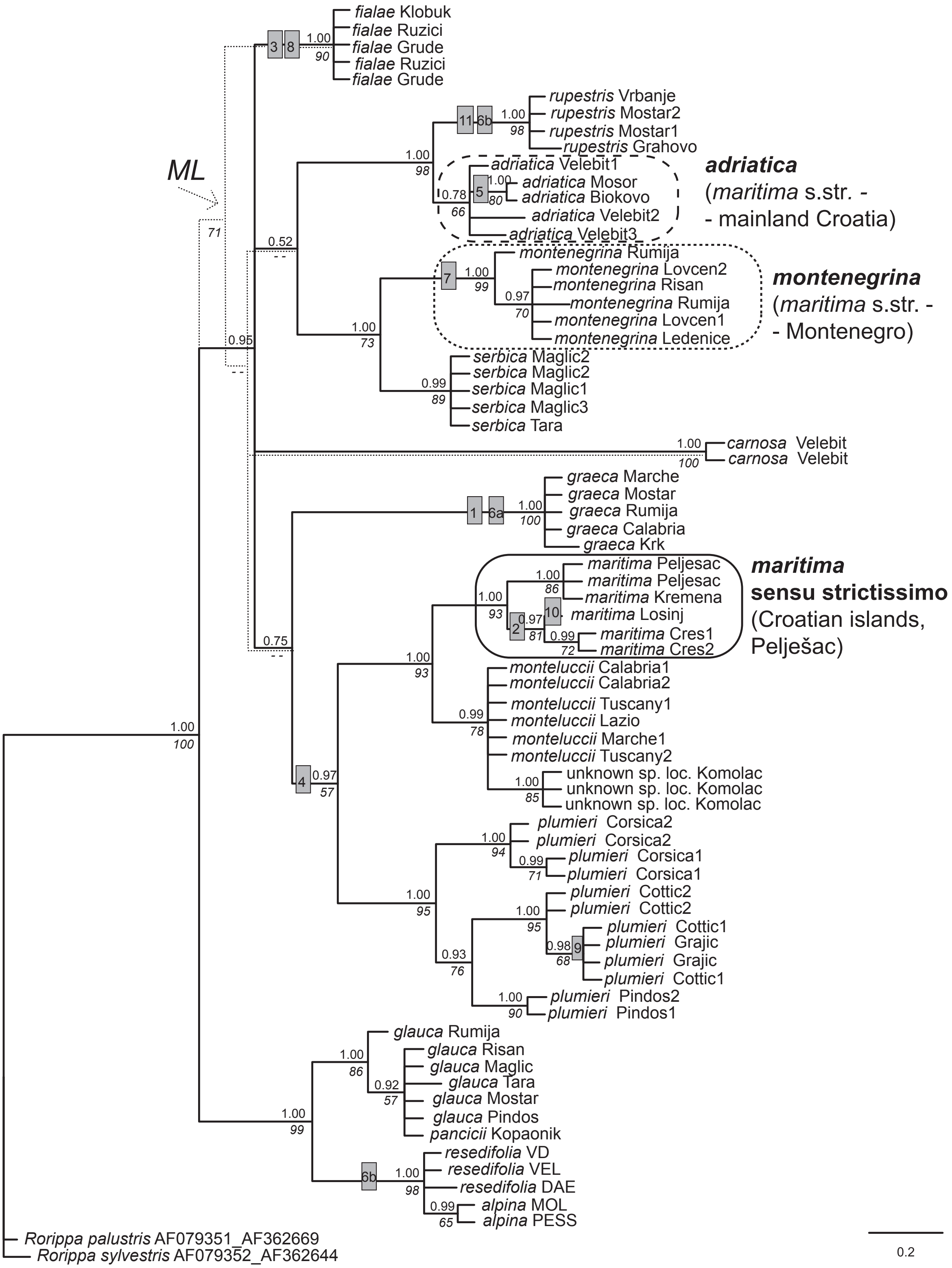

Fig. 2. Bayesian majority-rule consensus tree with mean branch lengths based on cpDNA sequences (combined alignments of *trnL* intron and *trnL-trnF* intergenic spacer) of the studied *Cardamine* species. The dotted lines and arrow indicate the topology as resolved in the maximum likelihood tree. Posterior probability values of the Bayesian consensus are indicated above the branches; bootstrap support as inferred for the maximum likelihood tree is shown below the branches (in italics, – means <50%). Gray bars with numbers 1–11 indicate indels >2 bp, specific to a certain clade or terminal sequence; 6a and 6b denote two different, but overlapping gaps. Accessions are denoted by the species epithet and accession code (according to Appendix 1). Clades corresponding to three allopatric and genetically distinct entities recognized within *C. maritima* s.str. are highlighted.

polytomy or clades with low PP and BS), but terminal parts showed a series of small, moderately to well-supported clades, mostly corresponding to individual species or allopatric entities. The following species/entities were supported as genetically distinct: *C. carnosa*, *C. fialae*, *C. graeca*, *C. monteluccii*, *C. plumieri*, *C. rupestris*, *C. serbica*, and three allopatric entities within *C. maritima* s.str. (accessions from Montenegro, mainland Croatia, and Croatian islands incl. Pelješac Peninsula). Samples from the Maglić Mts. (described as *C. maritima* var. *maglicensis*) displayed the same haplotype as the sample of *C. serbica* from the type locality (Tara Mts.). A morphologically peculiar population from southern Croatia (loc. Komolac) appeared here as being very close to *C. monteluccii.* A few sister-group relationships were suggested among the species/entities. Several of the clades or sister relationships were additionally supported by indels (see Fig. 2). Each of the eleven indels was found to be specific to a certain clade or terminal sequence, except for the indel no. 6b, which was shared by two distinct clades (*C. alpina* and *C. resedifolia* vs. *C. rupestris*).

ITS dataset. – Sequencing of the ITS region was straightforward, and resulted in completely homogenized sequences or sequences with only a few intra-individual single-site polymorphisms. These were mainly found in five accessions: two accesions of *C. fialae* (localities Grude, Klobuk), one of *C. maritima* sensu strictissimo from the Pelješac Peninsula (loc. Kremena), and two of *C. maritima* s.str. from mainland Croatia (loc. Mosor, Biokovo). The ITS alignment consisted of 71 ingroup sequences, and was 620 bp long (individual sequences spanning 604–619 bp) with only one indel >2 bp introduced (a 15-bp deletion was inferred in one sample of *C. glauca* from Mostar). The ITS dataset displayed 132 variable positions.

The Bayesian majority-rule consensus tree had the same topology in all four runs (with minor differences in PP values), and was also identical to the maximum likelihood tree (lnL = –2145.10) (Fig. 3). Two main clades were resolved, which, however, did not correspond to those recovered by cpDNA data. One clade comprised species that are considered to be relatives of the *C. maritima* group: *C. alpina*, *C. carnosa*, *C. glauca*, *C. graeca*, *C. pancicii*, *C. plumieri* and *C. resedifolia*. Each of the species (except *C. alpina* and *C. pancicii*, each represented only by one sample) formed its own clade and received strong support (PP = 0.99–1.00, BS = 81%–100%). Relationships among the species were generally well resolved (except for one internal clade with a low PP of 0.65 and BS of 61%). The clade identified in the cpDNA tree that included *C. alpina*, *C. glauca*, *C. pancicii* and *C. resedifolia* was observed also here. Otherwise the species relationships suggested here were in contrast with those resolved by cpDNA data. The other main clade of the ITS tree corresponded to the *C. maritima* group itself. Resolution within this clade, however, was weaker. One well-supported (PP = 1.00, BS = 98%) and two weakly supported (PP = 0.88, BS = 69%, and PP = 0.68, BS < 50%) clades were found in polytomy (denoted as I, II, III in Fig. 3), having little internal structure within each of them. Species-specific clades seen in the cpDNA tree were not so apparent here. In fact, the only two species with clearly distinct ITS sequences were *C. serbica* (incl. *C. maritima* var. *maglicensis*, clade with PP = 1.00, BS = 99%) and *C. monteluccii* incl. the population from Komolac (PP = 0.62, BS = 59%). If the Bayesian and maximum likelihood inferences were performed without the five accessions that contained intra-individual single-nucleotide polymorphisms (see above), the resolution of clade I significantly improved. Two other entities—*C. fialae* and *C. maritima* s.str. from mainland Croatia—were resolved in distinct clades as well (PP of 1.00, BS of 99%–100%), and support for the clade of *C. monteluccii* incl. the population from Komolac increased (PP = 1.00, BS = 87%; Fig. 3). The three clades I, II, III formed in polytomy, however, showed a NW-SE geographic pattern (see Fig. 1). Splits within *C. maritima* s.str. from Croatian islands and Pelješac, and within *C. maritima* s.str. from Montenegro also reflected this geographic structuring.

CHS *dataset.* – The *CHS* alignment comprised 70 ingroup sequences and was 906 bp long, without having indels. Most of the sequences gave single peaks throughout the sequence, implying homozygosity. SNP (single-nucleotide polymorphism) heterozygosities, however, were indicated by the presence of double peaks in several accessions of *C. fialae*, *C. glauca*, *C. maritima* s.str. and *C. rupestris*. The *CHS* alignment comprised 149 variable positions.

The Bayesian analysis resulted in a majority-rule consensus tree that had the same topology in all four replicate runs (with only minor differences in PP values). The maximum likelihood tree (lnL = –3067.97) differed from it by resolving one trichotomy, but with BS < 50% (Fig. 4). Two main clades were inferred, which corresponded to those recovered by ITS data. One clade comprised the relatives of the *C. maritima* group: *C. alpina*, *C. carnosa*, *C. glauca*, *C. graeca*, *C. plumieri* and *C. resedifolia* (accession of *C. pancicii* was not amplified for *CHS* successfully, and was omitted here). Each of the species formed its own clade and received strong support (PP = 1.00, BS = 96%–100%). Relationships among the species were well resolved (except for one internal clade with low PP of 0.73 and BS of 58%), and agreed with those recovered by the ITS tree except for the position of *C. graeca*.

