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Taxonomic position and circumscription of *Cardamine barbaraeoides* (Brassicaceae), a systematically challenging taxon from the Balkan Peninsula

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Abstract

The Balkan Peninsula, and the territory of Greece in particular, is a significant biodiversity hotspot in the Mediterranean that is rich in endemic plants. The focal species of this study, *Cardamine barbaraeoides*, is a narrow Greek endemic that has been confused taxonomically since its original description. Based on a detailed multivariate morphometric study, we provide here a set of morphological characters that enables the reliable identification of this species. In addition, we present an identification key to *C. barbaraeoides* and related taxa. We have revised herbarium specimens and literature data on the occurrence of this species in Greece and ascertained that it occurs only in the Pindos mountains (the Southern Pindos and partly the Northern Pindos floristic regions). All reports of this species from the Greek floristic regions of North Central, Sterea Ellas and partly also Northern Pindos were based on misidentifications of specimens of *C. acris*.

Introduction

The Balkan Peninsula, which is a significant biodiversity hotspot in the Mediterranean (Médail & Quézel 1997), is a geographically complex area with a rich and turbulent geological history (Griffiths *et al.* 2004). These factors have contributed considerably to its present plant diversity (Turrill 1929, Nieto Feliner 2014, Thompson 2020). The Balkan Peninsula, and Greece in particular, is rich in endemics. Out of the total amount of 5,800 species currently recognized as occurring in Greece, 15.5% are considered endemics (Georghiou & Delipetrou 2010). Endemic richness is connected with minimal extinctions in the Southern European montane and peninsular regions, as compared to events at higher latitudes during the Pleistocene climatic oscillations, as well as with the accumulation of diversity and an increased speciation rate in ecologically and topographically diverse landscapes (Nieto Feliner 2011). The Pindos Mts, which constitute one of the focal areas of this paper, are among the most important centres of Balkan plant diversity and endemism (Médail & Diadema 2009, Kougioumoutzis *et al.* 2021). Unlike in Central and Northern Europe, species in the Balkan Peninsula did not respond to glacial events by large range shifts in the south-north directions; instead, most migrations occurred on a small scale along elevational gradients, which also favoured secondary contacts of previously separated allied taxa and their consequent hybridization, often accompanied by allopolyploidization.

The genus *Cardamine* is a cosmopolitan genus, comprising almost 280 species (Marhold *et al.* 2021+). In the Balkan Peninsula it is represented mostly by diploids whereas polyploids occur there only exceptionally [e.g. *C. flexuosa* Withering (1796: 578) and species previously classified within the genus *Dentaria* Linnaeus (1753: 653)]. *Cardamine barbaraeoides* Halácsy (1894: 228) was described from Mt Peristeri near Syraku [Syrrako] (Lakmos Mts, Southern Pindos, NW Greece; Fig. 1). In the original description, Halácsy (1894: 228–229) already pointed out its similarity to another species from the Balkan Peninsula, *C. acris* Grisebach (1843: 153). He noticed that *C. barbaraeoides* differs from *C. acris* in the length of the rhizome, in the position of leaves, which are distributed equally on the stem, and in having leaves with three leaflets. Halácsy's description was based on plants in fruit. In a later publication (Halácsy

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1901), he completed the description, noting that this species possesses white petals and violet anthers, and suggested its proximity to *C. amara* Linnaeus (1753: 656), a widespread Eurasian species. In addition to localities from the Lakmos Mts in the Southern Pindos, he reported *C. barbaraeoides* also from Mt Smolikas in the Northern Pindos. Further authors either kept this taxon at the species level (Schulz 1903; for others see Table 1) or ranked it as a subspecies of *C. amara* [*C. amara* subsp. *barbaraeoides* (Halácsy) Maire & Petitmengin (1908: 26); Kit Tan (2002)] or of *C. raphanifolia* Pourret (1788: 310) [*C. raphanifolia* subsp. *barbaraeoides* (Halácsy) Strid (1986: 257), followed by Jones & Akeroyd (1993) and Jalas & Suominen (1994); Table 1].

TABLE 1. Synopsis of previous taxonomic treatments and given distribution areas of *Cardamine barbaraeoides*. Floristic regions of Greece (N Pindos, S Pindos, North Central, East Central, Sterea Ellas; see Fig. 1) follow Strid (1986).

Author	Treatment	Distribution
Halácsy 1894	*C. barbaraeoides*	Greece: Mt Peristeri (S Pindos: Lakmos Mts)
Halácsy 1901	*C. barbaraeoides*	Greece: Mt Peristeri and Mt Turnara (S Pindos: Lakmos Mts); Mt Smolikas[1] (N Pindos)
Schulz 1903	*C. barbaraeoides*	Greece: Mt Peristeri and Mt Turnara; Mt Zygos[2] (N Pindos)
Maire & Petitmengin 1908	*C. amara* subsp. *barbaraeoides*	Greece: Mt Zygos[2] (N Pindos)
Hayek 1925	*C. barbaraeoides*	Bulgaria[3]; Serbia[4]; Albania[5]; Greece: Thessaly and Epirus[2] (N Pindos, S Pindos, North Central, East Central)
Stojanov & Stefanov 1933, 1948	*C. amara* subsp. *barbaraeoides*	Bulgaria[3]: Central Balkan, Rodopi Mts, Mt Vitosha
Assenov 1970	*C. barbaraeoides*	Bulgaria[3]: W and Central Stara Planina; Osogovska planina; Mt Vitosha; Pirin; Rila; W and Central Rodopi Mts
Jovanović-Dunić 1972	*C. barbaraeoides*	Serbia[4]
Trinajstić 1976	*C. barbaraeoides*	Serbia[4]
Strid 1986	*C. raphanifolia* subsp. *barbaraeoides*	Greece: Sterea Ellas[1], S Pindos, N Pindos[2]; ?Albania[5]
Jones & Akeroyd 1993	*C. raphanifolia* subsp *barbaraeoides*	Central Greece[1], N Greece[2]; ?Albania[5]
Jalas & Suominen 1994	*C. raphanifolia* subsp. *barbaraeoides*	Central Greece[1], N Greece[2]
Kit Tan 2002	*C. amara* subsp. *barbaraeoides*	Greece: Sterea Ellas[1], S Pindos, N Pindos[2], North Central[1]; ?Albania[5]
Dimopoulos *et al.* 2013, 2017+	*C. barbaraeoides*	Greece: Sterea Ellas[1], S Pindos, N Pindos[2], North Central[1]

[1] Only specimens of *C. acris* are known from this area; the occurrence of *C. barbaraeoides* is highly doubtful.

[2] Both *C. barbaraeoides* and *C. acris* occur in this area.

[3] Populations from Bulgaria are now classified as *C. amara* subsp. *balcanica* (Marhold *et al.* 1996).

[4] From Serbia we have seen only specimens of *C. acris* and *C. amara* subsp. *balcanica*, but the occurrence of *C. barbaraeoides* is highly doubtful.

[5] From Albania we have seen only specimens of *C. acris*; the occurrence of *C. barbaraeoides* is highly doubtful.

In more recent studies, *C. barbaraeoides* has been tentatively assigned to the *C. raphanifolia* species complex (Lihová *et al.* 2004), but its taxonomic position and differentiation from close relatives remained unclear (Marhold *et al.* 2018). *Cardamine raphanifolia* was originally treated as a species with several subspecies, endemics of Southern Europe (Jones & Akeroyd 1993), which were all later elevated to the species level (Perný *et al.* 2004, 2005a,b). Therefore, *C. raphanifolia* in its strict sense occurs only in the Iberian Peninsula (Perný *et al.* 2005a,b). Apart from

the uncertain taxonomic position of *C. barbaraeoides*, reports about its distribution appeared highly confusing since early studies (Table 1). In contrast to Halácsy (1894, 1901) and Schulz (1903), Hayek (1925: 392–393) reported localities of *C. barbaraeoides* from a larger part of northwestern Greece [Thessaly ('The') and Epirus ('Ep'); see Table 1] and the Balkan Peninsula (Bulgaria, Serbia and Albania, regions 'Bu', 'Sb', 'A' by Hayek, not fully identical with these countries in their current borders). Further on, *C. barbaraeoides* (at the level of species or subspecies) was repeatedly reported from Bulgaria (Stojanov & Stefanov 1933, 1948, Assenov 1970), Serbia (Jovanović-Dunić 1972, Trinajstić 1976) and North Macedonia (Micevski 1995). However, Marhold *et al.* (1996) showed that populations from the Vitosha, Stara Planina, Rila, Pirin and Western Rodopi Mts (all in Bulgaria) that had been classified as *C. barbaraeoides* (or as *C. amara* subsp. *barbaraeoides*) in fact represent a different taxon and described these plants as *C. amara* subsp. *balcanica* Marhold, Ančev & Kit Tan (1996: 201). Marhold *et al.* (1996) reported *C. amara* subsp. *balcanica* also from the floristic region of North East (NE Greece), namely from the provinces of Dramas [Drama] and Serron [Serres], and Tomović *et al.* (2009) later reported it from Serbia and North Macedonia.

FIGURE 1. Map showing our sample sites and the localities of herbarium specimens from Greece revised by us. The sample sites used in the morphometric analyses are indicated by grey symbols: grey stars denote *Cardamine barbaraeoides* and grey crosses *C. acris* (see Table 2 for more details). The localities of the revised herbarium specimens are indicated by white symbols: white stars represent specimens confirmed as *C. barbaraeoides* (see Appendix 1) and white circles denote specimens of *C. acris* that were previously misidentified as *C. barbaraeoides* (see Appendix 2). Floristic regions of Greece according to Strid (1986) are delimited by dashed lines and named in italics.

TABLE 2. List of taxa studied and their accessions included in karyological and morphometric analyses: *Cardamine barbaraeoides*, *C. acris, C. amara* and *C. raphanifolia*. AD—Andorra, BG—Bulgaria, ES—Spain, FR—France, GR—Greece, PL—Poland, SK—Slovakia, UA—Ukraine. Collectors: JL—J. Zozomová-Lihová, KM—K. Marhold, MA—M. Anchev, MP—M. Perný, VK—V. Kučera. Chromosome numbers (2*n*) taken from other studies: [1] Perný *et al.* (2005b), [2]Perný *et al.* (2004), [3]Marhold (1992), [4]Marhold *et al.* (1996), [5]Perný *et al.* (2005a). Voucher specimens are deposited in SAV.

