

Taxonomic revision and conservation status of *Centaurea davidovii* (sect. *Lepteranthus*, Asteraceae)

Svetlana Bancheva & Rossen Gorgorov

Institute of Botany, Bulgarian Academy of Sciences, Acad. Georgi Bonchev St., bl. 23, 1113, Sofia, Bulgaria, e-mail: sbancheva@yahoo.com (author for correspondence)

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Abstract. *Centaurea davidovii* (sect. *Lepteranthus*) is a Bulgarian endemic species distributed in meadows and pastures only in the floristic region of Western and Central Balkan Range. Despite the fact that the taxon is represented by few and very fragmented populations, it is included neither in the Biological Diversity Act of Bulgaria, nor in the Red List of Bulgarian Vascular Plants (Petrova & Vladimirov 2009), because of its uncertain taxonomic status. The present biosystematic study is related to the work on the last, 12th volume of *Flora of R Bulgaria* and comprises data about the morphological variation, pollen morphology and structure, karyology and distribution patterns of *C. davidovii* and its closest relative *C. nervosa*. The population and conservation status of *C. davidovii* is discussed.

Key words: Asteraceae, Bulgarian flora, *Centaurea*, endemic and threatened plants, *Lepteranthus*, taxonomy

Introduction

Section *Lepteranthus* is the richest in species and subspecies in subgenus *Jacea*. It comprises over 20 taxa (Dostál 1976). On the basis of their chorology, they can be divided into the following groups: **1)** West European species related to *C. debeauxii* Gren. & Gord. and *C. nigra* L.; **2)** East European species forming a relation group around *C. phrygia* L., *C. pseudophrygia* C.A. Mey., *C. stenolepis* A. Kern., and various Carpathian, Caucasian and Balkan small endemic species, as well as the Alpine-Balkan species *C. uniflora* Turra s.l. (incl. *C. nervosa* Willd.); **3)** Some species with a Pontic-Central Asian area diagnosis, belonging to ser. *Trichocephalae* (Hayek) Dobroc., and some isolated species related to *C. rhaetica* Moritz.; **4)** A small number of Iberian and South European species. The Alpine-Balkan group of species related to *C. uniflora* includes *C. uniflora*, *C. nervosa* Willd. and *C. davidovii* Urum. *Centaurea uniflora* has a North Apennine//subalpine area diagnosis (Meusel & Jäger 1992), *C. nervosa* is its Eastern vicariant with a Balkan-East

Illyrian-South Carpathian-Southeast Alpine-(North Apennine) // subalpine area diagnosis, *C. davidovii* is a Bulgarian endemic from the Balkan Range (*Western & Central*). The latter taxon was described by the Bulgarian teacher Ivan Urumov (1906); it was considered subsequently as part of the infraspecific variation of either *C. nervosa* (Hayek 1925, 1931; Stojanov & Acharoff 1935; Delipavlov & Cheshmedzhiev 2003), or *C. uniflora* (Dostál 1976; Peev 1992).

The present biosystematic study is related to the work on the last, 12th volume of *Flora of R Bulgaria*. During the field investigations in 1996–1998 and again in 2008, only a few very fragmented populations of *C. davidovii* were found. The species is included neither in the *Biological Diversity Act* (2002), nor in the Red List of Bulgarian vascular plants (Petrova & Vladimirov 2009), or in the new edition of the *Red Data Book of Bulgaria* (Peev in press) because of its uncertain taxonomic status.

The aim of this study is to clarify the current taxonomic status of *C. davidovii*, as well as to elucidate the conservation conditions of this very rare Bulgarian endemic.

Material and methods

A comparative morphological approach, including investigation of overall morphology and pollen grains, along with karyological, ecological and geographical studies, as well as some observations on the population status were applied. The research was based on material collected from four floristic regions and subregions of Bulgaria, where the studied taxa *C. davidovii* (Fig. 1) and its closest relative *C. nervosa* (Fig. 2) occur naturally. Material from six localities was gathered (Table 1). A total of 137 samples were studied and their vouchers are kept in Bulgarian herbaria SO, SOM, SOA, Herbarium of the National History Museum in Plovdiv (NHU, Pl) and in the personal collection of the first author. Also, comparative material from the herbaria B and W was used.

Statistical variance analysis was carried out to determine variability within and between the populations of *C. nervosa* and *C. davidovii*. The morphological fea-



Fig. 1. *C. davidovii* in its natural habitat (photo S. Stoyanov).

Table 1. Localities of the studied populations of *C. davidovii* and *C. nervosa*.