Resolution within the second main clade, comprising the *C. maritima* group, was rather low. One weakly supported (PP = 0.59, BS < 50%) and two moderately to well-supported (PP = 0.92, BS = 64% and PP = 1.00, BS = 85%) clades were observed (denoted as a, b, c; see Fig. 4). They did not correspond to clades I–III of the ITS tree, but some congruence was apparent. Clades b and c corresponded to clade II, and part of clade a (subclades a1 and a2) corresponded to clade I. The following three species were distinct in their *CHS* sequences, forming species-specific clades: *C. fialae*, *C. monteluccii*, and *C. serbica* (incl. *C. maritima* var. *maglicensis*). The peculiar population from southern Croatia (loc. Komolac) was resolved to be close to *C. rupestris* and two accessions of *C. maritima* sensu strictissimo from the Pelješac Peninsula.

Morphometric analyses. — *Dataset of 'non-auriculate' populations.* – PCA based on individual plants of populations that were characterized by non-auriculate leaves resulted in three overlapping groupings, corresponding to *C. maritima* sensu strictissimo, *C. monteluccii*, and *C. rupestris*. The taxonomically unassigned population from Komolac was intermingled with the latter two groupings (figure not shown). CDA based on individual plants and the four above-mentioned pre-defined groups showed much better resolution than PCA (Fig. 5). *Cardamine monteluccii* was placed in a cluster that was well separated along the first axis, while *C. maritima* sensu strictissimo and *C. rupestris* were resolved as two groupings along the second axis and overlapped marginally. The population from Komolac was found at the variation margin of *C. maritima* sensu strictissimo. The first axis, contributing to the separation of *C. monteluccii*, was mostly correlated with the length of sepals (LBS, LSS), width of petals (WP), type of leaflet margin (SLM), and the number of teeth on the

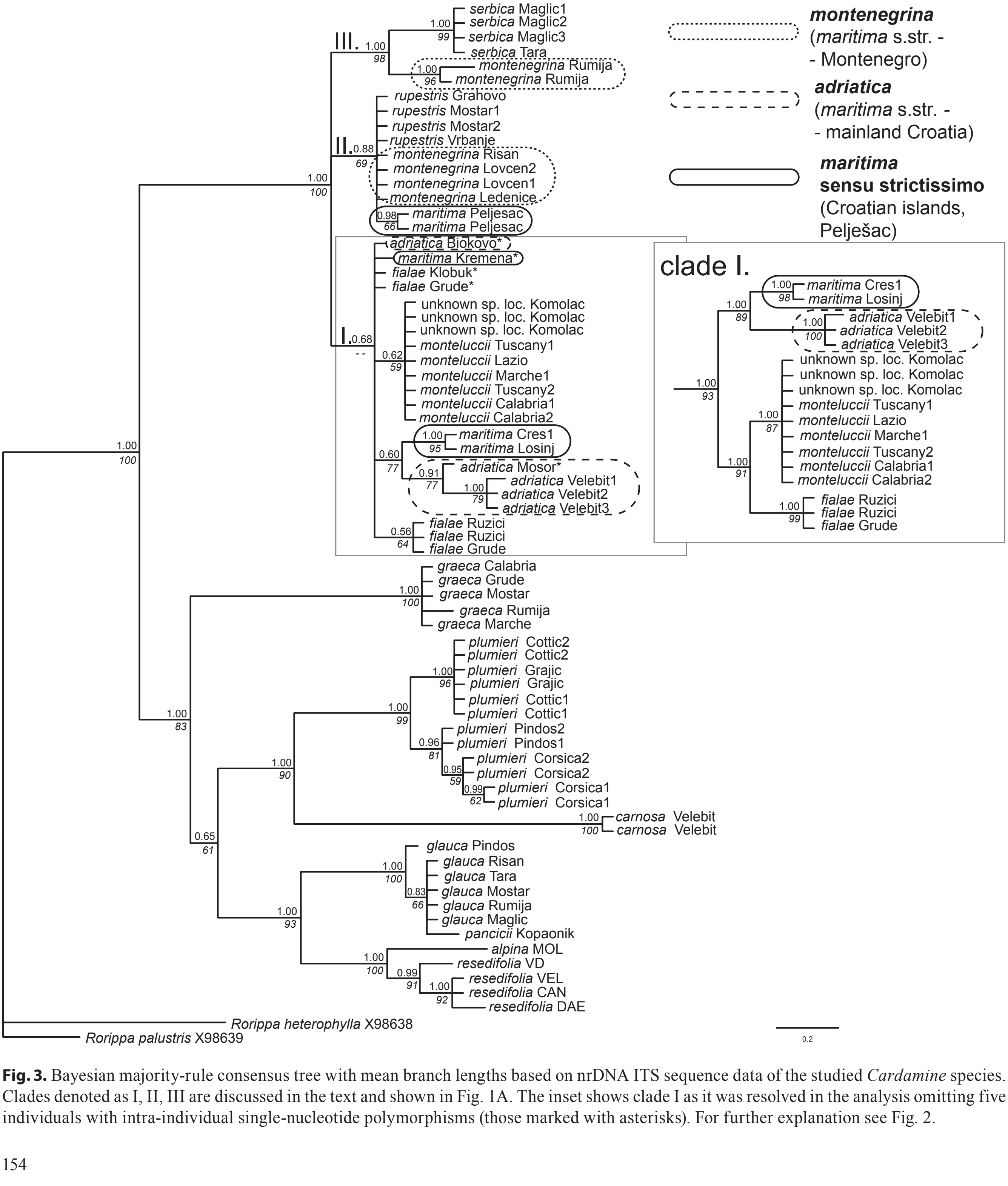

Fig. 3. Bayesian majority-rule consensus tree with mean branch lengths based on nrDNA ITS sequence data of the studied *Cardamine* species. Clades denoted as I, II, III are discussed in the text and shown in Fig. 1A. The inset shows clade I as it was resolved in the analysis omitting five individuals with intra-individual single-nucleotide polymorphisms (those marked with asterisks). For further explanation see Fig. 2.

most distal lateral leaflets of the middle stem leaves (NTLL; Table 2). *Cardamine maritima* sensu strictissimo and *C. rupestris* were differentiated from each other by the size (mainly the length) of the terminal and basal lateral leaflets of the middle stem leaves (LLL, LTL), length of stem (LS), width of stem base (WSB), and stem hairiness (HST) (characters correlated with the second canonical axis, see Table 2, and Table S1 in the Electronic Supplement).

Dataset of 'auriculate' populations. – PCA based on individual plants of the populations that are characterized by auriculate

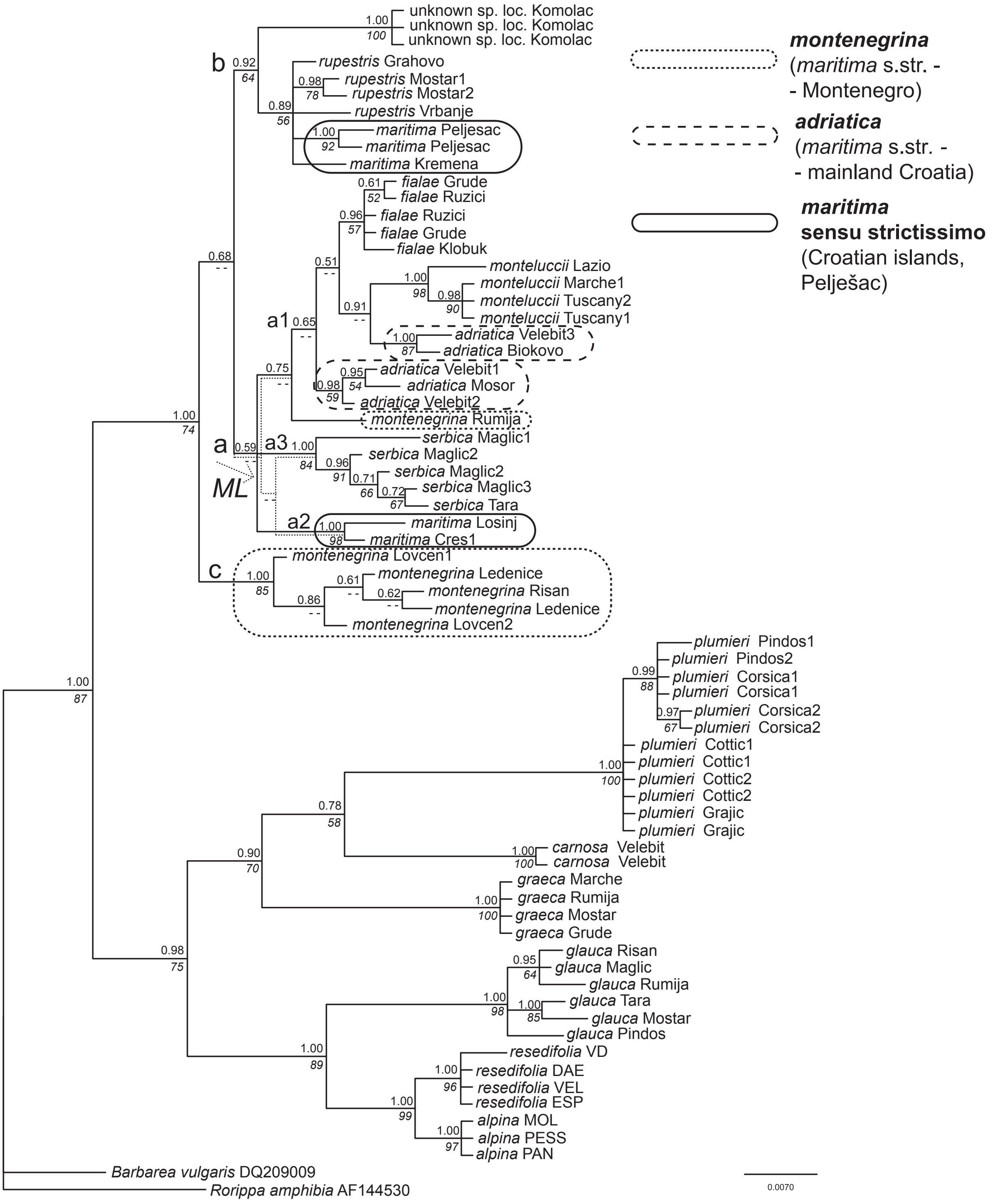

Fig. 4. Bayesian majority-rule consensus tree with mean branch lengths based on nuclear *CHS* (exon 2) sequence data of the studied *Cardamine* species. The dotted line and arrow indicate the topology as resolved in the maximum likelihood tree. For further explanation see Fig. 2 and text.

