# Chromosome numbers and taxonomic-chorological notes on selected species of *Hieracium* s. str. (*Asteraceae*) from Montenegro

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**Abstract.** The chromosome numbers for 15 species of *Hieracium* s.str. from Durmitor Mt (Montenegro) are reported, supplied by taxonomic and chorological notes. Eleven taxa (*H. blecicii* nom. prov., *H. calophyllum* s.l., *H. expallens*, *H. gymnocephalum*, *H. flexuosum*, *H. mirificissimum*, *H. naegelianum*, *H. paratrichum* nom. prov., *H. scheppigianum*, *H. spirocaule* nom. prov., *H. valdepilosum* s.l.) were found to be triploid, with 2n = 3x = 27, and four taxa (*H. durmitoricum*, *H. pallescens*, *H. pseudoshenkii*, *H. coloriscapum* s.l.) were tetraploid, with 2n = 4x = 36. For six species the chromosome number has not been reported so far. For the first time *H. mirificissimum* is reported for the flora of Montenegro and *H. expallens* for the floras of Serbia and Montenegro.

Key words: chorology, chromosome numbers, Durmitor Mt, Hieracium, Montenegro, ploidy levels, Serbia, taxonomy

#### Introduction

The genus *Hieracium* L. s.str. is notorious for its taxonomic complexity. According to Zahn (1921-23, 1922-38), within the territory of Serbia and Montenegro there are 79 recorded species and 213 subspecies. Afterwards, some 65 new taxa have been recorded or described. The greatest species diversity has been registered on the limestone terrains of the Southeast Dinarides (Durmitor Mt, Prokletije Mts). In the area of Durmitor Mt over 60 agamospecies have been recorded (Zahn 1921-23, 1922-38; Rohlena 1942). Particularly rich in species are the endemic *H*. sect. *Pannosa* (Zahn) Zahn and *H*. sect. *Glauciformia*  (Freyn) Zahn. Other important centres of diversity are Mt Šar and the Balkan Range (= Stara Planina), especially concerning the representatives of *H.* sect. *Cernua* R. Uechtr. (= *H.* sect. *Pseudostenotheca* (Fr.) Juxip, sensu Stace 1998), which usually grow on a silicate substrate.

The taxonomic complexity of *Hieracium* is due to the prevailing polyploidy and apomixis (Gustafsson 1946, 1947a,b). In comparison with the closely related genus *Pilosella* Hill, a recent natural hybridization in *Hieracium* seems to be strongly restricted (Mráz et al. 2005). While diploid taxa are sexual (Gustafsson 1946, 1947a,b; Chrtek 1997; Mráz 2003), polyploids reproduce apomictically (e.g. Gustafsson 1946, 1947a,b).

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Several approaches have been used to classify the diversity of the genus. The taxonomic concept of Nägeli and Peter (1885), later largely expanded by Zahn (1921-23, 1922-38), accepts the so-called '*species principales collectivae et species intermediae collectivae*,' with a huge number of infraspecific taxa. This approach was held mostly by the authors in Central and Southeast Europe, including Serbia and Montenegro (Rohlena 1942; Gajić 1975). The other approach held by the botanists in Great Britain, Western Europe, Scandinavia and Russia, accepts the 'narrow' species. In most cases these species correspond to the subspecies of the Central European school and are arranged in larger groups, many of which without valid taxonomic status.

Whenever possible, the 'narrow' species concept has been used in this paper. Some very complex groups, in which the taxonomic relations have not been acceptably resolved yet, were treated on aggregate level (s.l.). Stace's classification of sections was followed (Stace 1998), with the exception of *H.* sect. *Naegeliana* Zahn ex Szeląg, which has been recently described, and *H.* sect. *Cernua* (Szeląg 2003).