Origin and collection data	2*n*	Morphometrics No. of individuals
***Cardamine barbaraeoides* Halácsy**		
GR, Southern Pindos, Ioannina prov., Lakmos Mts, ca 6 km N of Kalarites (close to Mparos pass), in the stream above the road, 25 Jun 2001, *MP & VK*; 27 May 2002 *MP* (population 02KA in Perný *et al.* 2005b; population C007 in Šlenker *et al.* 2021a)	32[1]	42
GR, Southern Pindos, Ioannina prov., Lakmos Mts, ca 5 km N of Kalarites, in the stream above the road, 27 May 2002, *MP* (population 3KA in Perný *et al.* 2005b)	32[1]	14
GR, Southern Pindos, Ioannina prov., Lakmos Mts, ca 5 km S of Mt Peristeri (= Mt Lakmos), 7 Jul 2000, *MP & al.* (Lihová *et al.* 2006: 765, locality BR-1)	32	
GR, Southern Pindos, Ioannina prov., Lakmos Mts, ca 6 km S of Mt Peristeri (= Mt Lakmos), 7 Jul 2000, *MP & al.*	32	
GR, Southern Pindos, Ioannina prov., Lakmos Mts, ca 7.5 km S of Mt Peristeri (= Mt Lakmos), 7 Jul 2000, *MP & al.*	32	
Cardamine acris* Griseb. subsp. *acris		
BG, Stara planina, Tetevenska planina, NW of Mt Vezhen (2,198 m), 1,850 m, 21 Jun 2000, *MP & E. Georgieva* (population VN in Perný *et al.* 2004)	16[2]	25
BG, Stara planina, Tetevenska planina, Ribarishki prohod, 1,650 m, 22 Jun 2000, *MP & E. Georgieva* (population RP in Perný *et al.* 2004)	16[2]	38
BG, Osogovska planina, above Osogovo chalet, 28 Jun 2000, *MP & MA* (population OG in Perný *et al.* 2004)	16[2]	22
BG, Zapadni Rodopi, Pamporovo, Ardashla, 1,450 m, 25 Jun 2000, *MP* (population AR in Perný *et al.* 2004)	16[2]	30
GR, Northern Pindos, Ioannina/Kastoria prov., Gramos Mts, Aetomilitsa, SE slope of the valley ca 5 km N of the village, 19 Jun 2001, *MP & VK* (population 1AE in Perný *et al.* 2004)	16[2]	34
GR, North Central, Pella prov., Voras Mts, Voras Ski Resort, stream along the road above the resort, 21 Jun 2001, *MP & VK* (population 1VR in Perný *et al.* 2004)	16[2]	23
***Cardamine acris* subsp. *pindicola* Perný & Marhold**		
GR, Northern Pindos, Ioannina prov., Katara Pass above Metsovo, ca 2.5 km S of Mt Katara (1,820 m), before crossroad to Haliki, 18 Jun 2001, *MP & VK*; 29 May 2002, *MP* (population 2KP in Perný *et al.* 2004)	16[2]	22
***Cardamine acris* subsp. *vardousiae* Perný & Marhold**		
GR, Sterea Ellas, Evritania prov., Timfristos Mts, Karpenisi Ski Resort, stream ca 2.5 km NW of the resort, 24 Jun 2001, *MP & VK* (population TM in Perný *et al.* 2004)	16[2]	31
GR, Sterea Ellas, Fokida prov., Vardousia Mts, ca 6 km WSW of Athanasios Diakos, pass between the hills 2,340 m and 2,495 m, 23 Jun 2001, *MP & VK* (population 1VD in Perný *et al.* 2004)	16[2]	19
GR, Sterea Ellas, Fokida prov., Vardousia Mts, ca 6 km WSW of Athanasios Diakos, stream ca 500 m of subpopulation 1VD, 23 Jun 2001, *MP & VK* (population 2VD in Perný *et al.* 2004)	16[2]	24

.....continued on the next page

TABLE 2. (Continued)

Origin and collection data	2*n*	Morphometrics No. of individuals
Cardamine amara* L. subsp. *amara		
SK, Slanské vrchy Mts, Kokošovce, 0.5 km NE of the water reservoir, 500 m, 5 May 1987, *KM* (population no. 8 in Marhold 1992)	16[3]	18
SK, Muránska planina Plain, Tisovec, 440 m, 9 May 1986, *KM* (population no. 6 in Marhold 1992)	16[3]	21
SK, Spišské vrchy Mts, Starina, NE of the village, 460 m, 7 May 1987, *KM* (population no. 25 in Marhold 1992)	16[3]	20
UA, Schidni Beskydy i Nyz'ki Polonyny Mts, between the villages of Huklyvyi and Podobovets, 750 m, 7 May 1990, *KM, A. Kahalo & M. Zahulskii* (population no. 45 in Marhold 1992)	16[3]	22
UA, Prykarpattya Mts, Nyzhnii Synovydnyi, 380 m, 5 May 1988, *KM, A. Kahalo & M. Zahulskii* (population no. 42 in Marhold 1992)	16[3]	22
***Cardamine amara* L. subsp. *opicii* (J. Presl & C. Presl) Čelak.**		
SK, Vysoké Tatry Mts, Velická dolina Valley, near the Dlhé (Kvetnicové) pleso Lake, 1,750 m, 21 Jul 1986, *KM* (population no. 22 in Marhold 1992)	16[3]	25
SK, Nízke Tatry Mts, Štiavnica Valley, E of the Bocianske sedlo Saddle, 1,400 m, 16 Jul 1986, *KM* (population no. 19 in Marhold 1992)	16[3]	21
SK, Veľká Fatra Mts, Ľubochnianska dolina Valley, above the forester's house Raková, 850-900 m, 28 May 1986, *KM* (population no. 15 in Marhold 1992)	16[3]	20
PL, Tatry Wschodnie Mts, near the Litworowy Staw Lake, 1,620 m, 13 Jul 1989, *KM* (population no. 41 in Marhold 1992)	16[3]	18
UA, Chornohora Mts, Mt Hoverla, NW slope, 1,700 m, 17 Jul 1989, *KM, A. Kahalo & M. Zahulskii* (population no. 49 in Marhold 1992)	16[3]	20
***Cardamine amara* subsp. *balcanica* Marhold, Ančev & Kit Tan**		
BG, Zapadni Rodopi Mts, Beglika, natural reserve, near the right tributary of Semsiza River, 1,600–1,650 m, 20 Jun 1994, *KM* & *R. Vassilev* (population no. 67 in Marhold *et al.* 1996)	16[4]	26
BG, Rila Mts, Kostenets, Yurukova polyana, on the slope of Mt Sokolovets, 1,500 m, 22 Jun 1994, *KM & R. Vassilev* (population no. 65 in Marhold *et al.* 1996)	16[4]	12
BG, Vitosha Mts, 'Aleko', 1,800 m, 15 Jun 1994, *KM* & *MA* (population no. 59 in Marhold *et al.* 1996)	16[4]	25
BG, Rila Mts, Borovets, 1,300 m, 16 Jun 1994, *KM & MA* (population no. 63 in Marhold *et al.* 1996)	16[4]	17
BG, Pirin Mts, Demjanishka polyana, 1,620 m, 1 Jul 1995, *KM* & *MA* (population no. 60 in Marhold *et al.* 1996)	16[4]	26
BG, Rila Mts, Kompleks Maljovitsa, 3 Jul 1995, *KM* & *R. Vassilev* (population no. 66 in Marhold *et al.* 1996)	16[4]	30
***Cardamine raphanifolia* Pourr.**		
AD, Pyrenees, Ordino, stream in the village, 1,300 m, 8 Jun 2000, *MP* (population OR in Perný *et al.* 2005a)	48[5]	29
ES, Gerona, Pyrenees, Can Nofre, 1,200 m, 6 Jun 2000, *MP* & *J. Vicens* (population CN in Perný *et al.* 2005a)	48[5]	31
ES, Lérida, Pyrenees, Val d'Aran, S of Salardú, near Banhs de Tredós, Pónt dera Montanheta, Arriu d'Aiguamóg, 1,880 m, 26 Jun 2002, *JL* (population TRE in Perný *et al.* 2005a)	64[5]	24
ES, Navarra, Quinto Real, 2.2 km S of Irurito, 700 m, 8 May 2001, *JL* & *I. Biurrun* (population QRR in Perný *et al.* 2005a)	64[5]	20
ES, Cantabria, Puerto de Los Tornos, 800 m, 21 May 2001, *MP* & *M. Herrera* (population PL in Perný *et al.* 2005a)	48[5]	25
ES, Asturias, Pajares, below Puerto Pajares, near the road, 1,860 m, 24 May 2001, *MP* & *F. Llamas* (population AP in Perný *et al.* 2005a)	64[5]	20
FR, Pyrenees, Vallée d'Aspe, Les Forges d'Abel, S of Urdos, above north mouth of the Samport Tunnel, 1,200 m, 31 May 2001, *MP* & *L. Villar* (population LF in Perný *et al.* 2005a)	48[5]	32

Strid (1986) reported *C. barbaraeoides* (as *C. raphanifolia* subsp. *barbaraeoides*) from the floristic regions of Sterea Ellas, Southern Pindos and Northern Pindos in Greece whereas Kit Tan (2002) mentioned this taxon, treating it within *C. amara* as *C. amara* subsp. *barbaraeoides*, also from the region of North Central (Greece). Both authors excluded *C. barbaraeoides* from the areas of former Yugoslavia and Bulgaria. The occurrence of *C. barbaraeoides* in Albania, however, was considered possible by these authors. Nevertheless, neither *C. amara* nor *C. barbaraeoides* were included in the *Excursion flora of Albania* (Vangjeli 2015) or the *Flora Albanica atlas* (Vangjeli 2017). Dimopoulos *et al.* (2013, 2017+) reported *C. barbaraeoides*, in agreement with the earlier Greek flora by Kit Tan (2002), from the regions of North Central, Northern Pindos, Southern Pindos and Sterea Ellas. With regards to morphology, contrary to Halácsy (1901), Schulz (1903) and Kit Tan (2002), who reported that *C. barbaraeoides* has violet anthers, Strid (1986) mentioned the yellow colour of its anthers.

Our herbarium and field studies indicated that the plants from the areas reported by Strid (1986) and Kit Tan (2002), and identified as *C. barbaraeoides*, are morphologically highly heterogeneous and cannot be treated as a single taxon. They have apparently been confused with two other Balkan taxa, *C. acris* and *C. amara* subsp. *balcanica* (see above). Chromosome number counts and ploidy level estimates for plants from the Southern Pindos Mts, which morphologically correspond to the original material of the name *C. barbaraeoides* collected by Halácsy (see the section Taxonomic account), revealed the tetraploid level with 2n = 32 (Perný *et al.* 2005b, Šlenker *et al.* 2021a). By contrast, both *C. amara* subsp. *balcanica* and *C. acris* are diploids (Marhold *et al.* 1996, Perný *et al.* 2004). Based on target enrichment with genome skimming (Hyb-Seq) and genomic *in situ* hybridization (GISH), we inferred an allopolyploid origin of *C. barbaraeoides* from the Southern Pindos Mts. Our results suggested that the parental species of *C. barbaraeoides* were very likely the common ancestors of *C. amara* and *C. lazica* Boiss. & Balansa (in Boissier 1888: 31) on one side, and of *C. acris* and some western Anatolian taxon [currently ascribed to *C. uliginosa* Marschall von Bieberstein (1819: 438)] on the other (Šlenker *et al.* 2021a).