leaves resulted in three apparent groupings, corresponding to *C. fialae*, *C. maritima* s.str. from Montenegro and mainland Croatia, and to *C. serbica* and *C. maritima* var. *maglicensis* as one grouping (Fig. 6). There was no visible distinction between accessions of *C. maritima* s.str. from Montenegro and those from mainland Croatia in this analysis. Several characters were found to be strongly and almost equally correlated with the first component (i.e., contributing to the separation of *C. fialae*): the size of all floral characters (sepals, petals, filaments; LBS, WBS, LSS, WP, LP, LSF, LLF), hairiness of the sepals and stem (HST, HS), and the shape of the terminal and lateral leaflets of the stem leaves (LTL/WTL, LLL/WLL) (see Table 2, and Table S1 in the Electronic Supplement). Characters that have the highest correlations with the second component (i.e., contribute to the separation of *C. serbica* incl. *C. maritima* var. *maglicensis*) were the length of petals and filaments (LP, LLF, LSF), length of stem (LS), and width of the stem base (WSB).

Populations previously treated as Cardamine maritima *s.str. – Cardamine maritima* s.str. includes three genetically distinct and allopatric entities (see Fig. 2). Populations from Croatian islands incl. the Pelješac Peninsula (*C. maritima* sensu strictissimo) differ from the others at first sight by the absence of auricles on the base of the stem leaves. Morphometric analyses aimed to explore morphological differentiation among these entities, both at a population and individual plant level. PCA based on population averages resolved three clusters that are clearly separated along the first two axes (Fig. 7B), and correspond to the above-specified allopatric entities. Except for the presence/absence of auricles, several floral characters (size of sepals and petals, length of filaments: LBS, WBS, LSS, WP, LP, LLF; see Table 2, and Table S1 in the Electronic Supplement) as well as the width of stem base (WSB) were most important for their distinction. Although Montenegrin and mainland Croatian populations appeared

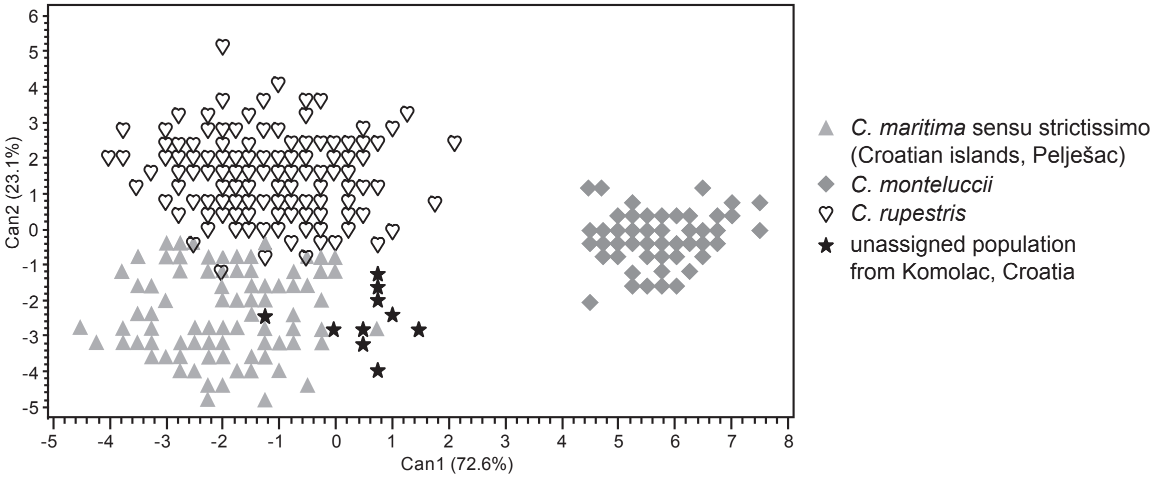

Fig. 5. Canonical discriminant analysis (CDA) of 417 individual plants of 'non-auriculate' populations of the *Cardamine maritima* group and four groups pre-defined, based on 24 morphological characters.

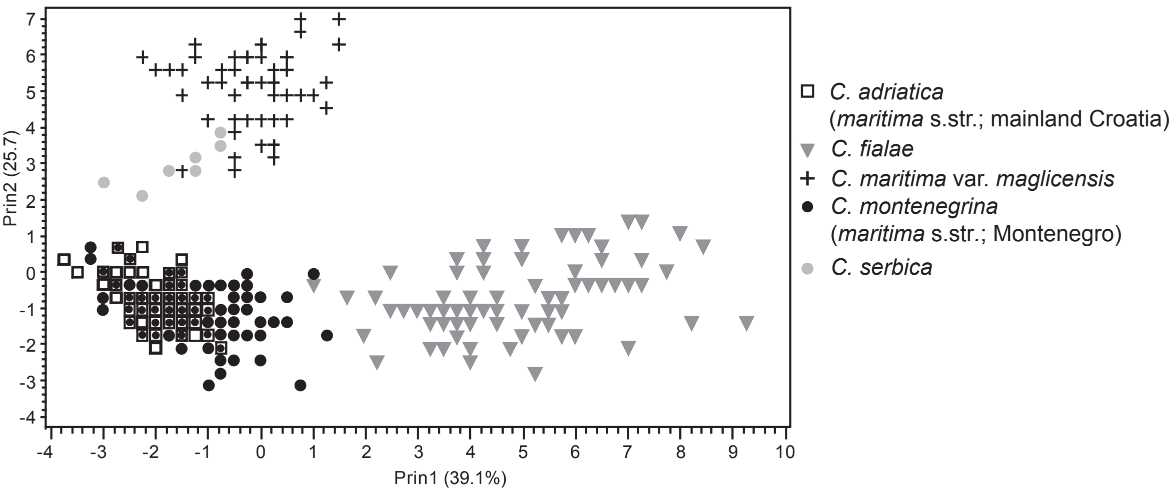

Fig. 6. Principal component analysis (PCA) of 399 individuals of 'auriculate' populations of the *Cardamine maritima* group, based on 20 morphological characters.

to be well separated at the population level, they were largely intermingled at the individual plant level (Fig. 7A). Next, CDA and classificatory discriminant analysis were performed at the individual plant level, and with the mainland Croatian and Montenegrin populations defined as two *a priori* groups. This was done to see the extent to which they could be distinguished when the characters were weighted and only these two groups were compared. The analyses resulted in much better differentiation than was shown by PCA, but some overlap is still apparent (Fig. 7C). In the classificatory discriminant analysis, 91% of individuals from mainland Croatia, and 94% of the Montenegrin individuals were correctly assigned (*k*-nearest non-parametric analysis with $k = 30$). CDA showed that floral characters in particular (size of petals and sepals (WBS, WP, LP), and the number of lateral stems and lateral inflorescences (NLI, NLS) contributed to their differentiation (Table 2, and Table S1 in the Electronic Supplement).

Analyses based on fruit characters. – PCA did not show any groupings in ordination graphs for fruit characters (figures not shown). Thus, we concluded that fruit characters do not contribute to the differentiation between the studied taxa.

■ DISCUSSION

Chloroplast vs. nuclear phylogenies—ambiguity in species relationships. — Taxonomic and phylogenetic studies of *Cardamine* are complicated due to the high frequency of polyploids and the reticulate evolutionary history. DNA sequence-based markers often showed low resolution and conflict, especially in recently evolved polyploid groups (Lihová & Marhold, 2006). The present study, on the other hand, exemplifies a case of diploid speciation and therefore, a rather straightforward evolutionary history and taxonomic solution was expected.

