Long neglected diversity in the Accursed Mountains of northern Albania: *Cerastium hekuravense* is genetically and morphologically divergent from *C. dinaricum*

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Abstract The Balkan Peninsula is a hotspot of European biotic diversity. One of its biogeographically most peculiar but poorly explored regions are the Albanian Alps (Alpet Shqiptare/Prokletije/Accursed Mountains) on the border between Albania, Kosovo and Montenegro, characterised by a high number of endemic species. A poorly known taxon from the Albanian Alps is *Cerastium hekuravense*, which was described from Mt. Maja Hekurave (Albania) in 1921, but later usually merged with *C. dinaricum*, a widespread endemic of the Dinaric Mountains, or connected with the arctic–alpine *C. alpinum*. Here, we used amplified fragment length polymorphisms to explore the phylogenetic position of *C. hekuravense* and particularly its relationship to *C. dinaricum*. Our data show that both species are genetically well differentiated, but their relation to other taxa remains unclear—they are either closely related to Alpine species of *C. ser. Latifolia* or to species co-occurring on the Balkan Peninsula, such as *C. banaticum* and *C. decalvans*. In addition, multivariate morphometric analyses show that *C. dinaricum* and *C. hekuravense* are morphologically well differentiated. Also their relative genome sizes, estimated

using flow cytometry, differ. We propose a taxonomic treatment with lectotype designation for both taxa and provide descriptions and an identification key. Last but not least, these cold-adapted species mostly growing on northerly exposed humid screes are highly threatened due to the global warming and should be ranked endangered according to IUCN criteria. *Cerastium hekuravense* known only from three localities is likely one of the most endangered mountain plant species of the Balkan Peninsula.

Keywords AFLP · Albanian Alps · Balkan Peninsula · Endemism · Genome size · IUCN · Taxonomy

Introduction

The Balkan Peninsula is a centre of plant species diversity and endemism; it is the floristically richest area in Europe harbouring about 6500 species, of which more than one-third (c. 2600–2700) are endemic and about 400 are considered to be local endemics (Horvat et al. 1974; Kryštufek and Reed 2004; Stevanović et al. 2007). These numbers are likely an underestimate, as only for Greece, including Crete and the islands, 5752 species, of which 1278 are endemic to this country, have been reported (Dimopoulos et al. 2013). In the western Balkan Peninsula, especially the high mountain system of the Dinarides (Dinaric Mountains) is highly diverse (Horvat et al. 1974; Redžić 2011). It spans from Slovenia in the North to northern Albania in the South, where the highest peak, Maja Jezercë (2694 m), is situated. This southernmost part of the Dinarides, which is known as Albanian Alps (Alpet Shqiptare/Bjeshkët e Namuna in Albanian) or Accursed Mountains (Prokletije in the southern Slavic languages), includes ca. 40 peaks over 2000 m and 17 peaks over 2500 m (Rakaj 2009).

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Roughly 20 plant species are endemic to the Albanian Alps in its broader definition, i.e. including Komovi and Visitor (Rakaj 2009 and references below). Several of them bear the specific epithet “bertisceus” (derived from the Ptolemean “Mons Bertiscus” for the Albanian Alps), pointing to their origin, e.g. *Crepis bertisceae* Jáv., *Draba bertisceae* D.Lakušić & Stevan., *Onobrychis bertisceae* Širj. & Rech.f. and *Valeriana bertisceae* Pančić. In the last decade, two new species endemic to this mountain range have been described, i.e. *Androsace komovensensis* Schönsw. & Schneew. (Schönswetter and Schneeweiss 2009; Frajman et al. 2014) and *Heliosperma oliverae* Niketić & Stevan. (Niketić and Stevanović 2007). In addition, several endemics, including *Edraianthus pilosulus* (Beck) Surina & Lakušić (Surina et al. 2009), *Heliosperma macranthum* Pančić (Frajman and Oxelman 2007; Frajman et al. 2009; see also Frajman et al. 2014) and *Wulfenia baldaccii* Degen (Surina et al. 2014) have been analysed phylogenetically, confirming their independent status. In contrast, *Campanula latifolioides* F.K.Mey., recently described by Meyer (2011), is likely a synonym of *Asyneuma pichleri* (Vis.) Lakušić & Conti (Pils 2016).