Still, the taxonomic position, circumscription and morphological differences of *C. barbaraeoides* from the related taxa remained unclear. Therefore, the aims of the current paper were to (1) revise the circumscription of *C. barbaraeoides*, define its actual range (distribution area), and ascertain its differentiation from closely related taxa occurring in the Balkan Peninsula, and to (2) resolve its taxonomic position with respect to *C. amara* and *C. raphanifolia*, to which *C. barbaraeoides* was previously subordinated (included as an infraspecific taxon). To address these issues, we undertook a multivariate morphometric approach combined with the study of original material of the name *C. barbaraeoides* and available herbarium specimens of related taxa in relevant herbarium collections.

Materials and methods

Plant material.—For the present morphometric study, we used plant material of *Cardamine barbaraeoides* and seven related taxa. We sampled 35 populations in the field, with 12 to 42 plants per population preserved as herbarium specimens and used for measurements (Table 2). Two population samples of *C. barbaraeoides* from the Lakmos Mts, from where this species was originally described, were collected in anthesis (altogether 56 plants). The samples of *C. acris*, *C. amara* and *C. raphanifolia* were taken from our previous studies (Marhold 1992, 1998, Perný *et al.* 2004, 2005a), covering a representative part of their distribution areas (Table 2). These species were included in the analyses because both *C. acris* and *C. amara* (specifically *C. amara* subsp. *balcanica*) were confused in the past with *C. barbaraeoides*, and *C. barbaraeoides* was also previously subordinate to *C. amara* or *C. raphanifolia*. Population samples of diploid *C. acris* (including three subspecies, namely *C. acris* subsp. *acris*, *C. acris* subsp. *pindicola* Perný & Marhold in Perný *et al.* 2004: 422, and *C. acris* subsp. *vardousiae* Perný & Marhold in Perný *et al.* 2004: 423) originated from six localities in Greece and four from Bulgaria. Sixteen population samples of diploid *C. amara* were used, including samples of *C. amara* subsp. *balcanica* from Bulgaria, and both *C. amara* subsp. *amara* and *C. amara* subsp. *opicii* (J. Presl & C. Presl 1819: 136) Čelakovský (1875: 449) from Central Europe (see Table 2). Finally, seven population samples of hexa- and octoploid *C. raphanifolia* originated from the Iberian Peninsula.

The distribution data for *C. barbaraeoides* are based on the study of specimens deposited in the herbaria B, BM, BP, C, JE, K, LD, PR, W and WU (acronyms according to Thiers 2008). The floristic division of mainland Greece into seven regions adopted here (North East, North Central, Northern Pindos, Southern Pindos, East Central, Sterea Ellas and Peloponnisos; Fig. 1) follows Strid (1986).

Chromosome counting.—Chromosomes of *C. barbaraeoides* were counted from mitotic metaphase plates observed in root tips obtained from cultivated plants. Chromosome spreads were prepared following Marhold *et al.* (2002), using the Giemsa stain.

Analyses of morphological characters.—For the scoring and measurements of vegetative morphological characters, herbarium specimens collected from the field were used. Floral parts from one randomly chosen well-developed flower per plant were attached by adhesive tape to a sheet of paper, dried and then scanned. Each flower organ was measured separately with the software Carnoy 2.0 (Schols *et al.* 2002) or QuickPHOTO Industrial v.2.3 (Promicra, Prague, Czechia). Twelve quantitative characters on the stem and leaves and five quantitative characters on flowers were measured, and ten ratios were derived (Table 3). From the character pairs used for computing the ratios, only one character out of each pair was retained in the final data matrix used in multivariate morphometric analyses. In total, nine measured characters and ten derived ratio characters were used in the subsequent multivariate analyses (Table 3). In addition to quantitative characters, five qualitative characters were scored. They included the presence of a basal leaf rosette, the type of indument on the stem, the colour of petals, the colour of anthers and stigma morphology (conspicuously enlarged or not, compared to the style). Because they did not vary within some of the taxa under study (which precludes their use in discriminant analyses) or could not be studied on each specimen, they were not used in the multivariate analyses but evaluated separately.

TABLE 3. List of quantitative characters measured for morphometric analyses.

Stem and leaf characters	
StemWidth	max. width of stem [mm]
StemHeight[1]	height of stem from its base to the base of the uppermost stem leaf [cm]
NoBranches	number of stem branches (including main stem)
NoLeaves	number of stem leaves
CongestLeaves	degree of congestion of leaves beneath the inflorescence, expressed by the number of leaves reaching the base of the uppermost stem leaf
MidStLeafLength[2]	length of middle stem leaf [cm]
UpStLeafLength[1]	length of the uppermost leaf [cm]
NoLeaflets[2]	number of pairs of lateral leaflets of the middle stem leaf
TermLflLength[1,2] and TermLflWidth[1,2]	length and width of terminal leaflet of the middle stem leaf [cm]
LatLflLength[1,2] and LatLflWidth[1,2]	length and width of first lateral leaflet of the middle stem leaf [cm]
Flower characters	
SepLength	average length of sepals [mm]
PetLength and PetWidth[1]	average length and width of petals [mm]
ShortFilLength[1] and LonFilLength	average length of longer and shorter filaments [mm]
Ratio characters	
NoLeaves/StemHeight, MidStLeafLength/StemHeight, UpStLeafLength/StemHeight, UpStLeafLength/MidStLeafLength, TermLflWidth/TermLflLength, LatLflWidth/LatLflLength, LatLflLength/TermLflLength, LatLflWidth/TermLflWidth, PetWidth/PetLength, ShortFilLength/LonFilLength	

[1] characters used only for computing ratios; [2] the leaf closest to the StemHeight/2 point

Pearson and Spearman correlation coefficients (Sneath & Sokal 1973) were computed in order to detect and eliminate pairs of highly correlated characters from further analyses. The Spearman coefficient was used because the characters used in the multivariate analyses did not have a normal distribution. First, we performed principal component analyses (PCA) based on all individuals and taxa sampled (Table 2) to visualize their overall morphological relationships and the position of tetraploid plants from the Lakmos Mts described as *C. barbaraeoides*. An R-type PCA (Sneath & Sokal 1973, Krzanowski 1990) was used based on correlation matrices with individual plants as OTUs (operational taxonomic units). As next, to examine the separation of *C. barbaraeoides* from the other taxa in more detail, several canonical discriminant analyses (CDA; Klecka 1980, Krzanowski 1990) were computed with individual plants as OTUs. Groups in the discriminant analyses were defined based on ploidy levels and geographic areas, and in agreement with previous molecular analyses (Perný *et al.* 2004, 2005a,b, Šlenker *et al.* 2021a). To determine the characters that contribute the most to the group separation in the ordination diagrams of CDA, the total canonical structure expressing the correlations of all characters with the canonical axes was analysed. Although several characters more or less deviated from normal distribution, CDA has proved to be considerably robust to such deviations

(Sneath & Sokal 1973, Thorpe 1976, Klecka 1980). Means, standard deviations and percentiles were computed for all quantitative characters. Boxplots with rectangles defined as interquartile ranges, and whiskers as the 5th and 95th percentiles, were plotted for the four species studied.

All statistical analyses were performed in R 4.0.0 software (R Core Team 2020) using the MorphoTools2 package (Šlenker *et al.* 2021b).

Results

In agreement with a previous study by Šlenker *et al.* (2021a), plants from populations from the Lakmos Mts, analysed karyologically here, have tetraploid chromosome numbers with 2n = 32.

Examination of qualitative characters not included in the multivariate morphometric analyses identified the following features of *C. barbaraeoides*: (1) exclusively white petals (like *C. amara*, in contrast to *C. raphanifolia* with pink to reddish-violet petals, and variable petal colour in *C. acris*); (2) mostly violet anthers, but also yellow ones, observed in a few plants within the populations studied here in detail (*C. amara* has exclusively violet anthers whereas plants of *C. acris* and *C. raphanifolia* have yellow anthers); (3) an inconspicuous stigma, not enlarged relative to the style (similar to that of *C. amara*, while plants of *C. acris* and *C. raphanifolia* have conspicuously enlarged stigmata); (4) basal leaves not forming a basal rosette (only loosely congested near the stem base; like in *C. amara* and *C. raphanifolia*, while *C. acris* forms a basal leaf rosette; (5) a glabrous stem (similarly to *C. acris* and in strong contrast particularly to *C. amara* subsp. *balcanica* having a densely hairy stem). It is therefore apparent that *C. barbaraeoides* from the Lakmos Mts can be distinguished from the related taxa by the combination of these several qualitative characters (see Table 4).

TABLE 4. States of qualitative morphological characters of *Cardamine barbaraeoides* and other taxa studied.

	C. barbaraeoides	*C. acris*	*C. amara*	*C. raphanifolia*
Petal colour	white	white, pale pink, reddish-violet	white	pink, reddish-violet
Anther colour	violet (rarely yellow)	yellow	violet	yellow
Stigma	inconspicuous	conspicuously enlarged	inconspicuous	conspicuously enlarged
Leaf rosette	absent (basal leaves loosely congested near the stem base)	present	absent	absent
Stem indument	glabrous	glabrous	densely hairy (subsp. *balcanica*) glabrous or sparsely hairy (subsp. *amara*) glabrous or densely hairy (subsp. *opicii*)	glabrous or sparsely hairy

Neither Pearson nor Spearman correlation coefficients, calculated from the matrix of nineteen quantitative characters used in the multivariate analyses, reached values exceeding 0.8; the highest value of the Pearson correlation coefficient was 0.776 (leavesReachingUppermostStLeaf vs stemLeaves/stemLengthToUppermostLeaf), that of the Spearman coefficient was 0.781 (LatLeafletLength/TerminalLeafletLength vs LatLeafletWidth/TerminalLeafletWidth). All nineteen characters as listed in Table 3 were therefore retained in the subsequent multivariate analyses.

On the PCA ordination diagram, based on the matrix of quantitative characters comprising all taxa (Fig. 2), plants of *C. barbaraeoides* are placed in its central part, in between plants of *C. amara* on one side and those of *C. acris* and *C. raphanifolia* on the other, with partial overlap on both sides. They are separated mostly along the first axis. From among the analysed subspecies of *C. amara*, the most distant from *C. barbaraeoides* is *C. amara* subsp. *opicii*, while *C. amara* subsp. *amara* and *C. amara* subsp. *balcanica* are closer to *C. barbaraeoides*. All subspecies of *C. acris* appear to be equally distant from *C. barbaraeoides*. The characters highly correlated with the first principal component axis (Table 5), and thus responsible for the separation of the analysed taxa, are the number of leaves and leaflets on middle stem leaves, the ratio of the number of stem leaves to stem height, congestion of leaves under the inflorescence and the ratio of lengths of short and long filaments.