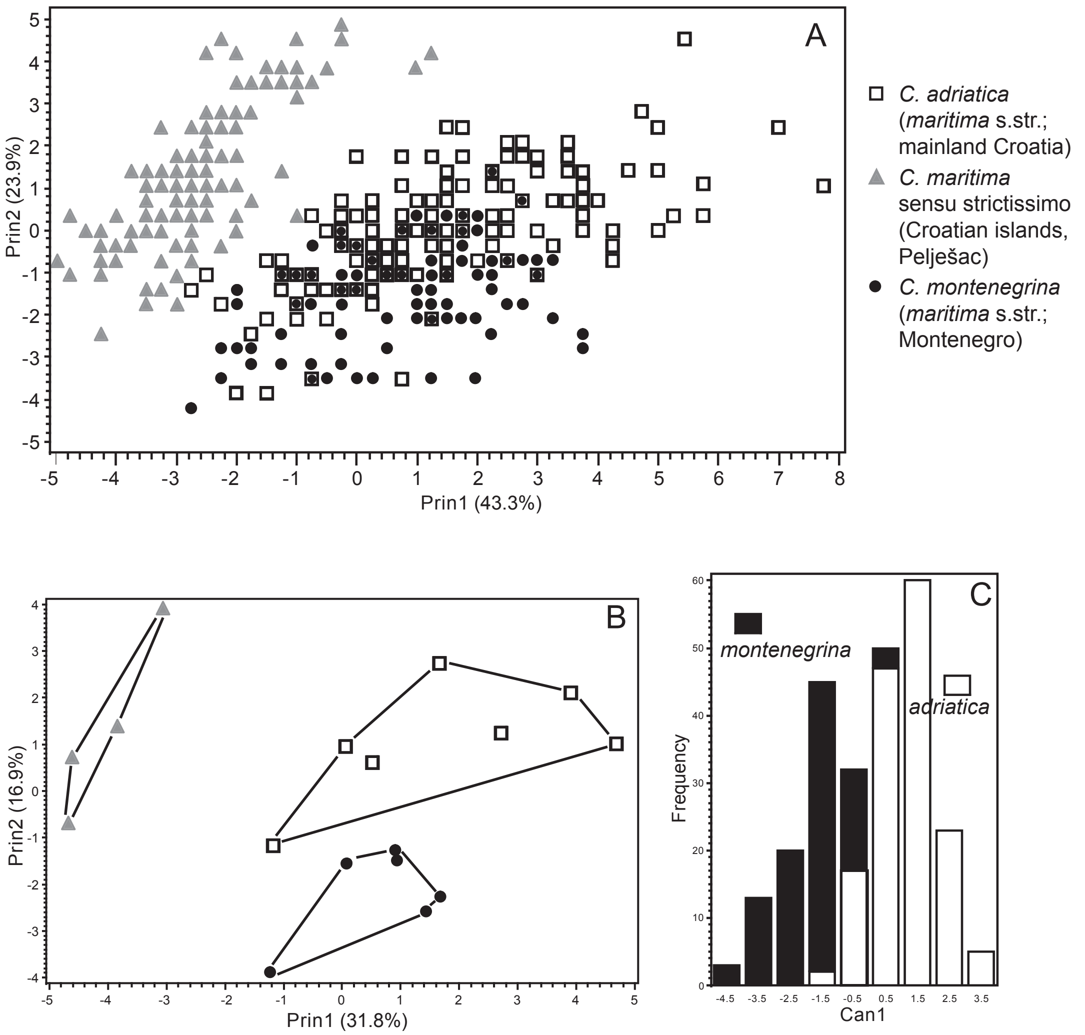

Fig. 7. A, B, Principal component analysis (PCA) of *Cardamine maritima* s.str. based on 18 morphological characters, and either individual plants (graph A) or populations (graph B) as objects; **C,** histogram of the canonical discriminant analysis (CDA) of individual plants of *C. montenegrina* (black) and *C. adriatica* (white) based on 24 morphological characters.

The phylogenetic analysis of cpDNA sequences resolved two main clades, and a series of small, moderately to well-supported terminal clades (Fig. 2). The latter clades were shown to be congruent with morphology and geographic areas, as well as with AFLP clusters (Kučera & al., 2008), and corresponded very well to previously described species. Nuclear DNA phylogenies (ITS, *CHS*) displayed two main clades as well (Figs. 3, 4), but they were different from those seen on the cpDNA tree. Resolution within the clade of the *C. maritima* group supported only some of these species, and indicated rather geographic correlations (Fig. 1).

Thus, incongruence between cpDNA and nuclear DNA data, as well as between two nuclear phylogenies, is obvious. It must be considered, however, that *Cardamine* is a fairly young genus and also one of the most species-rich genera in the Brassicaceae. Molecular data indicate that the lineages that gave rise to *Cardamine* and *Barbarea* diverged as late as ≈6 million years ago (Koch & al., 2000), and since then *Cardamine* diversified into as many as 200 species worldwide. The genus-wide phylogenetic reconstruction showed generally low resolution, regarding also deeper relationships, and even if high polyploids were omitted (Carlsen & al., 2009). It was hypothesized that the lack of resolution is primarily due to rapid initial diversification and speciation in the genus, rather than just breakdown of the phylogenetic signal by polyploidization and reticulation events. This conclusion is in agreement with AFLP variation (Kučera & al., 2008) and our present sequence data, which were derived from independent DNA markers with different inheritance and evolutionary dynamics.

Taxonomy of the *Cardamine maritima* group. — Our results of cpDNA sequencing and multivariate morphometric analyses, in concert with the previously published AFLP data (Kučera & al., 2008), lend strong support for the recognition of several species. While only two species, *C. maritima* and *C. monteluccii*, have been generally accepted in recent taxonomic treatments (Jones & Akeroyd, 1993; Jalas & Suominen, 1994), in this study we show that three other species, which were previously neglected or doubted, should be recognized: *C. fialae*, *C. serbica* and *C. rupestris*. We are able to attribute the resolved genetic entities to previously described species without any ambiguities, because we had material from type localities of several names (see Appendix 1) and the species were originally described and reported from restricted areas (such as certain mountain ranges). *Cardamine fialae* was confirmed to be a narrow endemic of the Ljubuški district in Bosnia and Herzegovina (see Appendix 2 in the Electronic Supplement; Fig. 1). The population of *C. serbica* from the type locality clearly fell within the variation of populations from Mratinje in Montenegro, described by Rohlena (1906) as *C. maritima* var. *maglicensis*. The name *C. serbica* should therefore also be applied to the latter populations. *Cardamine serbica*, as it is currently defined, occupies the area of the Tara Mts. in Serbia, and Maglić and Durmitor Mts. in Montenegro (see Appendix 2 in the Electronic Supplement; Fig. 1). *Cardamine monteluccii*, only recently described from Italy (Brilli-Cattarini & Gubellini, 1986), is clearly distinct from Balkan populations.

Morphological characters identified here as diagnostic are partly in accordance with those reported in floras (e.g., Trinajstić, 1976; Jones & Akeroyd, 1993). Nevertheless, several fruit characters, such as length of siliquae, styles and peduncles, reported by some authors (Pančić, 1884; Hayek, 1927; Trinajstić, 1976), are considerably variable even within the narrowly defined species, and are of little taxonomic use. To summarize the results of our morphometric analyses, an identification key to the species recognized is presented below.

Three entities within *Cardamine maritima* s.str. — The taxonomic solution for *C. maritima* s.str., spanning the area from the Kvarner bay in northern Croatia to Montenegro in the south, is more complicated. Three allopatric groupings are suggested here, although it may be a matter of discussion whether they deserve formal taxonomic recognition. The three entities harbour distinct cpDNA haplotypes, which form three clades that reside on distant parts of the phylogenetic tree (Fig. 2). Admittedly, cpDNA is not always the right marker to define species boundaries. Due to processes such as chloroplast capture or incomplete lineage sorting, the same haplotypes can be shared by different species, and cpDNA variation can display a geographic rather than a taxonomic structure (e.g., Wolf & al., 1997; Gutiérrez-Larena & al., 2002; Frajman & Oxelman, 2007; Koch & al., 2008). However, this is not the case with the present data, since all other clades retrieved in the cpDNA tree correspond well to morphology, and thus, cpDNA phylogeny appears to be a reliable predictor of species boundaries. In addition, the cpDNA pattern is supported by the recently published AFLP data (Kučera & al., 2008). From a morphological perspective, populations from Croatian islands and the Pelješac Peninsula differ from the other populations of *C. maritima* s.str. by the absence of auricles at leaf base, and several other, mainly floral, characters. These findings can be regarded as sound arguments in favour of recognizing three separate species within *C. maritima* s.str.

Nevertheless, arguments against this concept exist as well. The nuclear *CHS* and ITS phylogenetic trees seem to favour a broad concept of *C. maritima* that would include not only *C. maritima* s.str., but also *C. fialae*, *C. monteluccii*, *C. serbica* and *C. rupestris*, exhibiting specific geographic structure. The less pronounced morphological differentiation observed between the populations from Montenegro and Croatian mainland also might hamper their unequivocal determination. Despite these cons, we are convinced that the concept presented here, with three separate species within *C. maritima* s.str., more accurately reflects the evolutionary history of the group (see below), and avoids recognition of an apparently heterogeneous species both in respect of morphology and DNA data. Taxonomic separation of genetically and morphologically differentiated units also better serves conservation purposes. The studied group is almost exclusively composed of narrow endemics of the western Balkans, and as such it exemplifies high rates of speciation and floristic richness in this area, which has also been documented in other plant groups (e.g., Park & al., 2006; Stefanović & al., 2008).

According to the concept adopted here, the name *C. maritima* Port. ex DC. is applicable to populations from Croatian islands and the Pelješac Peninsula. The species is distributed in Croatia in two disjunct areas: the islands of the Kvarner bay (Cres and Lošinj), and on the Pelješac Peninsula and the adjacent islands of Mljet and Šipan (Appendix 2 in the Electronic Supplement; Fig. 1). Islands of central Dalmatia are typically open landscapes that are poorly covered by vegetation, so the lack of appropriate habitat explains its absence there.

Two names are available for the populations from mainland Croatia: *Pteroneurum microphyllum* J. Presl & C. Presl and *P. bipinnatum* Rchb. The type material of the former name has not been found, and is probably lost. The name can be assigned to this taxon based on the location of its type locality, given as Capo Cesto (today Primošten). Still, we did not find any herbarium specimens from this locality in the studied herbaria. Furthermore, the epithet "*microphyllum*" cannot be used in the genus *Cardamine*, as it would result in an illegitimate combination *Cardamine microphylla* (because of the earlier name *C. microphylla* Adams (1817)). The type specimen of the latter name, *Pteroneurum bipinnatum* Rchb., was most likely destroyed during the great Zwinger fire (Stafleu & Cowan, 1983). However, several specimens from the same locality ("Urulia" [near D. Brela village]) are deposited in other herbaria (Appendix 2 in the Electronic Supplement). One of our population samples was collected close to this site as well. We propose the neotype for this name here. Nevertheless, the epithet "*bipinnatum*" cannot be used in the genus *Cardamine*, as it would result in an illegitimate combination, because of the earlier name *C. bipinnata* (C.A. Mey.) O.E. Schulz (1903). The name *C. adriatica* Jar. Kučera, Lihová & Marhold is published here as a *nomen novum*. The distribution range of this species stretches through a relatively narrow strip along the Croatian coast, from sea level up to ca. 650 m of adjacent mountain chains (Velebit, Mosor, Biokovo Mts.; Fig. 1).