The chromosome number is a useful character for inferring the mode of reproduction of the studied taxa and, hence, for solving taxonomic problems. The basic chromosome number in the genus is x=9. So far all ploidy levels, from the diploid to the heptaploid, have been detected (e.g. see Schuhwerk 1996; Pulkina & Tupitzsyna 2000), though with a very different frequency of occurrence. Most common are the tri- and tetraploids, whereas penta-, hexa- and heptaploids have been seldom reported (Stace & al. 1995; Chrtek 1996; Pulkina & Tupitzsyna 2000). Diploids in Hieracium s.str. are not common, but the Balkan Peninsula has proved to be among the richest European areas in the number of diploid Hieracium species (Schuhwerk & Lippert 1998; Vladimirov 2000, 2003; Vladimirov & Szeląg 2001, 2006). Karyological studies of material from Serbia and Montenegro are very few (Schuhwerk & Lippert 1998; Niketić & al. 2003), which has encouraged us to carry out this study.

#### Material and methods

The herbarium material, seeds and living plants were collected from a single locality: Durmitor Mt, the slopes of Veliki Međed peak with southern exposition, on limestone screes within the zone of *Pinus mug*o, 1800–2000 m, 23.08.2001, coll. *M. Niketić* & *G. Tomović*. The voucher specimens are deposited in the herbarium of the Natural History Museum in Belgrade (BEO).

Two approaches to the determination of chromosome numbers were used.

- (i) The living plants were brought into cultivation in the vegetation house of the Institute of Botany in Sofia. Root tips were cut, pretreated with 0.01% colchicine solution for 90 min, fixed in 3:1 absolute ethanol-glacial acetic acid for at least 2h at room temperature, and stored in 96 % alcohol at -18 °C until needed. Then the root tips were hydrolysed in 1NHCl for 20 min at 60 °C, stained with Gomori's haematoxylin (Melander & Wingstrand 1953) for 30 min at 60 °C, and finally squashed in 45% acetic acid. Counting of the chromosomes was done on permanent slides. Usually 2-4 plants of each gathering were used for chromosome counting (method used by V.V.).
- (ii) Achenes were germinated in Petri dishes. Root tips of the seedlings were cut and pretreated with 0.5% colchicine solution for 1.5-3h at room temperature. Subsequently, root tips were fixed in a mixture of absolute ethanol and acetic acid (3:1) for at least 1 h and then hydrolysed for 5 min in 1N HCl at 60 °C. The squash and smear method with cellophane replacing the glass covers followed Murín (1960). Giemsa solution in phosphate buffer was used as a stain. Selected permanent slides have been stored at the Department of Botany, Institute of Biology & Ecology, P.J. Šafárik University, Košice. The chromosome number was counted in 1-4 germinated achenes from each species (method used by P.M.).

Taxonomic and chorological notes are based on the opinion of M.N. Herbarium specimens from the following herbaria have been studied: B, BM, BEO, BEOU, BP, BRNM, C, G, LJU, LY, PR, PRC, S, SKO, SO, SOM, W, WU, Z. The material was revised and compared with own collections from the Dinarides (BEO) and the living plants in the garden. For the representatives of *H.* sect. *Pannosa* morphometric analyses have been done (including canonical and correspondent analyses – unpublished data).

#### **Results and discussion**

#### Hieracium sect. Drepanoidea Monnier

H. pseudoshenkii (Rohlena & Zahn) Niketić (Fig. 1)
 Syn.: H. bupleuroides subsp. pseudoshenkii Rohlena & Zahn

2n = 4x = 36 (counted by P.M. & V.V.; Fig. 3A)

The species is endemic to the Southeast Dinarides: Durmitor Mt and the vicinity of Šavnik in Montenegro, and Prenj Mt in Herzegovina (Niketić & al. 2003). It grows in rocky habitats of the subalpine zone, in the openings of *Pinus mugo* communities. The chromosome number confirms an earlier count from Durmitor Mt (Niketić & al. 2003). The taxon belongs to the *H. bupleuroides* group (sensu Sell & West 1976) for which triploid (Christoff & Popoff 1933; Polatschek 1966; Murín & Uhríková 1970; Chrtek & al. 2004) and tetraploid (Chrtek & al. 2004; Szeląg & Vladimirov 2005) counts have been reported so far.