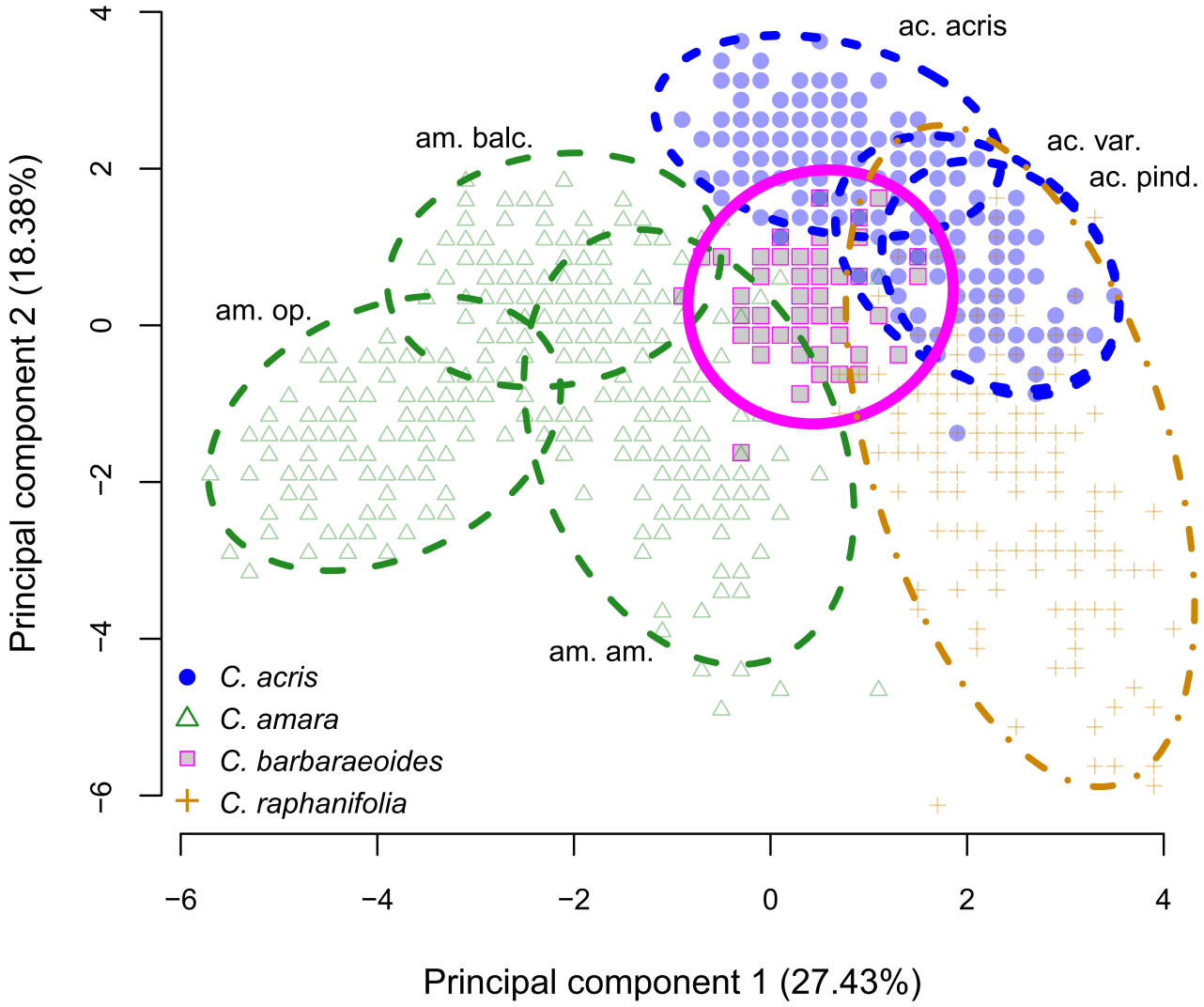

FIGURE 2. Principal component analysis of individuals of *Cardamine barbaraeoides* from the Lakmos Mts, *C. amara* subsp. *amara* (am. am.), *C. amara* subsp. *balcanica* (am. balc.), *C. amara* subsp. *opicii* (am. op.), *C. acris* subsp. *acris* (ac. acris), *C. acris* subsp. *pindicola* (ac. pind.), *C. acris* subsp. *vardousiae* (ac. var.), and *C. raphanifolia*, based on nine morphological characters and ten ratios. Prediction ellipses define the regions where any new independent observations from the respective taxa will fall with the probability of 95%.

We applied several canonical discriminant analyses (CDA) to different sets of taxa to examine the differentiation of *C. barbaraeoides* from the related species in more detail. The first canonical discriminant analysis comprised all analysed plants and taxa at the species level (*C. acris*, *C. amara*, *C. barbaraeoides* and *C. raphanifolia*) as groups (Fig. 3a,b). The two ordination diagrams show the separation of the groups along the first three canonical axes. The separation of the groups is much clearer compared with the ordination diagram of PCA, as the discriminant analysis weighs characters in favour of those that separate the predefined groups. Similarly to the results of PCA, the position of *C. barbaraeoides* along the first canonical axis is in between *C. amara* on one side and between *C. acris* and *C. raphanifolia* on the other. The second axis separates *C. acris* from *C. raphanifolia*, while along the third axis *C. barbaraeoides* is separated from all three taxa. Characters correlated strongly with the first axis were the number of leaves, the ratio of the number of stem leaves to stem height, congestion of leaves under the inflorescence, ratios of the lengths to the widths of lateral and terminal leaflets of middle stem leaves, and the ratio of the lengths of short and long filaments (Table 5). The second axis was strongly correlated with the length of longer filaments, petals and sepals, and with the ratio of length of the middle stem leaf to the height of the plant. Finally, the third axis was highly correlated with the ratio of the petal width to petal length, the number of leaflets on the middle stem leaf, and the ratio of the lengths of lateral and terminal leaflets of the middle stem leaf (Table 5).

Subsequently, we performed three CDAs with the aims to reveal the degree of morphological differentiation of *C. barbaraeoides* from each of the other species (*C. amara, C. acris* and *C. barbaraeoides*) separately and to identify the characters responsible for their separation. All three CDAs revealed unequivocal morphological differentiation of

C. barbaraeoides (Fig. 4). Although there is only minimal morphological overlap visible in the histogram of CDA with *C. amara* and *C. barbaraeoides* as two predefined groups (Fig. 4a), the separation of *C. acris* and *C. barbaraeoides* (Fig. 4b) and *C. raphanifolia* and *C. barbaraeoides* (Fig. 4c) in the CDA histograms are without any overlaps whatsoever. Total canonical structure coefficients (Table 5) indicate that three leaf characters best separate *C. amara* and *C. barbaraeoides*: the ratios of the lengths and widths of lateral and terminal leaflets, and the number of leaflets on middle stem leaves. Several leaf and flower characters differentiate between *C. acris* and *C. barbaraeoides:* the number of leaves, the number of leaflets of middle stem leaves, the ratio of the number of leaves to stem height, sepal and longer filament length, as well as the ratio of petal width to petal length and the ratio of the lengths of shorter and longer filaments. Finally, a number of characters contribute to the separation of *C. raphanifolia* from *C. barbaraeoides*, mainly the number of leaves, the ratio of the number of leaves to stem height, the width and length of the terminal leaflet of middle stem leaves as well as the ratio of petal width and length.

TABLE 5. Principal component analysis of *Cardamine barbaraeoides* and all other taxa studied: component loadings for the first and second components (PC1, PC2). Canonical discriminant analyses—correlation coefficients (total canonical structure) of morphological characters and canonical axes: CDA all—canonical discriminant analysis of samples of all taxa studied (Can1, Can2, Can3—correlations with the 1st, 2nd and 3rd canonical axes, respectively); am−barb—canonical discriminant analysis of samples of *C. amara* and *C. barbaraeoides*; acris−barb—canonical discriminant analysis of samples of *C. acris* and *C. barbaraeoides*; raph−barb—canonical discriminant analysis of samples of *C. raphanifolia* and *C. barbaraeoides.*

	PCA		CDA all			am−barb	acris−barb	raph−barb
Character	PC1	PC2	Can1	Can2	Can3	Can1	Can1	Can1
StemWidth	−0.102	0.116	0.037	−0.091	−0.208	0.221	0.226	0.322
NoLeaves	**−0.366**	0.043	**−0.737**	−0.12	0.117	0.231	**−0.752**	**−0.928**
NoBranches	0.023	0.243	0.316	−0.451	−0.078	−0.048	0.302	−0.043
CongestLeaves	**−0.346**	−0.118	**−0.641**	0.057	−0.055	0.305	−0.464	−0.412
NoLeaves/StemHeight	**−0.358**	−0.103	**−0.778**	−0.031	0.14	0.229	**−0.896**	**−0.922**
MidStLeafLength	−0.014	−0.137	0.122	0.385	−0.271	0.253	0.169	0.444
MidStLeafLength/StemHeight	−0.027	**−0.433**	−0.17	**0.566**	−0.239	0.303	−0.173	0.439
UpStLeafLength/StemHeight	0.146	**−0.395**	0.065	0.521	−0.117	0.054	−0.241	0.329
UpStLeafLength/MidStLeafLength	0.23	−0.222	0.198	0.314	0.109	−0.23	−0.242	0.098
NoLeaflets	**−0.299**	−0.03	−0.434	−0.289	**−0.464**	**0.602**	**0.575**	0.504
TermLflWidth/TermLflLength	0.242	0.095	0.58	0.258	−0.234	−0.063	0.365	**0.715**
LatLflWidth/LatLflLength	0.149	−0.035	0.288	0.415	−0.271	0.141	0.171	**0.625**
LatLflLength/TermLflLength	−0.226	−0.253	**−0.605**	0.19	**−0.404**	**0.674**	0.205	0.448
LatLflWidth/TermLflWidth	−0.252	**−0.309**	**−0.709**	0.256	−0.372	**0.677**	0.093	**0.548**
SepLength	0.104	**−0.356**	0.118	**0.698**	0.226	−0.453	**−0.636**	0.133
PetLength	0.286	−0.247	0.573	**0.553**	0.062	**−0.611**	−0.239	0.409
LonFilLength	0.173	−0.295	0.23	**0.807**	0.057	−0.398	**−0.575**	0.391
PetWidth/PetLength	0.189	−0.096	0.345	0.032	**−0.58**	0.415	**0.701**	**0.822**
ShortFilLength/LonFilLength	**−0.299**	−0.185	**−0.884**	0.202	0.096	**0.526**	**−0.71**	**−0.545**

Variation patterns of selected characters that contribute the most to the separation of *C. barbaraeoides* from its relatives, as identified in the above CDA, are presented as boxplot graphs in Fig. 5. Means, standard deviations, and the 5th and 95th percentiles for all quantitative characters are presented in Table 6. Morphological differences between *C. barbaraeoides, C. acris* and *C. amara* subsp. *balcanica* can be seen also in plant drawings in Fig. 6.