One of the poorly known taxa from the Albanian Alps is *Cerastium hekuravense* Jáv., which was described by Jávorka (1921) based on a specimen collected on Mt. Maja Hekurave in Albania. Despite the fact that Jávorka compared this species with representatives of *C. ser. Latifolia* Borza (*C. carinthiacum* Vest, *C. subtriflorum* Dalla Torre & Sarnth., *C. latifolium* L.), it was later commonly included in *C. alpinum* L. (Jalas 1964; Jalas and Suominen 1983; Jalas 1993; Euro + Med 2006). However, Merxmüller and Strid (1977) suggested that the species does not belong to the *C. alpinum* group and Niketić (1999) proposed that the glandular indumentum of the leaves is the only difference from *C. dinaricum* Beck & Szyszyl., an endemic species of the Dinaric Mountains included in *C. ser. Latifolia*. Moreover, the *C. alpinum* group only includes high polyploid species, from octo- to dodecaploids, whereas the species of *C. ser. Latifolia*, including *C. dinaricum*, are tetraploids (Brysting et al. 2011; Niketić et al. 2013). Niketić (1999) treated *C. hekuravense* as *C. dinaricum* var. *hekuravense* (Jáv.) Niketić and later (Niketić 2007) as *C. dinaricum* f. *hekuravense* (Jáv.) Niketić, which he suggested to occur scattered throughout the distribution range of *C. dinaricum*. However, a detailed study of *C. dinaricum* showed no phylogenetic differentiation between glandular and glabrous plants (Kutnjak et al. 2014). Yet, Kutnjak et al. (2014) did neither include the population from the *locus classicus* of *C. hekuravense*, Maja Hekurave, nor the only known Albanian population of *C. dinaricum* from Maja Kakisë east of Abat (Hayek 1924). For the sake of simplicity, we refer to the two focal taxa on the species level and their entirety is denoted as *C. dinaricum* s.l.

Here, we used amplified fragment length polymorphisms (AFLPs), relative genome size (RGS) measurements and morphometric analyses to elucidate the relationship between *C. hekuravense* from the Albanian Alps and the more widespread *C. dinaricum*. Specifically, we address the following questions: (1) Is *C. hekuravense* from the *locus classicus* (type locality) genetically differentiated from *C. dinaricum*? (2) If so, do the other known and recently discovered populations from the Albanian Alps cluster with *C. dinaricum* or with *C. hekuravense*? (3) Do eventually detected genetic groups differ in RGS and are they also morphologically differentiated? (4) Based on our results, we provide a taxonomic treatment of both taxa and evaluate their conservation status following the IUCN criteria.

Materials and methods

Plant material

Molecular analyses are based on silica gel-dried leaf material. We included four populations of *Cerastium dinaricum* previously analysed by Kutnjak et al. (2014), including the population from the type locality, and additionally sampled four populations from Albania tentatively ascribed to *C. dinaricum* s.l., including the population from the type locality of *C. hekuravense* (Table 1, Fig. 1). Additionally, we included all other species (Online Resource 1) classified within the *C. latifolium* aggregate (Niketić 2007)—*C. carinthiacum* (two populations), *C. latifolium* (two populations) and *C. uniflorum* Clairv. (one population), as well as the species distributed on the Balkan Peninsula, which include tetraploid populations (Niketić et al. 2013), i.e. *C. banaticum* (Rochel) Steud. (six populations), *C. decalvans* Schloss. & Vuk. (three populations), *C. eriophorum* Kit. (four populations) and *C. grandiflorum* Waldst. & Kit. (two populations), to infer the phylogenetic position of *C. dinaricum* and *C. hekuravense*.

AFLP analyses

Extraction of total genomic DNA was performed following the modified CTAB-protocol of Tel-Zur et al. (1999). AFLP fingerprinting was conducted as described by Kutnjak et al. (2014). Two blanks (DNA replaced by water) were included to test for contamination, and four samples were used as replicates between the two PCR batches to test the reproducibility. Numbers of sampled individuals per population are provided in Table 1.

Electropherograms were analysed with Peak Scanner version 1.0 (Applied Biosystems) using default peak detection parameters except employing light peak smoothing. The minimum fluorescent threshold was set to 100