#### H. sect. Hieracium

*H. pallescens* Waldst. & Kit. (Fig. 2) 2n = 4x = 36 (counted by P.M. & V.V.)

The species is distributed in the mountains of Central Europe, extending eastwards to Romania and south-eastwards to Bosnia, Montenegro and Southwest Serbia (Zahn 1935; Behr & al. 1937). According to Zahn, this taxon belongs to the *H. incisum* group (*H. bifidum-dentatum*), whereas Sell & West (1976) place it into the *H. bifidum* group. The chromosome number is reported here for the first time.

## H. sect. Naegeliana Zahn ex Szeląg H. naegelianum Pančić (Fig. 4) 2n=3x=27 (counted by V.V.; Fig. 3B)

The species is distributed in the high mountains of the Balkan Peninsula and Mt Abruzzo in Italy. It is characteristic for the limestone screes in the subalpine and alpine belts, at 1800–2500 m. The species was described from Komovi Mt in Montenegro (Pančić 1875). Several subspecies are known, but the collected specimens, as well as all the populations in Montenegro, belong to the nominal taxon.

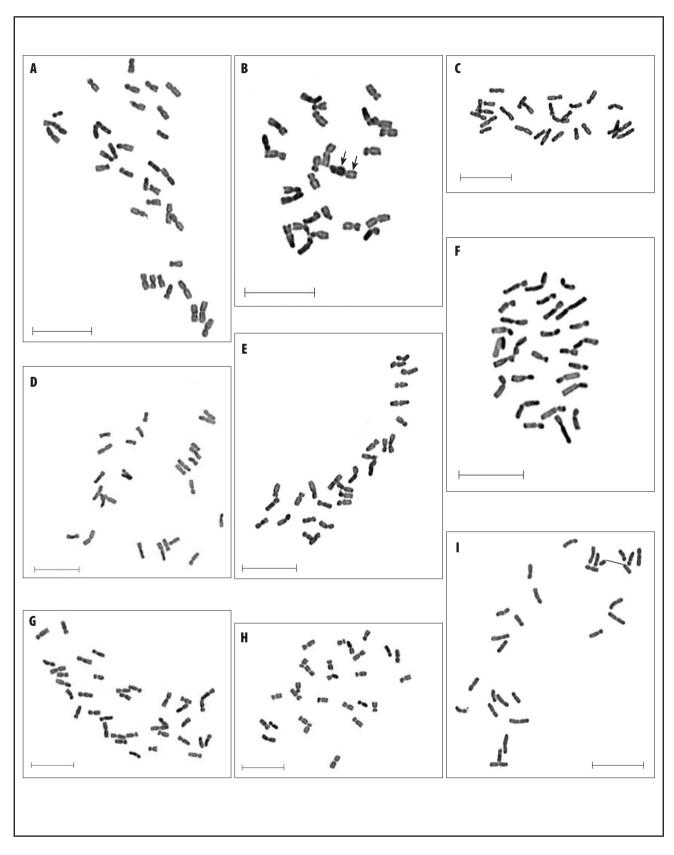
The chromosome number confirms the previous counts in specimens originating from Greece (Merxmüller 1975; Grau & Erben 1988; Franzén in Buttler 1991: 639) and Bulgaria (Vladimirov & Szeląg 2001).



Fig. 1. H. pseudoschenkii – herbarium specimen.



Fig. 2. H. pallescens - herbarium specimen.