Finally, there is no name available for the third segregant of *C. maritima* s.str. from Montenegro, which is described here as a new species *C. montenegrina* Jar. Kučera, Lihová & Marhold. It occurs in the Kotor, Bar and Ulcinj districts, and in the Podgorica district (canyon Morače), from sea level up to 920 m (Fig. 1).

A presumably hybrid population from southern Croatia. — A peculiar population from the Komolac village (Dubrovník district, southern Croatia) remains taxonomically unassigned. Thorough sampling and field research was conducted in the vicinity, but no similar populations were found. Morphologically it resembles *C. rupestris* and *C. maritima* sensu strictissimo (Fig. 5), in cpDNA and ITS phylogenies it is resolved in a clade together with *C. monteluccii* (Figs. 2, 3), while in the *CHS* tree it forms its own clade in a sister position to the clade of *C. rupestris* and *C. maritima* sensu strictissimo from the Pelješac Peninsula (Fig. 4). This may suggest its hybrid origin, with *C. monteluccii* and either *C. rupestris* or *C. maritima* sensu strictissimo as parental species. The distribution ranges of the two latter species overlap with the locality in Komolac, but affinity to the Italian species *C. monteluccii* is intriguing at first sight—it assumes contacts between areas east and west of the Adriatic coast. Indeed, in cold periods of the Pleistocene, sea level regression (Frenzel & al., 1992) enhanced the possibility of trans-Adriatic contacts and dispersal.

Evolutionary history and biogeography of the *Cardamine maritima* group. — Mutation rates in non-coding cpDNA regions are generally lower than in nrDNA (ITS) and low- and single-copy nuclear genes (Small & al., 2004), so we assume that the genetic structure resolved by cpDNA data reflects an older pattern, manifesting initial radiation of the ancestral lineage into a series of distinct species in the late Tertiary (see molecular dating above). Lack of resolution at internal nodes indicates that the radiation was rapid and most likely occurred in an allopatric manner, resulting in several narrow endemics. Ecological factors were probably not the major speciation force, since there is no apparent ecological differentiation between the species. The studied species can be found in shady sites in karstic regions, growing in gorges, on shady screes and slopes, and in deep rock crevices. Other factors, perhaps topographic heterogeneity (islands and mountains, forming geographic barriers to dispersal) and long-term environmental stability (refugial character of the region) of the Balkans, may have played a key role in promoting population isolation and gradual divergence at small geographic scales (see Thompson, 2005; Kryštufek & Reed, 2004). Due to a faster overall rate of evolution of nuclear ITS and *CHS* sequences, these data presumably recover more recent processes. Geographic structure in nuclear markers is rare, compared to uniparental and non-recombinating cpDNA markers, but has been observed, e.g., in Iberian *Armeria* Willd. species (Nieto Feliner & al., 2004). Exploration of contact zones between different ribotypes in *Armeria* suggested that gene-flow within and between species, and concerted evolution, caused this pattern, which was triggered by altitudinal migrations during climatic oscillations of the Pleistocene (Nieto Feliner & al., 2004). We assume that a very similar scenario can be suggested to explain the patterns of the *C. maritima* group. *CHS* and ITS sequences of *C. maritima* sensu strictissimo from the Pelješac Peninsula were more similar to those of the geographically close *C. rupestris*, than to the other conspecific accessions from the NW part of the range (Kvarner bay). Several other instances documenting this pattern can be seen in the present data. Thus, the geographic distribution of ITS variants and *CHS* alleles does not seem to be merely the result of random sorting of ancestral polymorphisms, but favours past interspecific gene flow and introgression. Increase of hierarchical structure in the ITS tree after removal of accessions with intra-individual single-site polymorphisms (Fig. 3) adds further evidence for hybridization as the major evolutionary process affecting nuclear DNA variation in this group.

Climatic changes during Quaternary glacial periods induced shifts in species distribution ranges and caused population extinctions. However, in contrast to northern territories, these were less severe in the Mediterranean. Topographical variability of this area allowed populations to migrate over short distances and along altitudinal belts, and inhabit suitable habitats (Hewitt, 2001; Tzedakis, 2004). As observed in southern Spain (Gutiérrez Larena & al., 2002; Nieto Feliner & al., 2004), we expect similar glacial-induced small-scale migrations along the Balkan coastline and in adjacent massifs. Habitats occupied by species of the studied group often have a typical refugial character (scree or rocky slopes facing to sea, deep sheltered valleys and river gorges; Thompson, 2005) supporting the view that at least some populations survived *in-situ* or were only marginally affected by glacial periods. Nevertheless, even slight alterations in species' distributions may have caused contacts between previously isolated populations, some of them resulting in interspecific gene flow and introgression, leaving traces in nuclear markers. Range shifts and migration apparently also involved trans-Adriatic contacts, as illustrated by the affinity of *C. monteluccii* to the NW clade (clade I in ITS, Fig. 3; or a1 and a2 in *CHS*, Fig. 4), and the presumably hybrid population in Komolac.

Finally, taxa regarded to be close relatives of the *C. maritima* group were genetically well supported. Their phylogenetic relationships, however, remained partly unresolved, since different branching patterns were suggested by cpDNA and nuclear markers. Still, nuclear data suggest that, in contrast to the *C. maritima* group, they were not affected by hybridization, but has probably evolved in a simpler divergence way.

In conclusion, this study illustrates that speciation on diploid level may be a challenging topic, and that the Mediterranean area represents an excellent place to study diverse evolutionary processes. In territories, such as the western Balkans, with relatively high environmental stability over long periods, and substantial topographic, climatic, edaphic or altitudinal heterogeneity, reconstructions of evolutionary history and biogeographic patterns do not often offer simple and straightforward scenaria.

■ TAXONOMIC CONCEPT OF THE *CARDAMINE MARITIMA* GROUP

Based on this study, we recognize the previously described species *C. fialae*, *C. rupestris* and *C. serbica*. *Cardamine maritima* var. *maglicensis* is considered to be a synonym of *C. serbica*. *Cardamine maritima* s.str. is segregated into three distinct species: *C. maritima* (sensu strictissimo), *C. montenegrina* sp. nov. and *C. adriatica* nom. nov. A list of the studied herbarium specimens is presented in the Appendix 2 in the Electronic Supplement; geographic distribution is depicted in Fig. 1. In both the key and morphological descriptions, value ranges of quantitative characters correspond to the 5th and 95th percentiles, with the 1st and 99th percentiles given in brackets.

Key to the species of the *Cardamine maritima* group

1 Plants with auricules on the base of stem leaves **2**

1 Plants without auricules on the base of stem leaves **5**

2 Main stem and sepals hairy, stem and rosette leaves bipinnate, margin of leaflets serrate **3**

2 Main stem and sepals glabrous, stem and rosette leaves pinnate, margin of leaflets coarsely crenate or entire . . . **4**

3 Longer sepals (5.1–)5.4–8.3(–9.5) mm long, petals (11.7–)12.8–17.9(–19.7) mm long, shorter and longer filaments (5.0–)5.5–8.3(–8.9) mm and (7.2–)7.7–10.8(–11.8) mm long, respectively, number of lateral stems 1–2. . . . **1. *C. fialae***

3 Longer sepals (1.4–)1.9–2.9(–3.1) mm long, petals (2.3–)2.7–8.7(–9.3) mm long, shorter and longer filaments (1.2–)1.3–2.6(–3.1) mm and (1.2–)1.3–2.6(–3.0) mm long, respectively, number of lateral stems 5(–7) only rarely 1 . . . . **2. *C. serbica***

4 Cushion-forming plants, petals (2.8–)3.3–5.7(–6.0) mm wide and (7.1–)7.5–11.0 mm long, inflorescences densely racemose . . . . **3. *C. adriatica***

4 Plants not forming cushions, petals (4.0–)6.0–7.5(–8.0) mm wide and (7.3–)10.3–12.3(–13.3) mm long, inflorescences loosely racemose . . . . **4. *C. montenegrina***

5 Main stem glabrous, stem and rosette leaves bipinnate, number of stem leaves (2–)3–9(–9), lateral leaflets of stem leaves larger, (0.3–)0.4–2.0(–2.7) cm long, (0.1–)0.15–1.2(–1.6) cm wide, terminal leaflets of stem leaves larger, (0.4–)0.5–2.7(–3.5) cm long, (0.1–)0.2–1.1(–1.3) cm wide, width of stem base (0.5–)0.8–2.5(–3.0) mm **6**

5 Main stem glabrous or hairy, stem and rosette leaves pinnate, number of stem leaves (2–)3–4(–5), lateral leaflets of stem leaves smaller, (0.2–)0.4–0.6(–0.7) cm long, (0.1–)0.3–0.5(–0.6) cm wide, terminal leaflets of stem leaves smaller, (0.2–)0.5–0.8(–0.9) cm long, (0.1–)0.2–0.5(–0.6) cm wide, width of stem base (0.4–)0.5–1.0(–1.2) mm. . . . . **5. *C. maritima* (sensu strictissimo)**

6 Stem horizontally spreading to ascending, flexuous, branched at base, rosette and stem leaves bipinnate, leaflets serrate, terminal leaflets of stem leaves ± of the same length as lateral leaflets, inflorescence densely racemose, longer sepals (1.9–)3.4–4.9(–5.4) mm long, petals (2.5–)2.6–6.1(–6.6) mm wide . . . . **6. *C. monteluccii***

6 Stem erect, non-flexuous, usually simple or branched in the upper part only, rosette leaves bipinnate, stem leaves pinnate, leaflets coarsely crenate or entire, terminal leaflets of stem leaves considerably longer than lateral leaflets, inflorescence loosely racemose, longer sepals (2.4–)2.6–4.0(–4.1) mm long, petals (4.3–)4.5–7.0(–7.5) mm wide . . . . **7. *C. rupestris***

1. ***Cardamine fialae*** Fritsch in Österr. Bot. Z. 47: 44. 1897 ≡ *C. maritima* var. *fialae* (Fritsch) Sagorski in Österr. Bot. Z. 61: 18. 1911 ≡ *C. maritima* subsp. *fialae* (Fritsch) Trinajstić, Suppl. Fl. Anal. Jugosl. 4: 8. 1976 – Ind. loc.: "Habitat in Hercegovina, ubi legit in rupium fissuris prope Klobuk 6.V.1892, F. Fiala" – Type: [label 1]: *Cardamine maritima* Port., In Felsritzen um Klobuk, bez. Ljubuški, Hercegovina, 6.V.1892, *F. Fiala s.n.*; [label 2]: *Cardamine fialae* sp. n., specimen originale!, Differt a *C. maritima* imprimis pubescentia!, Fritsch, 27.XII.1896 (lectotype designated here: WU!).