Table 1 Studied populations of *Cerastium dinaricum* and *C. hekuravense*

ID	Lab ID	Taxon	Sampling locality	Altitude (m a.s.l.)	Longitude (E)/Latitude (N)	Collectors (collection number)	Voucher	N _{AFLP}	RGS
1	C101	<i>C. dinaricum</i>	HR: Velebit, Mt. Vaganski vrh	1690	15.50419/44.365471	SB and IR	ZA-H-010-Cdin	3	0.306 ($\pm$ 0.002)
2	C018	<i>C. dinaricum</i>	BH: Prenj, Vjetrana brda ridge	1864	17.881667/43.54861	PS, BF and DK	IB	4	0.320 ($\pm$ 0.005)
3	C020	<i>C. dinaricum</i>	ME: Durmitor, valley of Škrčko jezero	1883	19.03/43.12833	PS, BF and DK	IB	5	0.323 ($\pm$ 0.004)
4	C022	<i>C. dinaricum</i>	ME: Komovi, summit of Mt. Kom Kučki	2394	19.6425/42.678611	PS, BF and DK	IB	3	0.323 ($\pm$ 0.003)
5	C466	<i>C. dinaricum</i>	AL: Alpet Shqiptare/Prokletije, summit of Maja Jezercë	2638	19.811/42.443056	PS, BF and MF	IB	5	0.318 ($\pm$ 0.002)
6	C499	<i>C. hekuravense</i>	AL: Alpet Shqiptare/Prokletije, Buni Jezercë	1850	19.81167/42.45944	DC and DS	IB	7	0.371 ($\pm$ 0.004)
7	C024	<i>C. hekuravense</i>	AL: Alpet Shqiptare/Prokletije, Maja Hekurave	2019	19.94694/42.3944	PS, BF and DK	IB	4	0.374 ($\pm$ 0.003)
8	C477	<i>C. hekuravense</i>	AL: Alpet Shqiptare/Prokletije, Maja Kakisë	1935	19.82388/42.35944	PS and BF	IB	5	0.361 ($\pm$ 0.003)

ID population identifier used throughout the paper. AL Albania, BH Bosnia and Hercegovina, HR Croatia, ME Montenegro. N_{AFLP} number of individuals investigated with amplified fragment length polymorphism. RGS relative genome size (mean $\pm$ standard deviation). Collectors: BF B. Frajman, DC D. Caković, DK D. Kutnjak, DS D. Stešević, IR I. Rešetnik, MF M. Falch, PS P. Schönswetter, SB S. Bogdanović

Fig. 1 Sampled populations of *Cerastium dinaricum* and *C. hekuravense*. Population numbers correspond to Table 1. Smaller symbols indicate non-sampled localities of *C. dinaricum*, where the species certainly occurs (see Kutnjak et al. 2014). The inset in the upper right

corner shows the position of the sampling area in Europe; the rectangle in the main part of the figure indicates the position of the Albanian Alps magnified in the inset in the lower left corner

relative fluorescence units. Automated binning and scoring of the AFLP fragments were performed using RawGeno 2.0-1 (Arrigo et al. 2009) for R 2.15.2 (R Development Core Team 2012) with the following settings: scoring range = 150–500 bp, minimum intensity = 100 relative fluorescence units (rfu), minimum bin width = 1 bp and maximum bin width = 1.5 bp. Fragments with a reproducibility lower than 85% based on sample–replicate comparisons were eliminated. Fragments present/absent in only one individual were excluded.

A Neighbour-joining (NJ) analysis based on Nei–Li genetic distances (Nei and Li 1979) was conducted and bootstrapped (2000 pseudo-replicates) with TREECON v.1.3b (van de Peer and De Wachter 1997). The tree was rooted with *C. grandiflorum* based on an ITS phylogeny (Frajman B., unpublished). Due to the simple structure in the data, no further analyses were conducted.

Genome size measurements

Flow cytometry (FCM) of 40,6-diamidino-2-phenylindole (DAPI)-stained nuclei was used to estimate relative genome size (RGS) of four newly sampled populations (all populations from Albania) as described by Kutnjak et al. (2014). The RGS was estimated for three to ten individuals per population.

Absolute genome size (AGS) was determined using FCM of propidium iodide (PI)-stained nuclei of two samples of *C. dinaricum* and one sample of *C. hekuravense* (Table 1) as described by Frajman et al. (2015), with the exception that *Pisum sativum* cv. Kleine Rheinländerin (2C = 8.84 pg; Greilhuber and Ebert 1994) was used as reference standard.

Morphometric analyses

Material for morphometric analyses included vouchers of all molecularly investigated populations of *C. dinaricum* s.l. (Table 1), supplemented with herbarium vouchers stored in the herbarium of the University of Innsbruck, IB (Frajman and Schönswetter 14631, 14632, 14633), totalling 54 individuals. Forty characters were measured or counted and 15 ratios were calculated (Table 2). Leaf characters were measured on the uppermost and one well-developed mid-stem leaf; the apex angle was measured only on the mid-stem leaf. Trichome characters were measured on the upper surface and the margin of a mid-stem leaf, as well as on the internode below the investigated leaf. Certain characters were missing in a few individuals, e.g. petals or fully developed fruits, and were thus replaced with mean values calculated for the other studied populations of the same species. Petal, sepal, bract, leaf and fruit characters were measured on images taken with a camera mounted on a Zeiss SteREO Discovery. V12 stereo

microscope at 8 × magnification. Characters of trichomes were measured on magnified images taken with an Olympus UC 30 wide zoom camera mounted on an Olympus SZX9 stereo microscope with 20 × magnification.