**Fig. 3.** Microphotographs of metaphase plates of: **A**, *H. pseudoschenkii*, 2n = 36; **B**, *H. naegelianum*, 2n = 27; **C**, *H. blecicii*, 2n = 27; **D**, *H. gymnocephalum*, 2n = 27; **E**, *H. paratrichum*, 2n = 27; **F**, *H. calophyllum*, 2n = 27; **G**, *H. durmitoricum*, 2n = 36; **H**, *H. scheppigianum*, 2n = 27; **I**, *H. valdepilosum*, 2n = 27. Scale bar – 10 µm.

*H.* sect. *Pannosa* (Zahn) Zahn
 *H.* blecicii Niketić, nom. in lit. 2002 (*H. gymnocephalum* s.l.)
 2n = 3x = 27 (counted by P.M. & V.V.; Fig. 3C)

This yet undescribed species (cf. Niketić 2002) is endemic to the mountains of West Montenegro and a small part of Southeast Bosnia. The analysis of the distribution of phenolic compounds pointed to very similar flavonoid and phenolic acid patterns with H. gymnocephalum s.str. However, some significant differences have been noted concerning the presence of certain compounds (Petrović & al. 1999). H. blecicii is characterized by relatively broad leaves and very thick plumose hairs with short branches. In the analyzed micropopulation only individuals with tubulose flowers were found.

This is the first record of the chromosome number of the species.

#### *H. gymnocephalum* Griseb. ex Pant. (Fig. 5)

2*n*=3*x*=27 (counted by P.M. & V.V.; Fig. 3D)

The species is an Illyrian-Scardo-Pindic element confined to the mountains of the Western Balkan Peninsula, ranging northwards from Herzegovina to Northwest Greece in the south. The most numerous populations were re-

corded in Montenegro. This species is a type representative of a triploid aggregate with the same name, and it is characterized by glabrous to very sparsely hairy acladium and involucre (in contrast to representatives of the other aggregates of *H.* sect. *Pannosa*). It is a very variable taxon, especially considering the hairiness of leaves and involucre and thus several



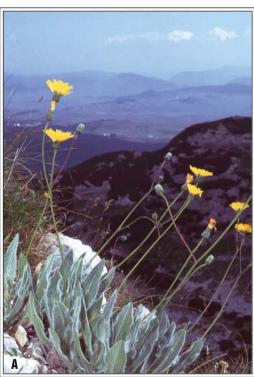




Fig. 4. *H. naegelianum*:A, whole plant;B, flower head before anthesis.



**Fig. 5.** *H. gymnocephalum*: **A,** whole plant; **B,** flower head before anthesis.

infraspecific taxa have been described. The plants collected from the population on Durmitor Mt are very similar to the type specimens from Komovi Mt (60 km south-eastwards) in terms of their morphological characters.

The chromosome number of the species is reported here for the first time.



Fig. 6. H. calophyllum - herbarium specimen.

- *H. paratrichum* Niketić, nom. in lit. 2003 (*H. gymnocephalum* s.l.)
- 2n = 3x = 27 (counted by V.V.; Fig. 3E)

This yet undescribed species is a member of the *H. gymnocephalum* group. It is distributed in some mountains of Montenegro and Herzegovina (Niketić & al. 2003). The species is characterized by scattered plumose hairs on the involucre and pedicels and by very thick plumose hairs with long branches on the leaves. The present chromosome count confirms an earlier report by Niketić & al. (2003) from Durmitor Mt.

### *H. spirocaule* Niketić, nom. in lit. 2002 (*H. gymnoce-phalum* s.l.)

#### 2n = 3x = 27 (counted by V.V.)

This yet undescribed species (cf. Niketić 2002) also belongs to the *H. gymnocephalum* complex. It is endemic to West Montenegro and a small part of Southeast Bosnia. The species is characterized by a very crowded, false basal rosette of narrow, acute bluish-green leaves with undulate margin and whitish to gray hairs throughout, and by 1-2(6) capitula.

The chromosome number is reported here for the first time.