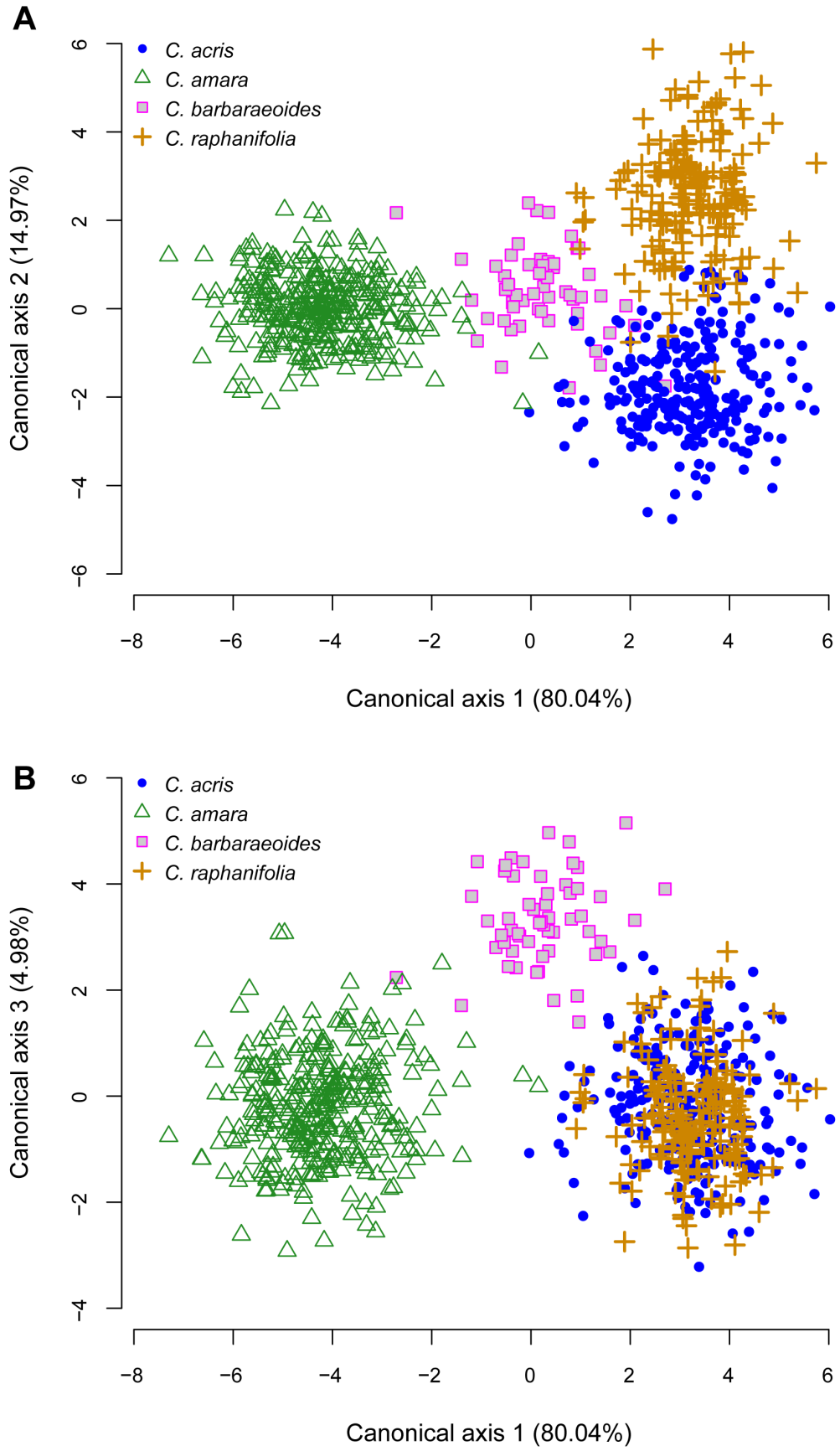

FIGURE 3. Canonical discriminant analysis of individuals of *Cardamine barbaraeoides*, *C. amara*, *C. acris* and *C. raphanifolia*, based on nine morphological characters and ten ratios.

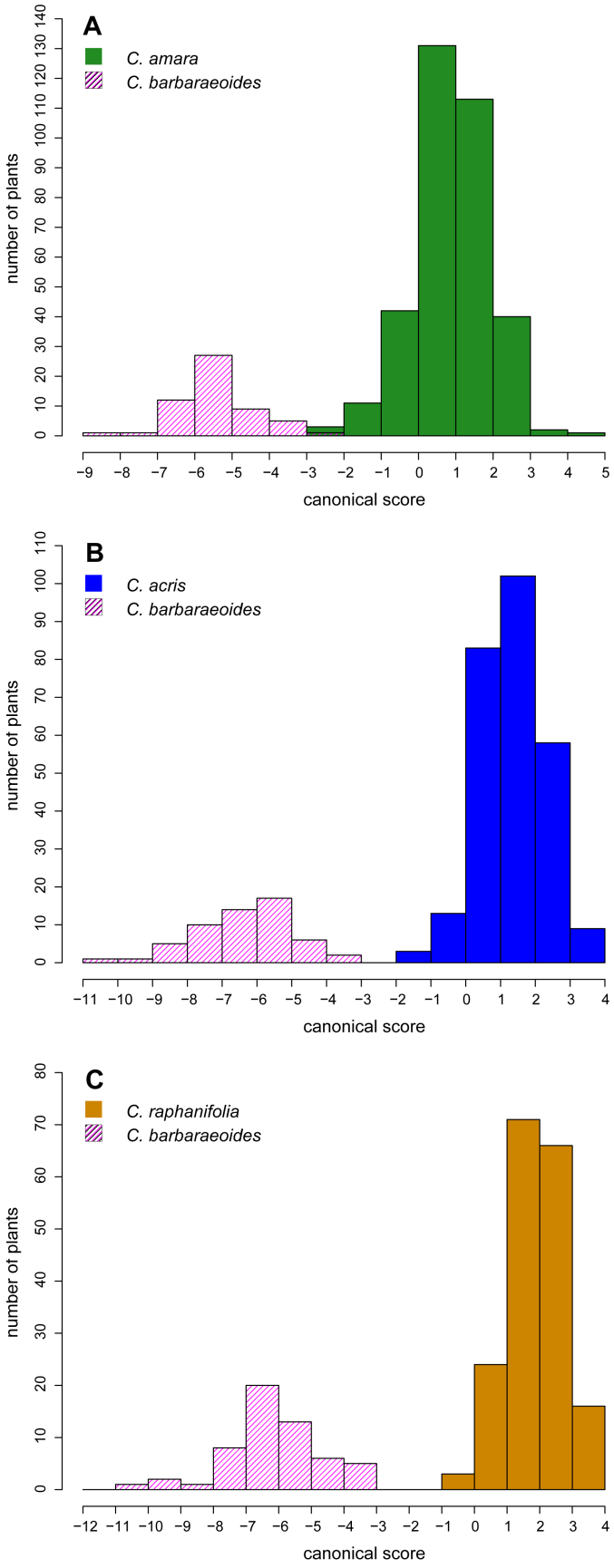

FIGURE 4. Canonical discriminant analyses based on individual plants and two predefined groups in each analysis: *Cardamine barbaraeoides* and *C. amara* (A), *C. barbaraeoides* and *C. acris* (B), and *C. barbaraeoides* and *C. raphanifolia* (C). The analyses were based on nine morphological characters and ten ratios.

TABLE 6. Descriptive statistics of quantitative characters of *Cardamine acris*, *C. amara*, *C. barbaraeoides* and *C. raphanifolia*. Means ± SD together with the 5th and 95th percentiles (in parentheses) are given; for character explanations see Table 3.

	C. acris	*C. amara*	*C. barbaraeoides*	*C. raphanifolia*
Number of plants measured	268	343	56	180
Stem and leaf characters				
StemWidth [mm]	4.16±2.07 (1.75–8)	3.91±1.51 (2–6.5)	3.05±1.06 (1.75–5)	3.86±1.06 (2.5–6)
StemHeight [cm]	45.42±20.2 (18.5–84)	31.73±16.73 (13.3–65)	31.04±8.22 (18.88–46.75)	33.15±10.6 (18–51.05)
NoBranches	4.75±3.73 (1–11)	1.8±1.5 (1–5)	2±2.12 (1–5.25)	1.86±1.22 (1–4)
NoLeaves	7.65±2.47 (4–12)	20.56±9.96 (5–34)	14.8±3.51 (10.5–21)	4.69±1.58 (3–8)
CongestLeaves	0.48±0.72 (0–2)	3.31±2.48 (0–8)	1.39±0.68 (0.75–2.25)	0.75±0.62 (0–2)
MidStLeafLength [cm]	6.1±2.91 (2.54–11.43)	6.54±2.45 (3.2–11.02)	4.93±1.74 (2.53–8.7)	9.21±4.32 (4–17.5)
UpStLeafLength [cm]	2.1±0.96 (0.9–4)	2.13±0.98 (0.86–4)	2.43±1.34 (0.75–4.47)	4.74±2.92 (1.5–9.8)
NoLeaflets	3.23±1.1 (2–5)	4.1±1.49 (2–7)	1.48±0.5 (1–2)	2.33±0.69 (1–3)
TermLflLength [cm]	2.42±1.08 (1.02–4.46)	2.17±0.72 (1.2–3.6)	2.81±0.78 (1.5–4.19)	3.29±1.11 (1.8–5.2)
TermLflWidth [cm]	2.16±1.12 (0.82–4.65)	1.54±0.72 (0.68–2.89)	2.04±0.67 (1.1–3.3)	3.37±1.24 (1.65–5.55)
LatLflLength [cm]	1.29±0.7 (0.45–2.7)	1.57±0.51 (0.88–2.52)	1.34±0.54 (0.59–2.35)	2±0.87 (0.9–3.3)
LatLfWidth [cm]	0.81±0.49 (0.25–1.9)	0.96±0.39 (0.45–1.7)	0.73±0.34 (0.3–1.35)	1.64±0.75 (0.65–3.05)
Flower characters				
SepLength [mm]	3.46±0.52 (2.71–4.39)	3.77±0.53 (2.87–4.68)	4.43±0.32 (3.95–5.02)	4.64±0.79 (3.19–5.85)
PetLength [mm]	9.31±1.3 (7.33–11.59)	8.02±1.1 (6.15–9.72)	10.07±0.85 (8.64–11.23)	12.04±2.21 (8.4–15.24)
PetWidth [mm]	5.06±1.06 (3.6–7.08)	3.93±1.09 (2.4–5.73)	3.86±0.42 (3.16–4.48)	6.66±1.34 (4.5–9.05)
ShortFilLength [mm]	3.03±0.56 (2.15–3.92)	4.78±0.71 (3.62–5.93)	4.82±0.9 (2.51–5.76)	4.97±1.15 (3.07–6.56)
LonFilLength [mm]	5.18±0.68 (4.12–6.06)	5.59±0.69 (4.51–6.72)	6.38±0.8 (5.05–7.27)	7.64±1.46 (5.58–10.24)
Ratio characters				
NoLeaves/StemHeight	0.19±0.06 (0.11–0.3)	0.68±0.33 (0.28–1.25)	0.49±0.12 (0.33–0.72)	0.15±0.06 (0.08–0.27)
MidStLeafLength/StemHeight	0.14±0.05 (0.08–0.22)	0.24±0.1 (0.11–0.42)	0.16±0.04 (0.1–0.21)	0.29±0.14 (0.14–0.52)