We tested correlation among metric characters employing Pearson or Spearman correlation coefficients dependent on character distribution. After standardization to zero mean and one unit variance, principal component analysis (PCA) was performed. As Tukey HSD Post hoc test showed no discriminatory power (*p* values between 0.33 and 0.98) for twelve characters (1, 2, 11, 12, 18, 19, 24, 30, 48, 49, 53 and 55) and three ratios (33, 36 and 39) we excluded them from the canonical discriminant analysis (CDA), which was applied to inspect the separation between *C. dinaricum* and *C. hekuravense* and the relative importance of characters as discriminators between them. Statistical analyses were performed using the package Statistica 5.1 (StatSoft 1996). Values presented in the species descriptions and in the identification key correspond to the 10 and 90% quantiles, supplemented by extreme values in parentheses.

Results

AFLP analyses

We scored 335 AFLP fragments for 96 individuals; 42 bands found in only one individual were excluded. For the 36 individuals of *Cerastium dinaricum* s.l. we scored 196 fragments, of which 43 found in only one individual were excluded from further analyses.

The neighbour-joining tree of AFLP profiles (Fig. 2) resulted in a strongly supported cluster (bootstrap support, BS 100) containing all species except *C. grandiflorum*, which was used for rooting. Within this cluster, three moderately to well-supported groups were resolved; the relationships among them were unresolved. One group included all accessions of *C. dinaricum* s.l. with strong support (BS 87), falling in two strongly supported clusters (both with BS 100). One of them contained accessions of *C. dinaricum* studied by Kutnjak et al. (2014) including the population from the *locus classicus*, as well as one newly sampled population from the summit of Maja Jezercë in Albania. The other cluster contained the population from Maja Hekurave, *locus classicus* of *C. hekuravense*, as well as the populations from Buni Jezercë and Maja Kakisë. The two remaining major groups contained species of *C. ser. Latifolia* from the Alps (*C. carinthiacum*, *C. latifolium*, *C. uniflorum*; BS 91) and Balkan accessions (BS 67) of species belonging to *C. ser. Alpina* (*C. decalvans* and *C. eriophorum*) and to *C. ser. Cerastium* (*C. banaticum*).

Table 2 Morphological characters studied

Char. No	Character	Abbreviations
1	Petal length, mm	PL
2	Petal width, mm	PW
3	Ratio of petal length and petal width	PL/PW
4	Distance from petal basis to incision, mm	LPBI
5	Ratio of distance from petal basis to incision and petal length	LPBI/PL
6	Sepal length, mm	CLL
7	Sepal width, mm	CLW
8	Ratio of sepal length and width	CLL/CLW
9	Distance from basis to widest part of sepal, mm	CLLMW
10	Ratio of distance from basis to widest part of sepal and sepal length	CLLMW/CLL
11	Width of hyaline margin of sepal, mm	CLHM
12	Bract length, mm	BL
13	Bract width, mm	BW
14	Ratio of bract length and width	BL/BW
15	Distance from basis to widest part of bract, mm	BLLMW
16	Ratio of distance from basis to widest part of bract and bract length	BLLMW/BLL
17	Width of the hyaline margin of bracts, mm	BHM
18	Length of uppermost leaves, mm	ULL
19	Width of uppermost leaves, mm	ULW
20	Ratio of length and width of uppermost leaves	ULL/ULW
21	Distance from basis to widest part of uppermost leaves, mm	ULLMW
22	Ratio of distance from basis to widest part of uppermost leaves and their length	ULLMW/ULL
23	Length of mid-stem leaves, mm	MLL
24	Width of mid-stem leaves, mm	MLW
25	Ratio of length and width of mid-stem leaves	MLL/MLW
26	Distance from basis to widest part of mid-stem leaves, mm	MLLMW
27	Ratio of distance from basis to widest part of mid-stem leaves and their length	MLLMW/MLL
28	Angle of the apex of mid-stem leaves, degree	ASL
29	Capsule length, mm	CL
30	Capsule width, mm	CW
31	Ratio of capsule length and width	CL/CW
32	Distance from basis to widest part of capsule, mm	CLMW
33	Ratio of distance from basis to widest part of the capsule and capsule length	CLMW/CL
34	Capsule teeth length, mm	CTL
35	Capsule teeth width, mm	CTW
36	Ratio of length and width of capsule teeth	CTL/CTW
37	Seed length, mm	SL
38	Seed width, mm	SW
39	Ratio of seed length and width	SL/SW
40	Stem length, mm	SH
41	Number of internodes	IN
42	Ratio of stem length and number of internodes	SH/IN
43	Length of internode adjacent to mid-stem leaf pair, mm	IL
44	Number of flowers per stem	FN
45	Length of peduncle of terminal flower, mm	PTFL
46	Length of inflorescence (from the terminal flower to the top), mm	IFL
47	Number of internodes in the longest inflorescence branch	IFLIN
48	Number of glandular hairs per mm ² on the upper epidermis of mid-stem leaves, calculated as the average of two squares with 1 mm ² each	GHLS