No	Taxon	Locality/ coll. S. Bancheva
C 1	<i>C. nervosa</i> (SSH 9624)	Balkan Range (<i>Central</i>), above Ribaritsa village, towards Vezhen peak, 09.07.1996
C 2	<i>C. nervosa</i> (SSH 9628)	Rila Mts, to the west of Granchar chalet, 2350 m, 11.07.1996
C 3	<i>C. davidovii</i> (SSH 9653)	Balkan Range (<i>Central</i>), Kozyata Stena chalet, 1660 m, 10.08.1996
C 4	<i>C. nervosa</i> (SSH 9528)	Mt Vitosh, Platoto, below Ushite peak, 1800 m, 20.07.1995
C 5	<i>C. nervosa</i> (SSH 9529)	Balkan Range (<i>Western</i>), above Midzhur peak, 1400–2160 m, 26.07.1995
C 6	<i>C. davidovii</i> (SSH 9638)	Balkan Range (<i>Central</i>), Eho chalet, 1650 m, 19.08.1995

tures of 30 individual plants from each population were measured; the plants were collected in their natural habitat at the time of flowering and fruiting. The trait matrix comprises 14 features (Table 2). The selection is based on the estimation of the taxonomic value of these features in some taxonomic works on *Centaurea* s.l. (Routsis & Georgiadis 1994), *Centaureinae* (Wagenitz & Hellwig 1996), *Cardueae* (Häffner 2000), and on personal judgment. The results are presented with: 1) a multiple box plot for graphic depiction of the distribution of morphometric features in the studied populations (Figs 3, 4); 2) cluster analysis of the population averages for determination of uniform groups; 3) linear discriminant analysis for grouping based on the degree of discreteness between the cluster-analysis-determined main groups, and for determination of morphometric features which considerably distinguish the discrete groups (Table 3). A sta-



Fig. 2. *C. nervosa* in its natural habitat (photo R. Gorgorov).

Table 2. Matrix of the morphological features used for the statistical variance analysis of the studied populations of *C. davidovii* and *C. nervosa*.

No	Features
V1	Length of the stem (cm)
V2	Ramification of the stem (number)
V3	Division of the leaf blade (non divided, pinnatilobed, pinnatisect)
V4	Length of the middle stem leaf (mm)
V5	Medial width of the middle stem leaf (mm)
V6	Length of the upper stem leaf (mm)
V7	Number of the capitula (number)
V8	Length of the involucre (mm)
V9	Width of the involucre (mm)
V10	Length of the appendage of the middle bract (mm)
V11	Number of the cilia on each side of the appendage (number)
V12	Length of the achene (mm)
V13	Width of the achene (mm)
V14	Length of the pappus (mm)

tistical analysis was carried out using the BMDP software package (Dixon 1990).

The pollen morphology was studied by light microscopy (LM), as well as by scanning and transmission electron microscopy (SEM and TEM). The material for light microscopy was prepared after Faegri & Iversen (1975). The feature matrix was in conformity with the matrix suggested by Leonardis & al. (1984). Preparation of the pollen grains for observation by SEM and TEM was accomplished according to Huttunen & Laine's (1983) and O'Brien & McCully's (1981) protocols. The observations were carried out with a SEM Leica S420 at 15 kV. The morphological terminology of Punt & al. (2007) has been used.

The conservation status was determined according to the *IUCN criteria* (2001).

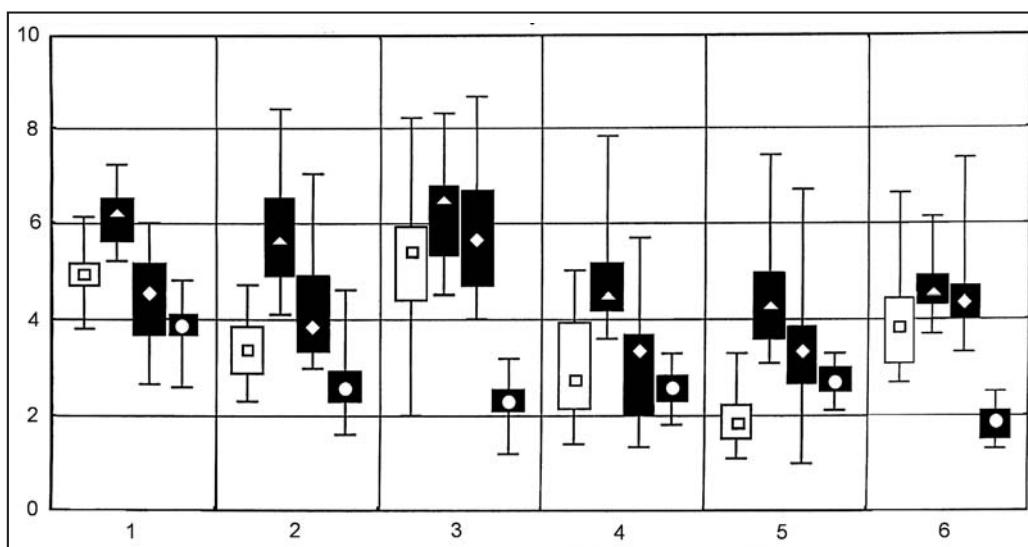


Fig. 3. Multiple box plot for graphic depiction of the distribution of selected morphometric features from Table 2: V1 (square), V4 (triangle), V5 (rhombus) and V6 (circle); Median, Box: 25%, 75%; whiskers: Min, Max.