2. ***Cardamine serbica*** Pančić, Dodatak Fl. Knež. Srbije: 111. 1884 ≡ *C. maritima* f. *serbica* (Pančić) Sagorski in Österr. Bot. Z. 61: 17. 1911 ≡ *C. maritima* prol. *serbica* (Pančić) O.E. Schulz in Bot. Jahrb. Syst. 32: 579. 1903 – Ind. loc.: "raste po kamenjarima u Podrinju između Perutsa [Perućac] i Dervente [Derventa]" – Type: An der Grenze von Bosnien und Serbien zwischen Derventa und Perutoče an d. Drina, V.1882, *Pančić s.n.* (lectotype designated here: WU!).

= *C. maritima* var. *maglicensis* Rohlena in Feddes Repert.

36/37: 145. 1906 ≡ *C. maritima* subsp. *maglicensis* (Rohlena) Trinajstić, Suppl. Fl. Anal. Jugosl. 4: 8. 1976 – Ind. loc.: “Montenegro: In rupestribus et lapidosis sub monte Maglić in valle fluminis Piva prope Mratinje, ca. 600–800 m” – Type: sub m. Maglić, pr. Mratinje, VIII.1905, *Jos. Rohlena s.n.* (lectotype designated here: PR!).

3. ***Cardamine adriatica*** Jar. Kučera, Lihová & Marhold, **nom. nov.** ≡ *Pteroneurum bipinnatum* Rchb., Fl. Germ. Excurs.: 676. 1830–1832 (non *C. bipinnata* (C.A. Mey.) O.E. Schulz 1903) ≡ *C. maritima* f. *bipinnata* (Rchb.) Sagorski in Österr. Bot. Z. 61: 17. 1911 – Ind. loc.: “Dalmatien “Urulia” [near D. Brela village] v. Welden” – Type: unknown—the original material was most likely destroyed in 1849 during the great Zwinger fire (Stafleu & Cowan, 1983). Neotype designated here: Croatia, Biokovo Mts., Baška Voda, village of Bast, 43°21′31″N, 16°59′18″E, 398 m a. s. l., 24.IV.2003, *J. Kučera & M. Kolník s.n.* (SAV!).

? = *Pteroneurum microphyllum* J. Presl & C. Presl, Delic. Prag. 1: 237. 1822 – Ind. loc.: “Hab. in arenosis maritimis Dalmatiae prope Capo Cesto [Primošten]” – Type: unknown.

– “*C. microphylla*” J. Presl & C. Presl, Delic. Prag. 1: 15. 1822 non Adams., nec (Willd.) O.E. Schulz, nom. inval. (Art. 34.1(a); McNeill & al., 2006).

4. ***Cardamine montenegrina*** Jar. Kučera, Lihová & Marhold, **sp. nov. –** Type: Montenegro, Mt. Lovćen, Kotor, village of Njegoši, 42°24′38″N, 18°47′23″E, 919 m, 4.V.2004, *J. Kučera & M. Kolník s.n.* (holotype: SAV).

Planta hibernans, annua raro biennis, (1.5–)7.0–19.0(–26.0) cm alta. Caulis 1–2(–4), pilosus erectus vel ascendens, ramosus, flexuosus, basi (0.5–)1.0–2.0(–2.3) mm latus. Folia rosulata 3–20(–24), pinnata. Folia caulina (2–)6–11(–14), alternata, glabra, non nisi margine sparse trichomata, pinnata, basi auriculata. Foliola (5–)7–8(–9), oblanceolata vel obovata, petiolulata vel subsessilata, profunde lobata aut obtusa. Lobi obtusi aut acre cuspidati. Margo foliolorum crasse crenatus aut integer. Foliola terminalia (0.2–)0.5–0.7(–0.9) cm lata, (0.3–)0.8–1.6(–1.9) cm longa, 3–4(–5) lobata. Foliola lateralia (0.2–)0.5–0.8(–1.0) cm lata, (0.3–)0.7–1.2(–1.4) cm longa, 3–5(–6) lobata. Inflorescentia racemosa cum 3–7(–12) inflorescentis lateralibus. Petala alba, (7.2–)10.4–12.6(–13.5) mm longa, (4.0–)6.0–7.4(–8.1) mm lata, obovata vel late obovata, unguis longus, apex sinuatus vel obcordatus. Sepala glabrata, anguste lanceolata vel anguste ovata, margine membranaceo, (3.0–)4.3–4.9(–5.3) mm longa, (1.0–)1.5–1.9(–2.0) mm lata. Stamina 6, tetradynama, antherae luteae. Stamina longiora (4.4–)6.2–7.4(–8.1) mm longa, breviora (2.4–)4.0–5.1(–5.5) mm longa. Stigma latior quam stylus. Siliquae erectae vel patentae, (14.5–)15.5–40.0(–44.0) mm longae, 2.0–3.5(–4.0) mm latae, pedunculi (6.0–)8.0–17.0(–20.0) mm longi, rostra (4.0–)5.0–11.0(–12.0) mm longa, glabrata, sparse pilosa.

Chromosome number. – $2n = 2x = 16$.

Habitats. – Calcareous rock crevices and shady screes.

Distribution area. – Montenegro: Kotor, Podgorica, Bar and Ulcinj districts (Fig. 1; Appendix 2 in the Electronic Supplement).

5. ***Cardamine maritima*** Port. ex DC., Syst. Nat. 2: 266. 1821 ≡ *Pteroneurum dalmaticum* Vis. in Flora 12: 17. 1829, nom. illeg. [Art. 52.1] ≡ *Pteroneurum maritimum* (Port. ex DC.) Rchb., Fl. Germ. Excurs.: 676. 1830–1832 ≡ *C. thalictroides* var. *maritima* (Port. ex DC.) Vis., Fl. Dalmat. 1: 128. 1842 – Ind. loc.: “in Dalmatiae peninsula Sabioncello (olim Hyllis) [Pelješac Peninsula] ad littora maris arenosa. Portenschlag (v.s.sp.)” – Type: In Dalmatia, peninsula Sabioncello ad littora arenosa maris, 1820, *Portenschlag s.n.* (holotype: G-DC!).

= *C. maritima* var. *pilosa* O.E. Schulz, Bot. Jahrb. Syst. 32: 580. 1903 – Ind. loc.: “Dalmatia: legg. Tommasini (H.N.) [US], Fr. Petter (H.B. etiam, H.D. etiam) [B!, G], Vruglia [near D. Brela village], leg. Visiani (H.Z. etiam) [Z!], pr. Spalato [Split] in m. Mossor legg. Maly 1869 (H.V.) [W!], in m. Radigne et Beljak, leg. Fr. Petter 1833, 1876 (H. var., etiam), pr. Ragusa [Dubrovnik] ad Garvosa et Lappad leg. Jabornegg 1863 (H. Aschers) [B!], pr. Cattaro [Kotor] leg. Petter (H.P.Ac.) [LE!], Ins. Lussin [Lošinj Island] in M. Ossero [Osoršcica Mt.] legg. Tommasini (H.V.) [WU!]” – Type: Montis Ossero, 2000 ft, *M. Tommasini s.n.* (lectotype designated here: WU!).

– “*C. maritima*” Port., Enum. Pl. Dalmat.: 15, Tab. 11. 1824, isonym [Art. 48.1].

– “*C. dalmatica*” Vis. ex Nyman, Consp. Fl. Eur.: 38. 1878, nom. inval., pro syn. [Art. 34.1].

– “*C. maritima* f. *vulgaris*” Beck in Glasn. Zemaljsk. Muz. Bosne Hercegovine Sarajevu. Prir. Nauke 2(7): 246, 1907, nom. inval. [Art. 24.3].

6. ***Cardamine monteluccii*** Brilli-Catt. & Gubellini in Webbia 39: 398. 1986 – Ind. loc.: “Marche, Gruppo del M. Gemmo: macereti, pendici detritiche, fruticeti e boschi xerofili radi nel versante SW del M. di Castel S. Maria sopra la Valle di Campobonomo, 800–850 m, suolo calcareo” – Type: Marche, Gruppo del M. Gemmo: macereti, pendici detritiche, fruticeti e boschi xerofili radi nel versante SW del M. di Castel S. Maria sopra la Valle di Campobonomo, 800–850 m, suolo calcareo, 17.V.1982, *A. Brilli-Cattarini & L. Gubellini s.n.* (holotype: PS!).