Table 2 (continued)

Char. No	Character	Abbreviations
49	Number of eglandular hairs per mm ² on the upper epidermis of mid-stem leaves, calculated as the average of two squares with 1 mm ² each	EHLS
50	Number of glandular hairs on the margin of mid-stem leaves along 1 mm just below the tip of the leaf	GHLM
51	Number of eglandular hairs on the margin of mid-stem leaves along 1 mm just below the tip of the leaf	EHLM
52	Length of the longest trichome on the margin of mid-stem leaves along 1 mm just below the tip of the leaf	TLM
53	Number of glandular hairs on the stem along 1 mm just below a mid-stem leaf pair	GHS
54	Number of eglandular hairs on the stem along 1 mm just below a mid-stem leaf pair	EHS
55	Length of the longest trichome on the stem along 1 mm just below a mid-stem leaf pair	TLS

Genome size of *Cerastium dinaricum* and *C. hekuravense*

Average RGS of *C. hekuravense* ranged from 0.361 in population 8 to 0.374 in population 7 and was distinctly different from the mean RGS of *C. dinaricum*, which ranged from 0.301 to 0.327 (Kutnjak et al. 2014); the average RGS of the population 5 from Maja Jezercë was 0.318 and thus within this range (Table 1, Fig. 3). The AGS of *C. dinaricum* ranged from 2.5674 pg (population 4 from the *locus classicus*) to 2.5713 pg (population 2), whereas the AGS of *C. hekuravense* (population 7 from the *locus classicus*) was 3.0311 pg.

Morphological differentiation between *Cerastium dinaricum* and *C. hekuravense*

The measured and counted values for the characters, as well as the calculated ratios, are presented in Online Resource 2. As the correlation coefficients did not exceed 0.9 for any character pair, we included all characters listed in Table 2 in further analyses.

The PCA (first three axes explaining 15.78, 10.98 and 8.06% of the total variation; Fig. 4a) showed a clear separation between *C. dinaricum* and *C. hekuravense* along the first two axes. The characters contributing most to the separation along the first axis, i.e. those having highest component scores in the coefficient matrix, describe habit (SH, SH/IN, IL), shape of the sepals (CLL/CLW), as well as shape of the middle leaves and bracts (MLL/MLW, BL/BW). Leaf, bract and trichome characters (ULLMW, MLLMW, MLLMW/MLL, BLMW, BLMW/BL, GHLM, EHLM) contributed most to the separation along the second axis. The CDA (Fig. 4b) showed a clear differentiation between the two species with the strongest contribution of the sepal characters (CLL/CLW, CLLMW, CLW, CLLMW/CL). Still, even for characters with high discriminatory power (Tukey HSD post hoc test, $P < 0.001$) boxplots (Fig. 5) show overlap in character states between both taxa.

Discussion

Cerastium hekuravense is a distinct species, which is genetically and morphologically clearly differentiated from *C. dinaricum* (Figs. 2, 4, 6, 7). The two species also have different genome sizes (Fig. 3) and even where they occur in relatively close vicinity, such as on the summit of Maja Jezercë (*C. dinaricum*) and close to Buni Jezercë (*C. hekuravense*) no traces of gene flow have been detected in the AFLP data. Despite the fact that Niketić (1999, 2007) treated *C. hekuravense* as a variety or even as a form of *C. dinaricum*, which he suggested to be scattered throughout the distribution range of the latter, our data clearly show that *C. hekuravense* is endemic to the Albanian Alps, thus additionally underlining the importance of this mountain range as an endemic-rich area (Rakaj 2009; see also Introduction).