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TABLE 6 (Continued**)**

	C. acris	*C. amara*	*C. barbaraeoides*	*C. raphanifolia*
Number of plants measured	268	343	56	180
UpStLeafLength/MidStLeafLength	0.38±0.16 (0.16–0.67)	0.36±0.2 (0.14–0.77)	0.48±0.17 (0.24–0.78)	0.53±0.22 (0.21–0.95)
TermLflWidth/TermLflLength	0.89±0.17 (0.61–1.14)	0.7±0.17 (0.44–1)	0.73±0.13 (0.54–0.94)	1.02±0.13 (0.77–1.22)
LatLflWidth/LatLflLength	0.64±0.19 (0.38–1)	0.61±0.13 (0.4–0.83)	0.56±0.16 (0.32–0.82)	0.82±0.15 (0.58–1.06)
LatLflLength/TermLflLength	0.52±0.11 (0.35–0.69)	0.74±0.12 (0.53–0.93)	0.47±0.11 (0.32–0.6)	0.6±0.12 (0.4–0.8)
LatLflWidth/TermLflWidth	0.37±0.08 (0.23–0.5)	0.65±0.14 (0.44–0.9)	0.35±0.08 (0.24–0.5)	0.48±0.09 (0.32–0.61)
PetWidth/PetLength	0.54±0.07 (0.42–0.66)	0.48±0.09 (0.36–0.64)	0.38±0.03 (0.34–0.44)	0.56±0.06 (0.45–0.65)
ShortFilLength/LonFilLength	0.58±0.07 (0.48–0.69)	0.86±0.07 (0.74–0.95)	0.75±0.08 (0.59–0.83)	0.65±0.07 (0.52–0.75)

The results of our study of herbarium specimens from numerous herbaria, belonging to *C. barbaraeoides* or originally identified as *C. barbaraeoides,* are summarized in Appendices 1 and 2, and depicted in Fig. 1. Based on these findings, the occurrence of *C. barbaraeoides* is confirmed only from the Lakmos Mts in the Southern Pindos and from one locality from the southern margin of the Northern Pindos (Mt Zygos, Fig. 1). All specimens identified as *C. barbaraeoides* from the Greek regions of North Central and Sterea Ellas, as well as most of such specimens from the Northern Pindos, belong to *C. acris*.

Discussion

Morphological distinction of *Cardamine barbaraeoides*.—The presented morphometric analyses demonstrate that *C. barbaraeoides* occupies an intermediate position between *C. amara* on one side and between *C. acris* and *C. raphanifolia* on the other. Probably because of this morphological intermediacy, *C. barbaraeoides* was regarded already by the author of its description as similar either to *C. acris* (Halácsy 1894) or to *C. amara* (Halácsy 1901), and later even classified within *C. amara* or *C. raphanifolia* (Table 1). Nevertheless, our analyses show that using a combination of several quantitative characters, *C. barbaraeoides* can clearly be discriminated from both *C. acris* and *C. raphanifolia*: It has more stem leaves, which are also more congested in the upper part of the stem, fewer leaflets on middle stem leaves, much shorter lateral leaflets compared to the terminal one, and narrower petals (Figs 5 and 6). *Cardamine barbaraeoides* can be distinguished also by certain qualitative characters (Table 5). Unlike *C. acris* and *C. raphanifolia*, it has an indistinct stigma and mostly violet anthers. In contrast to *C. acris*, it does not form a basal leaf rosette. Variation in the colour of anthers in *C. barbaraeoides* and in the colour of petals in *C. acris* might have contributed to the previous confusion of this taxon with *C. acris* or its classification within *C. raphanifolia*. *Cardamine barbaraeoides* has exclusively white petals, unlike *C. raphanifolia*, which has pink to reddish-violet petals; however, white to reddish-violet petals are present in *C. acris* (Perný *et al.* 2004, 2005a).

The presence of white flowers, the prevalence of violet anthers, an indistinct stigma and the absence of a basal leaf rosette, on the other hand, indicates a closer relationship of *C. barbaraeoides* to *C. amara*. Still, *C. barbaraeoides* is well delimited from *C. amara* by middle stem leaves with only 1–2 pairs of lateral leaflets and by a solely glabrous stem. Other quantitative characters (much enlarged terminal leaflets of middle stem leaves compared to lateral ones, fewer stem leaves, narrower petals, and longer petals, sepals and filaments) also jointly contribute to their separation, but their values sometimes partly overlap (Figs 5 and 6; see also the identification key below).

Plants from only two populations of *C. barbaraeoides* collected in the field were in flower and included in the morphometric analyses, but additional material from the other four localities was cultivated (see specimens collected by Perný in 2000 and referred to in Appendix 1). These plants showed constancy in the same distinguishing characters

as those observed in the field-collected plants. Taking into account the small distribution area of this taxon, we believe that the restricted amount of morphologically analysed plants was sufficient and representative to reflect reliably the morphological variation pattern among the populations and taxa under investigation.

Taxonomic treatment and evolutionary origin of *C. barbaraeoides*.—The hypothesis of a hybrid origin of *C. barbaraeoides* with the putative parents *C. amara* and *C. acris* (*C. raphanifolia* subsp. *acris*) was raised already by Schulz (1903: 314), based on morphological characters. The morphological intermediacy of *C. barbaraeoides* demonstrated here is, indeed, congruent with its allotetraploid origin inferred by Šlenker *et al.* (2021a). An allopolyploid origin clearly explains the morphological variation patterns observed in *C. barbaraeoides*, which shows closer resemblance in some characters to *C. acris* while in the other it is closer to *C. amara*, but it can be reliably differentiated from both species by a combination of characters. All lines of evidence gathered by Šlenker *et al.* (2021a) and within the current study support the recognition of *C. barbaraeoides* as a distinct taxon at the species level, and not as a subspecies of *C. amara* or *C. raphanifolia*. *Cardamine barbaraeoides* is tetraploid, in contrast to the diploid taxa *C. amara* subsp. *amara* (Marhold 1994), *C. amara* subsp. *balcanica* (Marhold *et al.* 1996) and *C. acris* (Ančev *et al.* 1997, Perný *et al.* 2004), and the hexa- and octoploid species *C. raphanifolia* (Perný *et al.* 2005a).

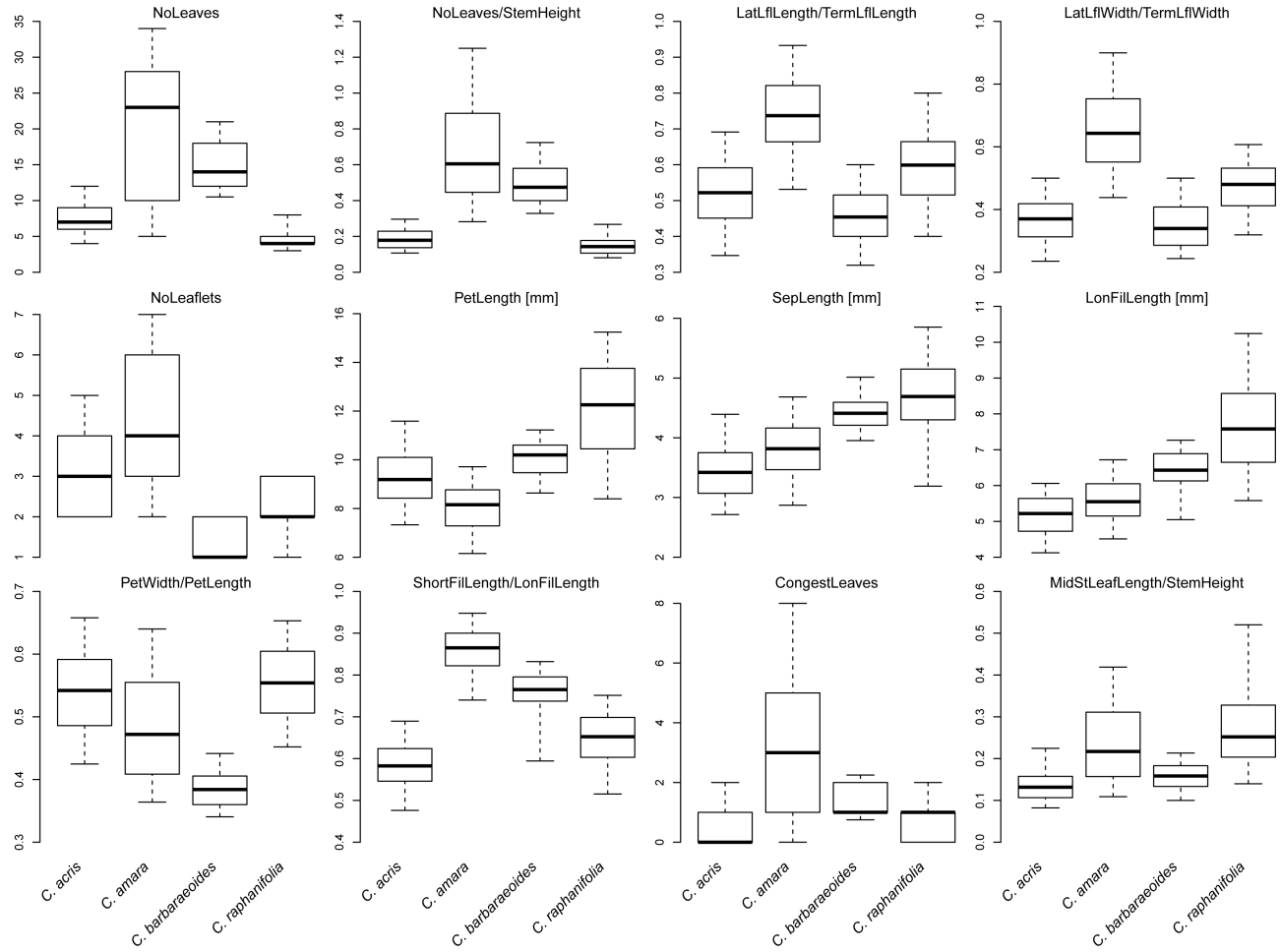

FIGURE 5. Box-and-whisker plots of the most important qualitative morphological characters discriminating between *Cardamine acris*, *C. amara*, *C. barbaraeoides* and *C. raphanifolia*.