Hieracium sect. Pannosa comprises remarkable, hairy-leaved taxa distributed in the Balkan Peninsula and Asia Minor, as well as on some East Mediterranean islands (Zahn 1921-23). Zahn (1921-23) designated three species principales in this section: H. gymnocephalum Griseb. ex Pant., H. waldsteinii Tausch and H. pannosum Boiss. The four species from this section that we have studied karyologically belong to the H. gymnocephalum group, whose centre of diversity is in the mountains of Montenegro (Niketić & al. 2003). So far only triploids have been found within this species group. In the H. pannosum and H. waldsteinii groups more ploidy levels have been detected, ranging from diploids (Schuhwerk & Lippert 1998; Vladimirov & Szelag 2006) to tri- and tetraploids (Christoff & Popoff 1933; Papanicolaou 1984; Schuhwerk & Lippert 1998; Vladimirov & Szelag 2001).

#### *H.* sect. *Pilosissima* Stace & P.D. Sell *H.* calophyllum R. Uechtr. s.l. (Fig. 6) 2n = 3x = 27 (counted by V.V.; Fig. 3F)

*H. calophyllum* group is endemic to the Western Balkan Peninsula with a centre of diversity in the mountains of Montenegro (Szeląg 2002; Niketić & al. 2003). The present count confirms an earlier report by Niketić & al. (2003).

#### *H. coloriscapum* Rohlena & Zahn s.l. (Fig. 7) 2n = 4x = 36 (counted by V.V.)

The species is an Illyrian-Scardo-Pindic element, inhabiting limestone screes in the subalpine and alpine mountain zones. It has probably derived from interbreeding of *H. gymnocephalum* and *H. naegelianum*. The plant has been recently recorded in Durmitor Mt. Beyond the territory of Montenegro, several distinct subspecies have also been described (Zahn 1936; Behr & al. 1939a, b).

The present count confirms an earlier report by Niketić & al. (2003).

### H. durmitoricum (Rohlena & Zahn) Niketić (Fig. 8) Syn.: H. scheppigianum subsp. durmitoricum Rohlena & Zahn

2n = 4x = 36 (counted by P.M. & V.V.; Fig. 3G)

The taxon is endemic to Durmitor Mt and several adjacent high mountains in the Southeast Dinarides (Niketić & al. 2003). The same chromosome number has already been reported for the species from Durmitor Mt (Niketić & al. 2003). Besides *H. gymno*-

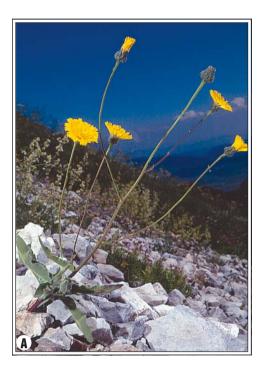




Fig. 7. H. coloriscapum: A, whole plant; B, flower head.



**Fig. 8.** *H. durmitoricum*: **A**, whole plant; **B**, flower head before anthesis.

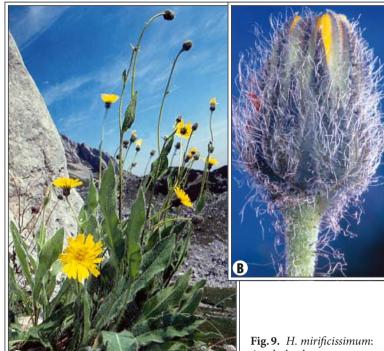
*cephalum*, another possible ancestor of this hybridogenous taxon is *H. willdenowianum* (Zahn) P.D. Sell & C. West from the *H. valdepilosum* group.

#### H. mirificissimum Rohlena & Zahn (Fig. 9)

Syn.: *H. flexicaule* Freyn & Vandas, nom. illeg., non Tausch; *H. guentheri-beckii* subsp. *portentosum* Hayek & Zahn.

2n = 3x = 27 (counted by P.M. & V.V.)