In spite of the peculiarity of its rich flora, Albania is one of the botanically least explored regions of Europe (Markgraf 1932; Frajman et al. 2014). After the last editions of the national flora (Paparisto et al. 1988; Qosja et al. 1992, 1996; Vangjeli et al. 2000) including 3758 taxa (3250 species) of vascular plants, a multitude of species new for Albania have been published (e.g. Barina and Pifkó 2008; Rakaj 2009; Ball 2011; Meyer 2011; Barina et al. 2013; Frajman et al. 2014). Even if *C. hekuravense* was described from the territory of Albania (Jávorka 1921), it was neglected in all recent Albanian floras, where only *C. dinaricum* was listed (Demiri 1983; Paparisto et al. 1988; Vangjeli 2003). In addition to clarifying the status of the known populations from Maja Hekurave and Maja Kakisë (the latter was published as *C. dinaricum* by Hayek 1924), we discovered an additional population of *C. hekuravense* close to Buni Jezercë (an indication of its occurrence there was kindly provided by M. Niketić; 10.10.2013, personal comm. with B. Frajman) as well as the only known population of *C. dinaricum* in Albania, on the western summit crest of Maja Jezercë.

Spatially explicit modelling of viable habitat for *C. dinaricum* suggested a decrease of about 37% by the year 2050 and 70% by the year 2080 due to global warming, and the

Fig. 2 Neighbour-joining tree derived from AFLP data. Population identifiers correspond to Table 1, Fig. 1 and Online Resource 1

Fig. 3 Relative genome size of *Cerastium dinaricum* and *C. hekuravense* as in Table 1. Population numbers correspond to Table 1 and Fig. 1. Circles represent mean values and whiskers the standard deviation

predicted habitat loss could result in range-wide extinction of the species in the very near future (Kutnjak et al. 2014). Thus, following the criterion B2 of the IUCN (2012) for endangered species, the following applies for *C. dinaricum*: (a) area of occupancy is estimated to be less than 500 km², (b) area of occupancy is severely fragmented and (c) continuing decline in the area, extent and/or quality of habitat has been inferred/projected. Therefore, we deem *C. dinaricum* endangered (EN) according to IUCN (2012).

The situation is similar, but likely more severe for *C. hekuravense*, which has a much narrower distribution and is currently known from only three localities, where it inhabits northerly exposed humid screes with extended snow cover. The ecology of *C. hekuravense* is thus similar to that of *C. dinaricum*, which, however, thrives also in rock crevices in the summit areas of some mountains (e.g. Kom Kučki in Montenegro and Maja Jezercë in Albania) and thus has a broader ecological niche. Field observations further suggest that *C. hekuravense* prefers more humid and colder screes than *C. dinaricum* (P. Schönschwetter and B. Frajman, personal observations). In all three localities the species was rare, the smallest population being that on Maja Kakisë, where only a few dozen individuals were found. Although we do not have climatic niche modelling data for *C. hekuravense* at hand, extrapolation of the results obtained for *C. dinaricum* suggests that the species should be treated at least as endangered (EN).

The AFLP data are inconclusive regarding the relationships of *C. dinaricum* and *C. hekuravense* with other tetraploid *Cerastium* species from the Alps and the Balkans (Niketić et al. 2013). It is clear that they do not belong to the *C. latifolium* aggregate, in which they were included in the past (Jávorka 1921; Niketić 2007). The *C. latifolium* aggregate thus likely includes only the Alpine broad-leaved species *C. carinthiacum*, *C. latifolium* and *C. uniflorum*, which form a separate lineage closely related to *C. dinaricum* and *C. hekuravense*. Another group of species closely related to our study taxa are *C. banaticum*, *C. decalvans* and *C. eriophorum*. They are mostly

Fig. 4 Morphological differentiation between *Cerastium dinaricum* (black) and *C. hekuravense* (grey). **a**, principal component analysis (PCA) based on 55 morphological characters, **b** histogram of canonical discriminant analysis (CDA) based on 40 morphological characters

Fig. 5 Boxplot diagrams of morphological characters discriminating between *Cerastium dinaricum* and *C. hekuravense*. Boxes define 25 and 75 percentiles; squares indicate medians; whiskers span the 5–95 percentiles and circles indicate outliers. Characters are explained in Table 2

distributed on the Balkan Peninsula and are all characterised by a more or less persistent indumentum. Even if they were classified in two different series by Niketić (2007)—*C. banaticum* in *C. ser. Cerastium*, *C. decalvans* and *C. eriophorum* in *C. ser. Alpina*—they together form a separate lineage, albeit with only moderate bootstrap support 67% (Fig. 2). Previous phylogenetic studies (Scheen et al. 2004; Brysting et al. 2007, 2011) mostly concentrated on the arctic-alpine members of *Cerastium* with focus on the origin of high polyploid species; several Balkan taxa, including *C. dinaricum* and *C. hekuravense*, were not sampled. Further phylogenetic studies using nuclear and plastid DNA sequences and broader taxon sampling are thus needed to finally clarify the phylogenetic position of *C. dinaricum* and *C. hekuravense* and to propose a revised infrageneric classification.