Distribution of *Cardamine barbaraeoides*.—Based on our field studies and herbarium evidence, the distribution area of *C. barbaraeoides* is restricted to the northern part of the Southern Pindos (the Lakmos Mts: Mt Peristeri near Syrrako, Mt Turnara and Mt Salatura near Chaliki) and the southern part of the Northern Pindos (Mt Zygos; see Fig. 1 and Appendix 1). According to our field observations, the species is common in springs and along small streams at elevations between 1,000 and 1,800 metres above sea level.

Reports of *C. barbaraeoides* from other areas in the Balkan Peninsula are based on misidentifications with *C. acris* and *C. amara* subsp. *balcanica* (see below, Table 1 and Appendix 2). A sympatric occurrence of *C. barbaraeoides* with *C. acris* is documented only at a single locality (Mt Zygos, see below) whereas *C. amara* subsp. *balcanica* is

only known to grow allopatrically, having its nearest localities in the region of North East Greece (Marhold *et al.* 1996). *Cardamine matthioli* Moretti (1847a,b), a morphologically distinct species from the *C. pratensis* group but once probably also misidentified as *C. barbaraeoides* (see also below), occurs in Greece at only a few localities (Marhold & Kit Tan 2000), all of which are outside the distribution range of *C. barbaraeoides*.

In recent Greek Floras and checklists (Strid 1986, Kit Tan 2002, Dimopoulos *et al.* 2013, 2017+; Table 1), *C. barbaraeoides* is reported from a much larger area in Greece than our results confirm. During our field trips in 2000–2002 and in 2019, we visited several localities in the regions of Northern Pindos (Katara Pass, Mt Vasilitsa), North Central and Sterea Ellas, from where *C. barbaraeoides* was reported, but found only diploid *C. acris* there (Perný *et al.* 2004). It is not surprising that populations of *C. acris* were in several cases confused with *C. barbaraeoides*, as they appear extremely variable throughout their distribution area in Greece, especially in the colour of petals (see above). More details on *C. acris* and its three subspecies in Greece are provided by Perný *et al.* (2004). In the following text, we briefly report on previous misidentifications, focusing on localities visited and herbarium specimens revised by us, arranged in geographic order (see also Appendix 2).

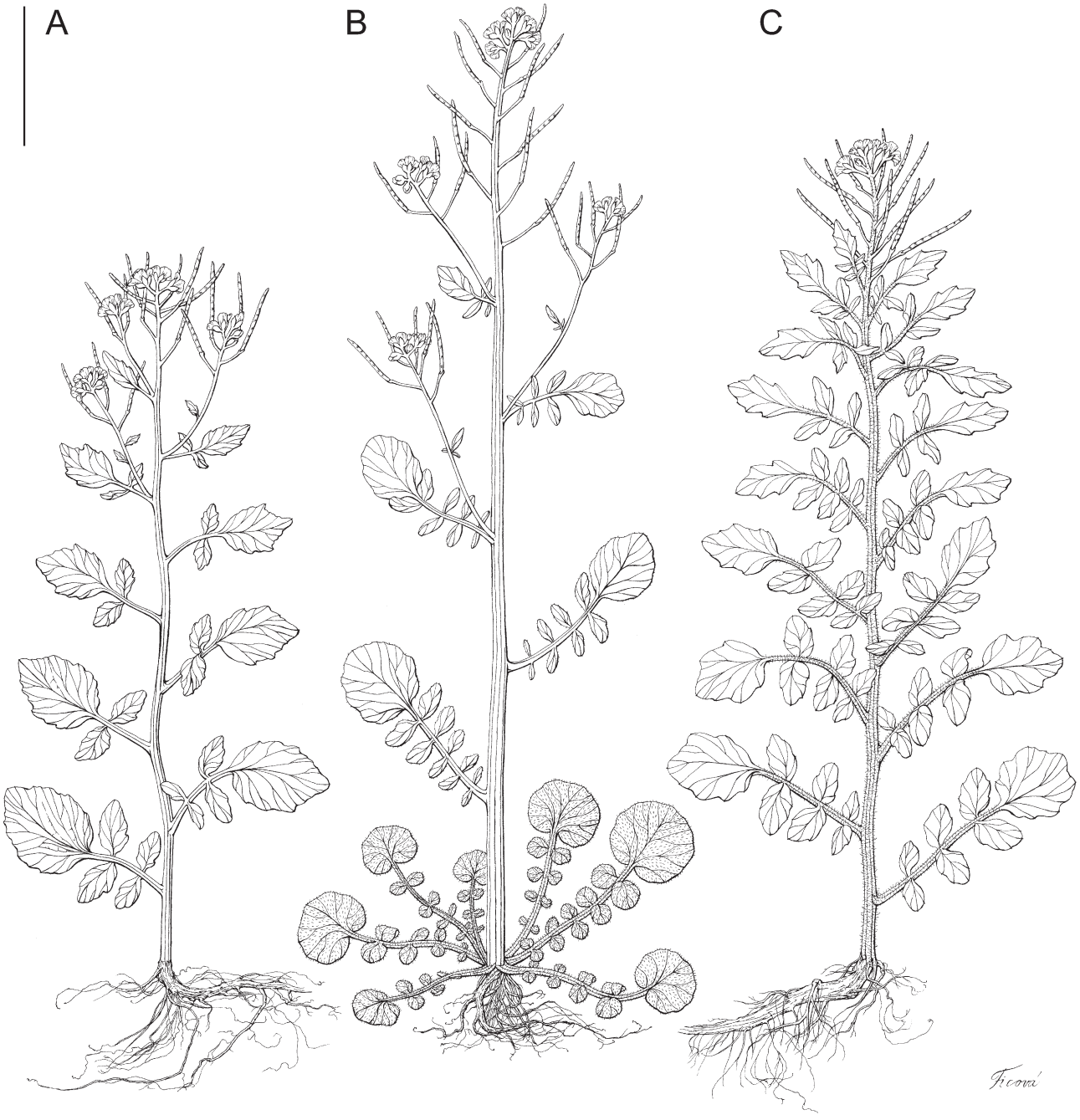

FIGURE 6. Drawings of *Cardamine barbaraeoides* (A), *C. acris* subsp. *acris* (B), and *C. amara* subsp. *balcanica* (C). Scale bar − 5 cm. Drawn by Jana Ficová.

(1) In the Northern Pindos (northwestern Greece), *C. barbaraeoides* was first reported by Halácsy (1901) from Mt Smolikas. This was based on a specimen (*Baldacci 1896, Iter Albanicum (Epiroticum) Quartum no. 208*, WU-Hal) that belongs to *C. acris*. A specimen from Mt Zygos (*Haussknecht, Iter Graecum 1885*, JE) reported by Schulz (1903) also represents *C. acris*, although other specimens from this locality [*Sintenis, Iter Thessalicum 1896, no. 792,* B 100000945, JE; also reported by Schulz (1903)] belong partly to *C. barbaraeoides* (on the herbarium sheet from JE, one plant is *C. acris* and the other is *C. barbaraeoides*). Most specimens from the Northern Pindos cited as *C. raphanifolia* subsp. *barbaraeoides* by Strid (1986) [Mt Smolikas (*Hartvig et al. 5890* C; *Baldacci 1896, Iter Albanicum (Epiroticum) Quartum no. 208*, WU-Hal); Katara pass (*Strid 24579* C; *Aldén 1436* C); Valley between Mt Gomara and Mt Vasilitsa (*Hartvig et al. 5275* C); SW of Souflikas (*Hartvig et al. 6983* C)] belong to *C. acris*. In the *Flora Hellenica* (Kit Tan, 2002), a detailed distribution map of *C. barbaraeoides* (as *C. amara* subsp. *barbaraeoides*) is presented, however, without providing any list of localities. Inferring from our herbarium and field studies, we assume that most records from the Northern Pindos in fact refer to *C. acris*. The only exception is Mt Zygos, where *C. barbaraeoides* and *C. acris* co-occur. Strid (1986) did not mention *C. acris* from the Northern Pindos, and Kit Tan (2002) gave only one locality of this species in this area.

(2) All specimens cited from the northern part of the Southern Pindos (the Lakmos Mts) by Halácsy (1894, 1901), Schulz (1903) and Strid (1986) belong to *C. barbaraeoides* (Appendix 1, Table 1). Kit Tan (2002) reported *C. barbaraeoides* also from the southern part of the Southern Pindos, but at least one locality, namely that on Mt Karava, is most likely based on misidentification with *C. matthioli* (Kit Tan 2003; in litt.).

(3) Kit Tan (2002) and Dimopoulos *et al.* (2013, 2017+) reported *C. barbaraeoides* also from the region of North Central (Greece). We have seen only one specimen identified as *C. barbaraoides* from this region (close to Pisoderi, *Lippert 18409* M), which undoubtedly belongs to *C. acris*. Based on our herbarium and field studies in 2001, only *C. acris* occurs in this region (see Table 1, Fig. 1, and Perný *et al.* 2004).

(4) Strid (1986) was the first who reported *C. barbaraeoides* from Sterea Ellas (Mt Timfristos, central Greece). We have not seen any specimen from this locality, but during our field excursion in 2001 we only found *C. acris* there. There are two localities from Sterea Ellas on the map presented by Kit Tan (2002). The first of them is on Mt Timfristos, the second one is on Mt Iti. From Mt Iti we have seen only specimens of *C. acris* (*Hagemann et al. 208* B 100127684, C), which were originally determined as *C. barbaraeoides* but later revised as *C. raphanifolia* subsp. *acris*. It should be noted that Strid (1986), Kit Tan (2002) as well as Dimopoulos *et al.* (2013, 2017+) reported along with *C. barbaraeoides* also *C. acris* [Strid (1986) as *C. raphanifolia* subsp. *acris* (Griseb.) O.E.Schulz] from the regions of Sterea Ellas and North Central (Greece).

Taxonomy

Identification key to *Cardamine barbaraeoides*, *C. raphanifolia*, *C. acris* and all Balkan representatives of the *C. amara* and *C. pratensis* groups.