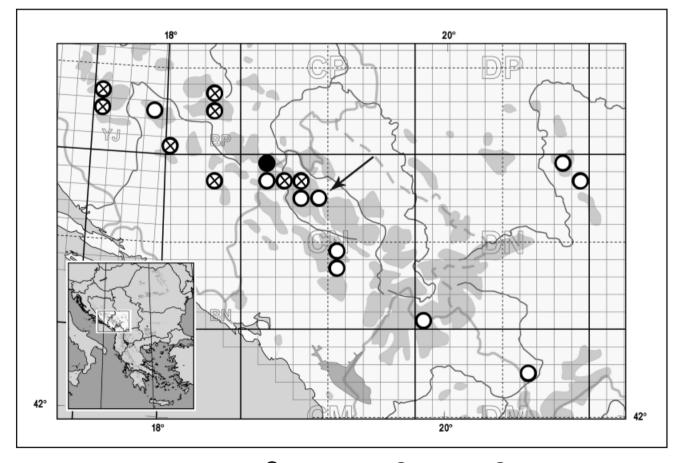
The species is endemic to Southeast Dinarides – Montenegro, Bosnia and Herzegovina, and to Central and Southwest Serbia (Niketić 2003). It is considered a hybridogenous species, probably originating from interbreeding between *H. gymnocephalum* and *H. scorzonerifolium* Vill. Morphologically, it is very similar to the related species *H. guentheri-beckii* Zahn, with which it often forms mixed populations. The species is also very similar to *H. durmitoricum* and



**Fig. 9.** *H. mirificissimum*: **A**, whole plant; **B**, flower head before anthesis.

*H. scheppigianum* Freyn. It is recorded for the first time for the flora of Serbia in the following localities:

- DN-98 **Serbia,** Mt Kopaonik, "In gramin. m. Treska", July 1903, coll. *O. Bierbach*, det. *L. Adamović* sub *H. calvescens* Adamović (BM); 1600 m, on limestone, 14.08.2004, coll. *M. Niketić* & *G. Tomović* (BEO);
- DN-89 **Serbia,** Mt Kopaonik, Bele Stene peak, 1600 m, on limestone, 12.09.1991, coll. *M. Niketić* (BEO);
- DM-67 **Serbia,** Mt Paštrik, "An felsen der Gipfelregion", 1700 m, 27.07.1918, coll. *I. Dörfler*, sub *H. guentheribeckii* subsp. *portentosum* (no. 877) (BP, W, WU, Z).



**Fig. 10.** *H. mirificissimum*: total distribution range, **O** – new herbarium data; **O** – literature data; **O** – both literature and herbarium data. Arrow points to *locus classicus* of the species, Durmitor Mt, from where the plants for the karyological studies have been collected.

New localities of the species have been found on the territory of Montenegro and Herzegovina during the field research in the past few years (Fig. 10):

- CN-18 **Montenegro,** Mt Volujak, Vilište peak (1960 m), 17.08.2000, coll. *M. Niketić* (BEO);
- CN-37 **Montenegro,** Durmitor Mt, Planinica peak – Škrka lakes, 1800–1900 m, 17.08.1993, coll. *M. Niketić* (BEO);
- CN-47 **Montenegro,** Durmitor Mt, Minin Bogaz peak, 2200–2300 m, 13.08.1994, coll. *M. Niketić* (BEO);
- CN-53 **Montenegro**, Mt Maganik, Petrov peak, 1800–2100 m, 09.08.2000, coll. *M. Niketić* (BEO);
- CN-54 **Montenegro,** Mt Moračke Planine, Mt Stožac, Jablanovac peak, 1900 m, 07.08.2000, coll. *M. Niketić* (BEO),
- DN-00 **Montenegro**, Mt Prokletije, Bjelič, on rocky ground, limestone, 1800– 1900 m, 09.07.1994, coll. *M. Niketić* (BEO),



YJ-32 **Bosnia & Hercegovina,** Mt Prenj, Pod Sivadijom – Jezera, 1650–1700 m, 07.08.2001, coll. *M. Niketić* (BEO).