7. ***Cardamine rupestris*** (O.E. Schulz) K. Malý in Glasn. Zemaljsk. Muz. Bosne Hercegovine Sarajevu. Prir. Nauke 23: 588. 1911 ≡ *C. maritima* prol. *rupestris* O.E. Schulz, Bot. Jahrb. Syst. 32: 580. 1903 – Ind. loc.: “Ins. Lussin [Lošinj Island] in M. Ossero [Mt. Osoršcica] legg. Tommasini (H.Boiss., H.C., H.V.) [G, GH, W!], Raimann 1887 (H.V.) [W!], A. Haračić 1895 (H.Behr.) [B!]; Hercegovina pr. Mostar in m. Stolac 200 m legg. H. Raap 1895, F. Fiala 1896 (H.V.U.) [WU!], C. Baenitz 1897 (H.P.Ac., H.V., H.Vr., H.Z.) [LE!, W!, WRSL, Z!], 1898 (H.Z.) [Z!]” – Type: [label 1]: *C. thalictroides* All., Flora Bosniaca, Stolac, Hercegovina, in Felsritzen, IV.1896, *F. Fiala s.n.*; [label 2]: *Cardamine maritima* Portenschlg. prol *rupestris* O.E. Schulz, O.E. Schulz! [written by O.E. Schulz], Fritsch! [written by K. Fritsch] (lectotype designated here: WU!).

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Appendix 1. Plant material analyzed in the present study. Taxa names, localities and collection details are listed, followed by chromosome number records, number of plants investigated in morphometrics, and GenBank accession numbers. Chromosome numbers were determined by J. Kučera. Collector abbreviations: *JK*, J. Kučera; *JL*, J. Lihová; *KM*, K. Marhold; *MK*, M. Kolník. Accession codes correspond to those used in the studies Kučera & al. (2008) and Lihová & al. (2008). Dashes indicate missing or inapplicable data. All collectors lacking collection numbers are *sine numero*.

Species, accession code—country, region, locality, altitude (m a.s.l.), collection date (day.month.year), *collector(s)* (herbarium acronym): **$2n$ =** [new chromosome record published here], morph # [number of specimens used in morphometric analyses], *CHS* exon 2, *trnL* intron, *trnL-trnF* intergenic spacer, ITS GenBank accession numbers.

***Cardamine maritima* group**

Cardamine adriatica Jar. Kučera, Lihová & Marhold (= *C. maritima* s.str. from mainland Croatia), **Velebit 1**—Croatia, Velebit Mts., Starigrad, Velika Paklenica gorge, ca. 60 m, 18.IV.2003, *JK & MK* (SAV): –, morph 9, DQ208973, FJ384297, DQ209139, DQ209124; **Velebit 2**—Croatia, Velebit Mts., Porat, Tribanjska draga, 11 m, 18.IV.2003, *JK & MK* (SAV): –, morph 3, FJ384167, FJ384300, FJ384359, FJ384222; **Velebit 3**—Croatia, Velebit Mts., Starigrad, Seline, Mala Paklenica gorge, ca. 250 m, l. & 19.IV.2003, *JK & MK* (SAV): –, morph 5, FJ384165, FJ384301, FJ384360, FJ384223; **Mosor—**Croatia, Mosor Mts., Gornje Sitno village, 649 m, 21.IV.2003, *JK & MK* (SAV): **$2n = 16$**, morph 29, FJ384164, FJ384298, FJ384357, FJ384217; **Velebit 4**—Croatia, Velebit Mts., Karlobag, between the villages of Vidovac and Sušanj, 436 m, 25.IV.2003, *JK & MK* (SAV): **$2n = 16$**, morph 25, –, –, –, –; **Biokovo—**Croatia, Biokovo Mts., Baška voda, Bast, 398 m, 24.IV.2003, *JK & MK* (SAV): –, morph 26, FJ384166, FJ384299, FJ384358, FJ384215. ***Cardamine fialae*** Fritsch, **Ružići**—Bosnia and Herzegovina, Grude, near the village of Ružići, 287 m, 23.IV.2003, *JK & MK* (SAV): **$2n = 16$**, morph 26, FJ384130 & FJ384131, FJ384276 & FJ384278, FJ384338 & FJ384340, FJ384227 & FJ384228; **Klobuk**—Bosnia and Herzegovina, Ljubuški, near the village of Klobuk, 285 m, 23.IV.2003, *JK & MK* (SAV) **(type locality of *C. fialae*)**: –, morph 26, DQ208995, FJ384275, DQ209137, DQ209122; **Grude**—Bosnia and Herzegovina, near the town of Grude, 298 m, 23.IV.2003, *JK & MK* (SAV): –, morph 30, FJ384129 & FJ384132, FJ384277 & FJ384279, FJ384339 & FJ384341, FJ384229 & FJ384230. ***Cardamine maritima*** Port. ex DC. (= *C. maritima* sensu strictissimo; Pelješac Peninsula and Croatian islands), **Kremena**—Croatia, S Dalmacia, village of Kremena, coast opposite Pelješac Peninsula, 40 m, 25.IV.2005, *JK & MK* (SAV): **$2n = 16$**, morph 28, FJ384161, FJ384295, FJ384355, FJ384216; **Lošinj**—Croatia, Lošinj Island, Mt. Televrina, 528 m, 27.IV.2005, *JK & MK* (SAV): –, morph 30, FJ384162, FJ384292, FJ384352, FJ384219; **Pelješac**—Croatia, Pelješac Peninsula, above the village of Orebić, 50 m, 25.IV.2005, *JK & MK* (SAV) **(type locality of *C. maritima*)**: **$2n = 16$**, morph 26, FJ384159 & FJ384160, FJ384290 & FJ384291, FJ384350 & FJ384351, FJ384224 & FJ384225; **Cres 1**—Croatia, Cres Island, ca. 1 km W of village of Lubenice, 94 m, 26.IV.2005, *JK & MK* (SAV): **$2n = 16$**, morph 29, FJ384163, FJ384293, FJ384353, FJ384218; **Cres 2**—Croatia, Cres Island, village of Lubenice, 140 m, 30.V.1997, *M. Magnes* (GZU): –, –, –, FJ384294, FJ384354, –. ***Cardamine monteluccii*** Brilli-Catt. & Gubellini, **Lazio—**Italy, Lazio, Monti Lepini, Carpineto, Fontanille dell' Occhio di Bue, 685 m, 6.V.2003, *JK & MK* (SAV): –, –, FJ384148, FJ384251, FJ384317, FJ384204; **Tuscany 1—**Italy, Tuscany, Roccalbegna, Pescinello, 740 m, 8.V.2003, *JK*, *MK & F. Selvi* (SAV): **$2n = 16$**, morph 29, DQ208986, FJ384250, DQ209140, DQ209123; **Tuscany 2**—Italy, Tuscany, Semproniano, settlement of Rocchete, 505 m, 8.V.2003, *JK & MK* (SAV): –, morph 11, FJ384150, FJ384253, FJ384319, FJ384206; **Marche 1**—Italy, Marche, near the town of Pióraco, 475 m, 9.V.2003,

Appendix 1. Continued.

JK & MK (SAV): **$2n = 16$**, morph 28, FJ384149, FJ384252, FJ384318, FJ384205; **Marche 2**—Italy, Marche, Mt. San Vicino, Valle dell Aquarella, 682 m, 9.V.2003, *JK & MK* (SAV): –, morph 12, –, –, –, –; **Calabria 1**—Italy, Calabria, Pollino Mts., Morano Calabro, UTM: WE 94, 1100 m, 12 & 15.IV.1994, *L. Bernardo & P. Calvosa* (CLU 5465): –, –, –, FJ384249, FJ384316, FJ384207; **Calabria 2**—Italy, Calabria, Timpe di Cassano, 10.V.1997, *L. Bernardo* (CLU 5466): –, –, –, FJ384248, FJ384315, FJ384208. ***Cardamine montenegrina*** Jar. Kučera, Lihová & Marhold (= *C. maritima* s.str. from Montenegro), **Risan**—Montenegro, above the village of Risan, 504 m, 22.IV.2003 & 4.V.2004, *JK & MK* (SAV): **$2n = 16$**, morph 36, FJ384170, FJ384286, FJ384347, FJ384220; **Ledeniće**—Montenegro, village of Ledeniće, 695 m, 22.IV.2003, *JK & MK* (SAV): **$2n = 16$**, morph 30, DQ208998 & FJ384169, FJ384289, DQ209138, DQ209125; **Lovćen 1**—Montenegro, Mt. Lovćen, near the road from Kotor to the village of Krstac, 423 m, 22.IV.2003 & 4.V.2004, *JK & MK* (SAV): **$2n = 16$**, morph 34, FJ384168, FJ384288, FJ384349, FJ384226; **Lovćen 2**—Montenegro, Mt. Lovćen, Kotor, village of Njegoši, 919 m, 4.V.2004, *JK & MK* (SAV): **$2n = 16$**, morph 23, FJ384172, FJ384285, FJ384346, FJ384221; **Rumija**—Montenegro, Rumija Mts., saddle above the town of Bar, 23.IV.2005, *JK & MK* (SAV): **$2n = 16$**, morph 31, FJ384171 & FJ384173, FJ384284 & FJ384287, FJ384348 & FJ384345, FJ384213 & FJ384214. ***Cardamine rupestris*** (O.E. Schulz) K. Malý, **Čevo 1**—Montenegro, Cetinje, ca. 2 km S of village of Čevo, 817 m, 4.V.2004, *JK & MK* (SAV): **$2n = 16$**, morph 30, –, –, –, –; **Čevo 2**—Montenegro, Cetinje, ca. 2.5 km N of village of Čevo, 643 m, 4.V.2004, *JK & MK* (SAV): **$2n = 16$**, morph 28, –, –, –, –; **Grahovo**—Montenegro, Nikšić, village of Grahovo, 689 m, 5.V.2004, *JK & MK* (SAV): **$2n = 16$**, morph 29, DQ208984, FJ384283, DQ209141, DQ209126; **Vrbanje**—Montenegro, Herceg Novi, near village of Vrbanje, 490 m, 22.IV.2005, *JK & MK* (SAV): **$2n = 16$**, morph 28, FJ384157, FJ384280, FJ384342, FJ384200; **Komarno**—Montenegro, Podgorica, between villages of Riječani and Komarno, 147 m, 23.IV.2005, *JK & MK* (SAV): –, morph 26, –, –, –, –; **Krašići—**Montenegro, Kotor, near village of Krašići, 12 m, 24.IV.2005, *JK & MK* (SAV): –, morph 22, –, –, –, –; **Mostar 1**—Bosnia and Herzegovina, Mostar, Podvelež hill, ca. 800 m, 21.IV.2005, *JK & MK* (SAV): –, morph 30, FJ384155, FJ384282, FJ384344, FJ384198; **Mostar 2**—Bosnia and Herzegovina, Mostar, village of Stolac, 117 m, 22.IV.2005, *JK & MK* (SAV) **(type locality of *C. maritima* prol. *rupestris*)**: –, morph 21, FJ384158, FJ384281, FJ384343, FJ384199. ***Cardamine serbica*** Pančić (incl. *C. maritima* var. *maglicensis* Rohlena marked by asterisk), **Maglić 1**—Montenegro, Maglić Mts., N of the town of Plužine, 700 m, 15.VII.2003, *JK & MK* (SAV): –, morph 17, FJ384151, FJ384272, FJ384335, FJ384209; **Maglić 2**—Montenegro, Maglić Mts., Plužine, village of Mratinje, 708 m, 15.VII.2003, *JK & MK* (SAV) **(type locality of *C. maritima* var. *maglicensis*)**: **$2n = 16$**, morph 25, DQ208974 & FJ384152, FJ384270 & FJ384271, DQ209136 & FJ384334, FJ384210; **Maglić 3**—Montenegro, Maglić Mts., town of Plužine, 708 m, 5.V.2004, *JK & MK* (SAV): –, morph 14, FJ384153, FJ384273, FJ384336, FJ384211; **Tara**—Serbia, Tara Mts., Bajina Bašta, village of Perućac, 285 m, 22.V.2004, *JK & MK* (SAV) **(type locality of *C. serbica*)**: –, morph 7, FJ384154, FJ384274, FJ384337, FJ384212. ***Cardamine*** sp., **Komolac**—Croatia, Dubrovačko-Neretvanska županija, near the village of Komolac, 14 m, 24.IV.2005, *JK & MK* (SAV): –, morph 10, FJ384127–FJ384128 & FJ384156, FJ384254–FJ384256, FJ384320–FJ384322, FJ384201–FJ384203.