1	Leaves forming a basal rosette	2 (*C. acris*, *C. pratensis* group)
-	Leaves not forming a basal rosette	8
2	Anthers purplish before dehiscence	*C. rivularis* (*C. pratensis* group)
-	Anthers yellow before dehiscence	3
3	Terminal leaflet of stem leaves much larger than lateral ones, middle stem leaves pinnate, rarely pinnatisect, with 1–5(–6) pairs of lateral leaflets or segments	4. (*C. acris*)
-	Terminal leaflet of stem leaves approximately of the same size as lateral ones, middle stem leaves pinnatisect, with (2–)4–10(–14) pairs of lateral segments	7
4	Petals white	5
-	Petals pale pink to reddish-violet, exceptionally white	6
5	Number of leaflets of the rosette leaves 5–9, number of leaflets of the middle stem leaf 3–7, length of stem between the base and uppermost leaf 26–55 cm; endemic to the Northern Pindos Mts in northwestern Greece	*C. acris* subsp. *pindicola*
-	Number of leaflets of the rosette leaves 7–11, number of leaflets of the middle stem leaf 5–9, length of stem between the base and uppermost leaf 16–38 cm; endemic to central Greece	*C. acris* subsp. *vardousiae*
6	Stem unbranched or with up to 2 branches, length of the stem between the base and the uppermost leaf 16–38 cm, maximum width of the stem 1.3–4.0 mm, petals 8.1–12.2 mm long; endemic to central Greece	*C. acris* subsp. *vardousiae*
-	Stem with 1–11 branches, rarely unbranched, length of the stem between the base and uppermost leaf 29–84 cm, maximum width of the stem 2.0–7.8 mm, petals 7.2–10.7 mm long; widespread in the Balkan Peninsula, absent in central Greece and in the Northern Pindos Mts in northwestern Greece	*C. acris* subsp. *acris*
7	Rhizome without globular tubers, lower segments of middle stem leaves somewhat deflexed	

..... *C. matthioli* (*C. pratensis* group)
- Rhizome usually with several globular tubers, lower segments or leaflets of middle stem leaves slightly ascending..... ..... *C. penzesii* (*C. pratensis* group)
8 Petals pink to reddish-violet, stigma conspicuous, much wider than style, anthers yellow..... *C. raphanifolia*
- Petals white, stigma indistinct, as wide as style, anthers violet (only in *C. barbaraeoides* rarely yellow) 9
9 Middle stem leaves with 1–2 pairs of lateral leaflets, stem glabrous..... *C. barbaraeoides*
- Middle stem leaves with (2–)3–5(–7) pairs of lateral leaflets, stem glabrous or sparsely to densely hairy.....10 (*C. amara*)
10 Stem glabrous or sparsely hairy, with (2–)3–14(–23) leaves ..... *C. amara* subsp. *amara*
- Stem densely hairy, with (15–)17–34(–37) leaves ..... *C. amara* subsp. *balcanica*

Taxonomic account

Cardamine barbaraeoides Halácsy (1894: 228–229).

Ind. loc.: 'An einem Giessbache der oberen Region des Peristeri oberhalb des Dorfes Syraku [Syrrako]. Höhe 1500 m.'

Type:—[Greece] Dr. E. von Halácsy, Iter graecum secundum a. 1893, Epirus boreali-orientalis, Gregarie ad rivulos regionis montis Peristeri supra pagum Syraku [Syrrako], Alt. 1500, 14. 6. 1893, *E. v. Halácsy s.n.* [Lectotype, designated here WU-Hal 0075941! (https://wu.jacq.org/WU0075941)], Isolectotypes: B!, BP!, C!, LD 1045685! PRC!, WU-Hal 0075939! (https://wu.jacq.org/WU0075939), WU-Hal 0075939! (https://wu.jacq.org/WU0075940).

Note:—There are three specimens of the original collection of *C. barbaraeoides* in Halácsy's herbarium at WU. In addition, further duplicates were distributed to many other herbaria. Therefore, no holotype of this name is available and a lectotype had to be selected. Although Strid (1986) cited original material of this name from the herbaria LD and WU-Hal (as 'Type'), he did not choose a lectotype. Kit Tan (2002) indicated the specimen from the herbarium WU-Hal as a 'type', but she failed to comply with the Arts. 7.11, 9.23 of the ICN in designating a lectotype (Turland *et al.* 2018).

≡ *Cardamine amara* subsp. *barbaraeoides* (Halácsy) Maire & Petitmengin (1908: 26).

≡ *Cardamine raphanifolia* subsp. *barbaraeoides* (Halácsy) Strid (1986: 257).

Description:—Perennial herb, (15–)20–51(–56) cm tall (up to the lowest flower/fruit peduncle); rhizome prostrate or ascending; stem ascending, simple or rarely branched above, with up to 6(–12) branches, glabrous, up to (1.0–)1.8–5.0(–7.0) mm wide at base. Leaves not forming a basal rosette. Basal leaves glabrous, pinnate, with 1–2 pairs of lateral leaflets, but shrivelled at anthesis; their terminal leaflet much larger than the lateral ones, orbicular to broadly circular; lateral leaflets sessile, ovate or elliptic to orbicular. Stem leaves 9–21(–23), equally distributed on the stem, with a weak tendency to be congested under the inflorescence (0–3 leaves reaching the base of the uppermost stem leaf), pinnate, similar to basal leaves, glabrous; middle stem leaves (2.1–)2.3–9.0(–9.7) cm long, with 1–2 pairs of lateral leaflets; terminal leaflet rhombic, broadly elliptic to almost circular, 1.5–4.3(–5.0) cm long, (0.9–)1.1–3.3(–4.1) cm wide, much larger than lateral ones; lateral leaflets elliptic, first lateral leaflets (0.5–)0.6–2.5(–2.9) cm long, (0.2–)0.3–1.5(–1.6) cm wide. Inflorescence racemose, peduncles glabrous. Sepals ovate with membranous margins, (3.8–)3.9–5.0(–5.2) mm long. Petals white, narrowly obovate, (8.0–)8.6–11.4(–12.2) mm long, 3.0–4.6(–4.7) mm wide, with short claw, apex truncate to emarginate, glabrous. Stamens 6, tetradynamous, shorter filaments (2.3–)2.4–5.8(–6.2) mm long, longer filaments (3.5–)4.4–7.4(–7.7) mm long; anthers violet, or rarely yellow. Stigma indistinct, as wide as the style. $2n = 4x = 32$.

Distribution and habitat:—Greece. Lakmos Mts (northern part of the Southern Pindos), Mt Zygos (southern part of the Northern Pindos). Streams and stream banks, wet rock faces, springs on wet meadows and pastures. 1,000–1,800 m a.s.l.

Illustration: Fig. 6

Data availability

The data matrix of measured morphological characters generated for this study can be found in an online repository at https://data.mendeley.com/datasets/6yy8yjyrpy/1 (DOI: 10.17632/6yy8yjyrpy.1).

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Appendix 1

List of other specimens of ***Cardamine barbaraeoides*** studied
Greece. Southern Pindos: [Lakmos Mts], Mt Peristeri, supra pagum Syraku [Syrrako], 1,500 m, 14 Jun 1893, *E. de Halácsy, Iter graecum secundum a. 1893* (lectotypus: WU-Hal, isolectotypes: B 100000944, B 100000946, BP, K, PR, WU-Hal); Sirako [Syrrako], NW edge of the village, 39.598˚ N, 21.105˚ E, 8 Jul 2000, M. *Perný & al.* (SAV) (locus classicus; see also Šlenker *et al.* 2021a, collection code C008, 2n = 4x = 32; Lihová *et al.* 2006: 765, locality BR-2); E of Syrrako, tourist path from Syrrako to Kalarites, 39.595˚ N, 21.112˚ E, 1,018 m, 21 Jun 2019, *M. Šlenker* (SAV) (Šlenker *et al.* 2021a, collection code C009, 2n ≈ 4x); E of Syrrako, between Syrrako and Kalarites, Chrousias river, 39.597˚ N, 21.113˚ E, 950 m, 21 Jun 2019, *M. Šlenker* (SAV) (Šlenker *et al.* 2021a, collection code C010, 2n = 4x = 32); NE of Matsouki, 39.568˚ N, 21.169˚ E, 1,058 m, 21 Jun 2019, *M. Šlenker* (SAV) (Šlenker *et al.* 2021a, collection code C011, 2n ≈ 4x); Chaliki, Turnara, 26 Jun 1896, *P. Sintenis, Iter thessalicum 1896, No. 793* (WU-Hal); Chaliky, Mt Salatura, 26 Jun 1896, *P. Sintenis, Iter thessalicum 1896, No. 793* (JE). **Northern Pindos:** Zygos, 27 Jun 1896, *P. Sintenis, Iter thessalicum 1896, No. 792* [B 100000945, BP, JE (see Discussion)].
Note: The symbol '≈' indicates DNA ploidy levels estimated by flow cytometry.

Appendix 2

List of the examined specimens of ***C. acris*** misidentified as ***C. barbaraeoides***
Greece. Northern Pindos: Mt Grammos, ravines SW of Souflikas, 5 km N of the village Aetomilitsa, 17 Jul 1977, *P. Hartvig, P. Kjaer & B. Christensen 6983* (C); Mt Smolika[s], supra Kerasovo, 19 Jul 1896, *Baldacci, 1896, Iter Albanicum (Epiroticum) Quartum no. 208* (BM000758495, K, PR, WU-Hal); Mt Smolikas, 1,800 m, 9 Jul 1958, *K. H. Rechinger, Iter balcanico-mediterraenum 1958 (Iter graecum IX.), No. 21076* (C, K, W); supra Kerasovo, ad fontem "Thisino", 9 Jul 1958. *K. H. Rechinger, Iter balcanico-mediterraenum 1958 (Iter graecum IX.), No. 20823* (B 10000947, W); Mt Smolikas, 10 km W of Samarina, 5 km S of the village Paraskevi, 15 Jun 1976, *P. Hartvig, C. Baden & S. G. Christiansen 5890* (C); Valley between Mt Gomara and Mt Vasilitsa, ca 7 km SW of Samarina, 24 Jun 1976, *P. Hartvig, C. Baden & S. G. Christiansen 5275* (C); in jugo Katara supra Metsovo, 29 Jul 1956, *K. H. Rechinger, Iter Graecum VIII., 1956, No. 18399* (K, W); Katara, 4 km NE of Metsovo, 3 Jul 1971, *B. Aldén 1436* (C); E of Katara Pass, c. 4 km E of the summit, 19 May 1985, *Strid 24579* (C); in summo montis Zygos supra Metsovo, Jul 1885, *C. Haussknecht, Iter Graecum 1885* (JE). **North Central (Greece):** Florina, Aufstieg zur Kiwaniza (östlicher Vorgipfel des Kalo Nero = Bela Voda) vom Kataphygion des Alpine Club Florina bei Pisoderi, 1,800–2,130 m, 8 Jul 1982 *W. Lippert 18409* (M). **Sterea Ellas:** Nom. Phthiotidos, Mt Iti, Aufstieg von Neochorion zum Berg Petrotos, 1,700 m, 16 Jun 1982 *I. Hagemann, H. Scholz & W. Schwarz 208* (B 100127684, C).