After an examination of the above-mentioned herbarium material stored in BM, it was concluded that the literature report of the Apennine hybridogenous species *H. portanum* Belli (*H. gymnocephalum-heterogynum*, Zahn 1921-23) for Kopaonik Mt (det. *Adamović* sub *H. calvescens*) was incorrect and actually represents *H. mirificissimum*. At the same locality (Treska), only individuals of *H. mirificissimum* (coll. *M. Niketić & G. Tomović*, BEO) were recorded. The indumentum of the leaves and involucre at first glance was similar to that of *H. portanum* from Calabria, but the latter species probably originated from interbreeding between *H. gymnocephalum* and *H. heterogynum* (Froel.) Gutermann. Thus, *H. portanum* is endemic to South Italy and has never been recorded in the flora of the Balkan Peninsula.

Furthermore, examination of the herbarium specimens determined as *H. guentheri-beckii* subsp. *portentosum* Hayek & Zahn from the Paštrik Mt in Kosovo (BP, W, WU, Z) has shown no significant morphological differences from *H. mirificissimum*. This was further supported by their original descriptions (Zahn 1909, 1921-23), which were essentially very similar. Therefore, *H. guentheri-beckii* subsp. *portentosum* can be considered as a synonym of *H. mirificissimum*.

This chromosome number is reported for the first time for the species.

#### H. scheppigianum Freyn (Fig. 11)

Syn.: H. agastum Rech. f. & Zahn

2n = 3x = 27 (counted by P.M & V.V.; Fig. 3H)

The species is confined to the limestone areas in some mountains of Bosnia, Montenegro and Kosovo (Niketić & al. 2003). According to Zahn's classification, *H. scheppigianum* s.l. was divided into several subspecies (Zahn 1936). Niketić & al. (2003) concluded that it was a polyphyletic aggregate, and its type representative probably originated from interbreeding between *H. gymnocephalum* and a representative of *H. bupleuroides* aggregate.

The present chromosome number confirms an earlier report by Niketić & al. (2003), also from Durmitor Mt.



**Fig. 11.** *H. scheppigianum*: **A,** whole plant; **B,** flower head.

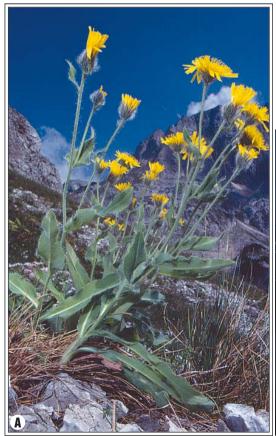
H. sect. Villosa (Griseb.) Gremli

H. expallens (Fr.) Arv.-Touv. (Fig. 12)

Syn.: *H. dentatum* var. *expallens* Fr.; *H. dentatum* subsp. *subexpallens* Zahn, nom. illeg.

2n = 2x = 27 (counted by V.V.)

The Hieracium dentatum group comprises taxa that are morphologically intermediate and have possibly derived from hybridization between the taxa of H. bifidum and H. villosum groups (sensu Sell & West 1976). It is distributed in the Jura, Alps, Carpathians, Central Apennines and mountains of the Northwestern Balkan Peninsula. So far about 50 subspecies have been described (Zahn 1930), all very similar morphologically. Sell & West (1976) have recognized certain taxa as distinct microspecies. Only one literary source (Behr & al. 1937) refers to *H. expallens* on the border of Albania and Serbia (Mt Paštrik). Representatives of the same taxon were recorded for the first time in the floras of Serbia and Montenegro in the following localities (Fig. 13):