Taxonomic treatment

Cerastium dinaricum G.Beck & Szysz., Rozpr. Akad. Um. (Mat.-Przyr.) 19: 62. 1889.—TYPE: “Kom Kucki”, *I. Szyszylowicz*, Iter Montenegrinum 1886 (lectotype **designated here**: PRC 452158!).

= *Cerastium dinaricum* f. *velebiticum* Degen & Lengyel, Magyar Bot. Lapok 6: 126. 1907. ≡ *Cerastium dinaricum* var. *velebiticum* (Degen & Lengyel) Graebner & Corr. in Aschers. & Graebner, Syn. Mitteleur. Fl. 5(1): 628. 1918.—TYPE: “Croatia. Velebit. In lapidosis alpinis montis Malovan supra Raduč, 1500–1700”, 5 Aug 1906, *A. de Degen*, plantae Hungariae exsiccatae (lectotype **designated here**: JE 00007505!).

Description: Densely caespitose perennial, with ascending stems (4)7–20(29) mm long and having (4)5–8(11)

Fig. 6 *Cerastium dinaricum* (above) and *C. hekuravense* (below) and their habitats. Photos by B. Frajman and P. Schönswetter

internodes. Leaves sessile, ovate, (9.0)12.5–17.8(20.0) mm long and (2.1)2.6–5.6(7.2) mm wide, (2.3)2.8–5.2(6.1) times longer than wide, widest at (0.1)0.3–0.5(0.6) of the length, apex (17.2)26.4–69.6(91.0)°, upper surface of the leaves glabrous or with indumentum of 2–11(14) glandular and 1–9(10) eglandular hairs per mm². Bracts (2.2)2.4–5.3(7.8) × (0.4)0.7–1.9(3.0) mm, (1.6)2.3–4.3(5.9) times longer than wide. Flowers (1)2–5(7) per stem. Sepals (2.8)5.0–6.9(7.2) mm long and (1.6)1.7–2.7(3.2) mm wide, (1.3)2.0–3.6(3.8) times longer than wide, widest at (0.2)0.3–0.6(0.9) of

the length. Petals (6.3)7.0–10.3(10.8) mm long and (2.7)3.8–6.3(7.0) mm wide, (1.3)1.4–2.2(2.6) times longer than wide, incision (1.6)2.–3.7(3.9) mm deep, (0.2)0.3–0.4(0.5) of the total petal length. Capsules (7.1)8.8–11.7(12.6) × (2.1)2.9–4.8(5.5) mm, (1.7)2.0–3.4(3.9) times longer than wide, widest at (0.2)0.3–0.5(0.6) of the length, (1.1)1.4–2.1(3.5) times longer than the sepals. Seeds (0.1)1.4–1.9(2.0) mm long and (0.5)1.1–1.6(1.8) mm wide, (0.2)1.0–1.5(1.6) times longer than wide. $2n = 36 + 1$ (Niketić et al. 2013).

Fig. 7 Iconography of *Cerastium hekuravense* (a–d) and *C. dinaricum* (e–h). a, e whole plant; b, h stem leaf; d, g sepal with indicated hyaline margin; c, f petal; in g and h the leaf and sepal are divided to indicate that the plant can be glabrous or pubescent. Drawings by M. Magauer

Distribution: Disjunct distribution in the Dinaric Mountains from Mt. Snežnik in Slovenia over Velebit and Dinara in Croatia, Prenj and Volujak in Bosnia and Hercegovina, Durmitor, Komovi and Žijevo in Montenegro to Maja Jezercë in Albania.

Habitat: Usually northerly exposed humid screes and rock crevices mostly in summit areas; the habitat in Slovenia, where it grows on the bottom of a karstic doline with temperature and vegetation inversion, is an exception.

Conservation status: Endangered (EN).

Note: In the protologue also a collection from Malovan (Velebit) is given, but the specimen has not been seen.