Close relatives of the *Cardamine maritima* group

Cardamine alpina Willd., **MOL**—Spain, C Pyrenees, Valle de Molières, Estanhòts de Molières, 2370 m, 2.VII. 2004, *JL & M. Slovák* (SAV): –, –, FJ384139, FJ384231, DQ209206, DQ209115; **PAN**—Spain, C Pyrenees, Balneario de Panticosa, Ibones Azules, 2350 m, 19.VII.2004, *JL & M. Slovák* (SAV): –, –, DQ208972, –, –, –; **PESS**—Andorra, E Pyrenees, Circ dels Pessons, 2500 m, 25.VII.2004, *JL & M. Slovák* (SAV): –, –, FJ384140, FJ384234, FJ384302, –. ***Cardamine carnosa*** Waldst. & Kit., **Velebit—**Croatia, Velebit Mts., NP Paklenica, 1470–1700 m, 22.VI.2001, *G. Schneeweiss, P. Schönswetter & A. Tribsch 6257* (WU 004365): –, –, FJ384141 & FJ384142, FJ384268 & FJ384269, FJ384332 & FJ384333, FJ384180 & FJ384181. ***Cardamine glauca*** Spreng. ex DC., **Maglić**—Montenegro, Maglić Mts., Mratinje, 708 m, 15.VII.2003 & 5.V.2004, *JK & MK* (SAV): –, –, FJ384143, FJ384258, FJ384323, FJ384178; **Rumija**—Montenegro, Rumija Mts., above the town of Bar, 499 m, 22.IV.2005, *JK & MK* (SAV): –, –, FJ384146, FJ384260, FJ384325, FJ384177; **Risan—**Montenegro, above the village of Risan, 504 m, 4.V.2004, *JK & MK* (SAV): **$2n = 16$**, –, DQ208985, FJ384257, DQ209203, DQ209120; **Tara**—Serbia, Tara Mts., village of Perućac, 285 m, 22.V.2004, *JK & MK* (SAV): –, –, FJ384144, FJ384259, FJ384324, FJ384175; **Mostar**—Bosnia and Herzegovina, Mostar, gorge of the river Neretva, 220 m, 21.IV.2005, *JK & MK* (SAV): –, –, FJ384145, FJ384261, FJ384326, FJ384176; **Pindos**—Greece, N Pindos, Katára, 5 km WSW Malakási, 1690–1715 m, 19.V.2000, *W. Gutermann & al. 35053* (herbarium Walter Gutermann, Vienna): –, –, FJ384147, FJ384262, FJ384327, FJ384174. ***Cardamine graeca*** L., **Calabria—**Italy, Calabria, Pollino Mts., Timpa di Porace, –, 17.IV.2002, *L. Peruzzi, D. Gargano & G. Cesca* (CLU 5464): –, –, –, FJ384267, FJ384331, FJ384194; **Marche**—Italy, Marche, Pióraco, 475 m, 9.V.2003, *JK & MK* (SAV): **$2n = 16$**, –, DQ208996, FJ384264, DQ209202, DQ209121; **Krk**—Croatia, Krk Island, W of the town of Krk, –, 6.IV.1985, *E. Habeler* (GZU): –, –, –, FJ384296, FJ384356, –; **Rumija**—Montenegro, Rumija Mts., above town of Bar, 499 m, 22.IV.2005, *JK & MK* (SAV): –, –, FJ384133, FJ384266, FJ384330, FJ384197; **Mostar—**Bosnia and Herzegovina, Mostar, village of Grabovica, 144 m, 21.IV.2005, *JK & MK* (SAV): –, –, FJ384134, FJ384265, FJ384329, FJ384196; **Grude**—Bosnia and Herzegovina, village of Grude, 298 m, 23.IV.2003, *JK & MK* (SAV): –, –, FJ384135, –, –, FJ384195. ***Cardamine pancicii*** Hayek, **Kopaonik**—Serbia, Mt. Kopaonik, Suvo Rudište, 25.VI.2003, *Lakušić 17161* (BEOU, SAV) **(type locality of *C. pancicii*)**: –, –, –, FJ384263, FJ384328, FJ384179. ***Cardamine plumieri*** Vill., **Cottic 1**—Italy, Cottic Alps, Monte Orsiera, NE Fenestrelle, 2600–2878 m, 17.VII.1998, *P. Schönswetter & A. Tribsch* (WU 030839 & WU 030840): –, –, FJ384117 & FJ384118, FJ384244 & FJ384245, FJ384311 & FJ384312, FJ384192 & FJ384193; **Cottic 2**—Italy, Cottic Alps, source of the Po river, Lago Fiorenza, 2100 m, 16.VII. 2006, *JL & KM* (SAV): –, –, FJ384121 & FJ384122, FJ384238 & FJ384239, FJ384305 & FJ384306, FJ384188 & FJ384189; **Grajic**—Italy, Grajic Alps, Valle del Chisone, Truc del Cuculo, 2090 m, 18.VII. 2006, *JL & KM* (SAV): –, –, FJ384123 & FJ384126, FJ384236 & FJ384237, FJ384303 & FJ384304, FJ384190 & FJ384191; **Pindos 1**—Greece, N Pindos, Katára, 5 km WSW Malakási, 1690–1715 m, 19.V.2000, *W. Gutermann & al. 35053bis* (herbarium Walter Gutermann, Vienna): –, –, FJ384115, FJ384247, FJ384314, FJ384183; **Pindos 2**—Greece, distr. Grevena, from Kataphygion Grevena to Vasilitsa, 1800–2349 m, 10.VII.1982, *W. Lippert 18658* (W 2005-06854): –, –, FJ384116, FJ384246, FJ384313, FJ384182; **Corsica 1**—France, Corsica, from Haut Asco towards Mt. Cinto, ca. 2500 m, 5.VII. 2006, *JL & KM* (SAV): –, –, FJ384119 & FJ384120, FJ384240 & FJ384241, FJ384307 & FJ384308, FJ384186 & FJ384187; **Corsica 2**—France, Corsica, E slopes of Monte d'Oro, 1930 m, 7.VII.2006, *JL & KM* (SAV): –, –, FJ384124 & FJ384125, FJ384242 & FJ384243, FJ384309 & FJ384310, FJ384184 & FJ384185. ***Cardamine resedifolia*** L., **VD**—Romania, Făgăraş Mts., Saua Caprei, 2274 m, 10.VII.2003, *JK & MK* (SAV): –, –, FJ384136, FJ384235, DQ209208, DQ209119; **DAE**—Italy, Bergamo Alps, Val di Daone, Lago di Malga Bissina, 1780 m, 8.VI.2003. *JL* et al. (SAV): –, –, FJ384137, FJ384232, DQ209204, DQ209118; **VEL**—Spain, Sierra Nevada, NW of Veleta peak, 2850 m, 17.VII.2004, *JL & M. Slovák* (SAV): –, –, FJ384138, FJ384233, DQ209205, DQ209116; **CAN—**Spain, C Pyrenees, Candanchú, Collado de Tortiellas, 1990 m, 20.VII.2004, *JL & M. Slovák* (SAV): –, –, –, –, –, DQ209117; **ESP—**Spain, C Pyrenees, Espot, Coma dels Estanyets, 2100 m, 23.VII.2004, *JL & M. Slovák* (SAV): –, –, DQ208999, –, –, –.