- CN-19 **Montenegro,** Mt Maglić, 1800–2000 m, 19.08.2000, coll. *M. Niketić* (BEO);
- CN-47 **Montenegro,** Durmitor Mt, the slopes of Veliki Međed peak, 1850 m, 14.08.1992, coll. *M. Niketić* (BEO);
- CN-48 **Montenegro**, Durmitor Mt, Crvena Greda peak, 1800– 1900 m, 20.08.1996, coll. *M. Niketić* (BEO);
- BN-91 **Montenegro,** Mt Orjen, peak, 1800 m, 22.08.1998, coll. *M. Niketić* (BEO);
- CN-53 **Montenegro,** Mt Maganik, Petrov Vrh peak, 1800–2100 m, 09.08.2000, coll. *M. Niketić* (BEO);
- CN-54 **Montenegro,** Mt Moračke Planine, Vragodol valley, 2100 m, 26.08.1998, coll. *M. Niketić* (BEO);
- DM-74 **Serbia,** Mt Šar Planina, Brod village, Gradski Kamen hill, 1450–1650 m, 30.09.1991, coll. *M. Niketić* (BEO);
- DN-22 **Serbia**, Mt Prokletije, Nedžinat lakes, 1500–1600 m, 02.09.1997, coll. *M. Niketić* (BEO).

The chromosome number for the species is reported here for the first time.

#### H. flexuosum Waldst. & Kit. ex Willd. (Fig. 14)

Syn.: *H. scorzonerifolium* subsp. *flexuosum* (Waldst. & Kit. ex Willd.) Nägeli & Peter

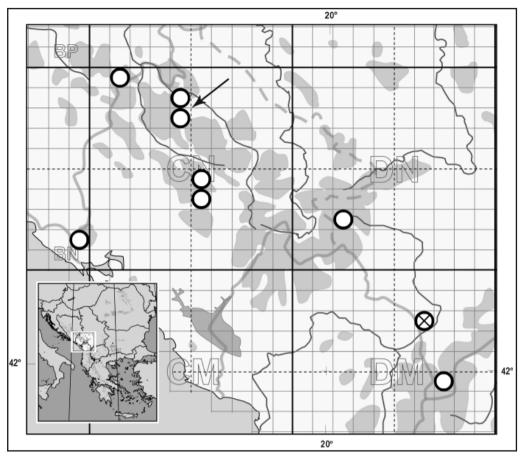
2n = 3x = 27 (counted by P.M.)

This hybridogenous species is distributed in the Alps and the Dinaric Alps, up to the Prokletije Mts in Montenegro and Kosovo. It is very closely related to *H. scorzonerifolium* Vill.

The present count confirms an earlier report by Niketić & al. (2003).



Fig. 12. *H. expallens*:A, whole plant;B, flower head before anthesis.



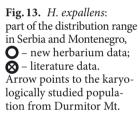






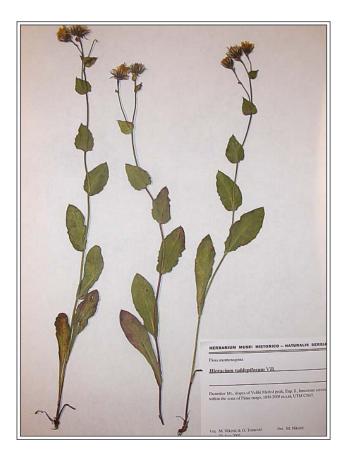
Fig. 14. *H. flexuosum*:A, whole plant;B, flower head.

#### H. valdepilosum Vill. s.l. (Fig. 15)

2n = 3x = 27 (counted by P.M. & V.V.; Fig. 3I)

The main taxonomic diversity of this species group in the sense of *Flora Europaea* (Sell & West 1976) is confined to the Alps, whereas the Balkan Peninsula shelters only a few isolated taxa. The studied microspecies definitely belongs to the *H. valdepilosum* group, but we were unable to determine its exact name. It has been recently discovered and reported for the first time for Serbia and Montenegro (Niketić & al. 2003). The triploid chromosome number has already been reported by Niketić & al. (2003) for the same taxon and is congruent with a count published earlier for a possibly different microspecies from the same species group (Auquier & Renard 1979).

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Fig. 15. H. valdepilosum – herbarium specimen.

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