Cerastium hekuravense Jáv., Bot. Közl. 19: 18. 1921. $\equiv$ *Cerastium dinaricum* var. *hekuravense*, (Jáv.) Niketić, Glasn. Prir. muz., Ser. B 49–50: 48. 1999. $\equiv$ *Cerastium dinaricum* f. *hekuravense* (Jáv.) Niketić, Endem. predst. roda *Cerastium* II Evr.: 52. 2007.—TYPE: “Montes Albaniae borealis versus opp. Djakova extensi: Montes Hekurave. In glareosis calc. sub rupe Maja Drošks supra pag. Dragobija—alt. ca. 1700 m., ad nivem perpetuam”, 30 Aug 1918, S. Jávorka, (lectotype **designated here**: PRM 357775!).

Description: Laxly caespitose perennial, with decumbent stems (10)17–27(30) mm long and having (6)6–8(8) internodes. Leaves sessile, ovate, (8.8)10.1–15.6(17) long and (2.4)2.7–5.9(7.0) mm wide, (1.7)2.0–4.0(5.6) times longer than wide, widest at (0.1)0.2–0.4(0.6) of the length, apex (23.3)30.4–89.5(97.1)°, upper surface of the leaves glabrous or with indumentum of 1–4 glandular and 1–9(10) eglandular hairs per mm². Bracts (2.4)2.5–5.6(6.4) $\times$ (1.2)1.3–2.4(2.9) mm, (1.7)1.8–2.7(3.0) times longer than wide. Flowers 2–5(7) per stem. Sepals (5.3)5.4–7.9(8.0) mm long and (2.0)2.2–3.1(3.6) mm wide, (2.0)2.1–2.9(3.2) times longer than wide, widest at (0.2)0.3–0.5(0.6) of the length. Petals (6.2)8.0–10.4(11.4) mm long and (3.0)3.5–6.0(6.8) mm wide, (1.3)1.4–2.4(2.7) times longer than wide, incision (0.8)1.4–3.1(3.6) mm deep, (0.1)0.1–0.3(0.4) of the total petal length. Capsules (5.3)6.1–11.4(12.1) $\times$ (3.1)3.7–4.4(5.0) mm, (1.4)1.5–2.7(3.2) times longer than wide, widest at (1.8)1.9–4.0(5.4) mm distance from the basis, widest at (0.2)0.3–0.5(0.6) of the length, (0.8)0.9–1.8(1.9) times longer than the sepals. Seeds (0.4)0.8–1.6(2.2) mm long and (0.3)0.6–1.3(1.8) mm wide, 1.1–1.4(1.5) times

longer than wide. Chromosome number unknown, but based on the relative genome size $2n = 36$ is expected.

Distribution: Endemic to the Albanian Alps (Alpet Shqipëtare), where it is only known from three localities—Buni Jezercë, Maja Hekurave and Maja Kakisë.

Habitat: Northerly exposed humid screes with extended snow cover.

Conservation status: Endangered (EN).

Key to the species of *Cerastium dinaricum* s.l

Even if there is a strong overlap in character states between both species, it is possible to discriminate between them using a combination of characters given in the key. The most discriminating character, although not always distinct on herbarium specimens, is bold. Both species and their habitats are shown in Figs. 6 and 7.

1a. Densely caespitose perennial with ascending stems and (4)7–20(29) mm long internodes. Plants variable in indumentum, from completely glabrous to densely hairy. Leaves (2.3)2.8–5.2(6.1) times longer than wide. Sepals (2.8)5.0–6.9(7.2) mm long and (1.6)1.7–2.7(3.2) mm wide. Capsules (7.1)8.8–11.7(12.6) × (2.1)2.9–4.8(5.5) mm, (1.7)2.0–3.4(3.9) times longer than wide. Seeds (0.1)1.4–1.9(2.0) long and (0.5)1.1–1.6(1.8) wide *C. dinaricum*

1b. Laxly caespitose perennial with decumbent stems and (10)17–27(30) mm long internodes. Plants with at least some glandular hairs. Leaves (1.7)2.0–4.0(5.6) times longer than wide. Sepals (5.3)5.4–7.9(8.0) mm long and (2.0)2.2–3.1(3.6) mm wide. Capsules (5.3)6.1–11.4(12.1) × (3.1)3.7–4.4(5.0) mm, (1.4)1.5–2.7(3.2) times longer than wide. Seeds (0.4)0.8–1.6(2.2) long and (0.3)0.6–1.3(1.8) wide, (1.1)1.1–1.4(1.5) times longer than wide *C. hekuravense*

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Information on Electronic Supplementary Material

Online Resource 1. Voucher information about the outgroup taxa included in the AFLP analyses. Each population of each species has its specific population ID.

Online Resource 2. Character states of *Cerastium dinaricum* and *C. hekuravense* from morphometric analyses. For explanation of character abbreviations see Table 2.

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