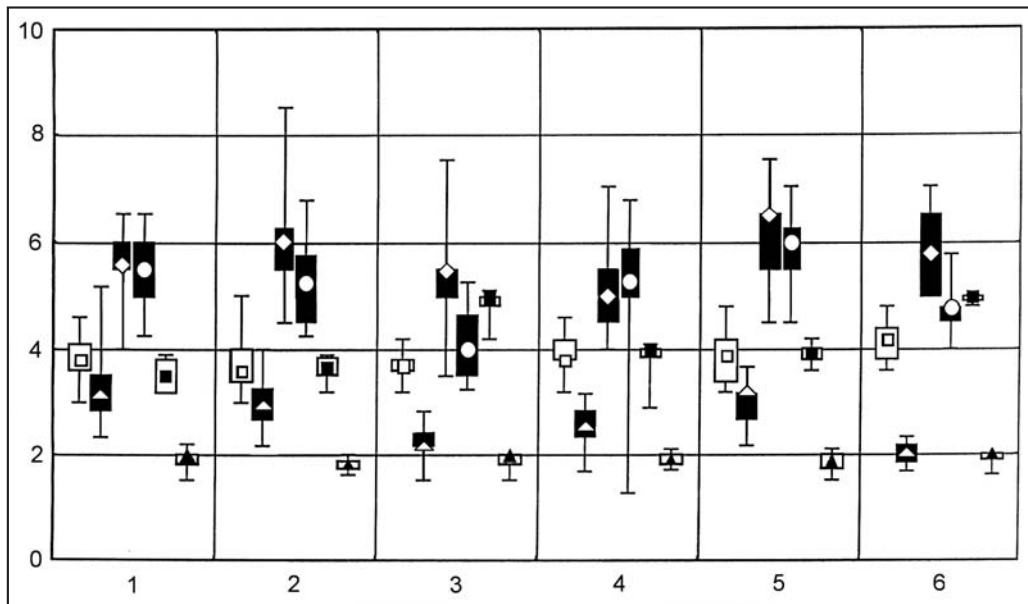


Fig. 4. Multiple box plot for graphic depiction of the distribution of selected morphometric features from Table 2: V8 (white square), V9 (white triangle), V10 (white rhombus), V11 (white circle), V12 (black square) and V13 (black triangle); Median, Box: 25%, 75%; whiskers: Min, Max.

Table 3. Discriminant analysis for even grouping.

Sample	%	Number of the cases classified between the groups		Informative features for the discrete grouping
		G1	G2	
G1 Balkan Range (Central): Ribaritsa chalet Rila Mts: Granchar chalet Mt Vitosha: loc. Ushite Balkan Range (Western): Midzhur peak	100	118	0	Width of the achene Length of the stem Width of the involucre Division of the leaf blade Medial width of the middle stem leaf
G2 Balkan Range (Central): Kozyata Stena Balkan Range (Central): Eho chalet.	97.9	1	47	Length of the upper stem leaf
Total	99.4	119	47	

Results and discussion

Variation-statistical analysis

A variation-statistical approach was used to specify inter- and infra-population variation of *C. nervosa* and *C. davidovii*, and to support determination of the taxonomic status of *C. davidovii*. Four populations of *C. nervosa* and two of *C. davidovii* have been studied (Table 1).

Two groups were established by cluster analysis (Fig. 5). The first one (G1) includes the populations of *C. nervosa* (C1, C2, C4 and C5) (Table 1). The most similar populations in terms of morphology are the ones from Rila Mts (C2) and Mt Vitosha (C4). They are characterized by the longest appendages of the involucre. The population from the Western Balkan Range has the lowest heights of the aboveground part of the stem. The medial width of the middle-stem leaves demonstrates the highest variability. The length of the middle-stem leaves is most constant in the population from the Central Balkan Range. That

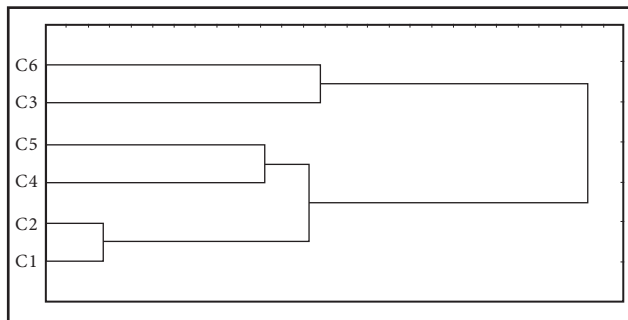


Fig. 5. Cluster analysis by population averages for determination of the uniform groups in *C. davidovii* and *C. nervosa*: C1, C2, C3, C4, C5 and C6 – population averages of the studied populations (see Table 1).

population also has the longest upper-stem leaves.

The overall variability of individual plants is high, both within populations and between them, but there is no discreteness in the metric characteristics of the studied traits.

The second well-established group (G2) includes the populations of *C. davidovii* (C3 and C6). The discriminant analysis showed that the achene length has the highest informative value for distinguishing the two groups. This length was 3–4 mm in *C. nervosa*,

and 5 mm in *C. davidovii*. Other important characteristics for the discrete grouping are: width of the involucre, medial length of middle-stem leaves, length of the uppermost leaf, and number of cilia on each side of the middle bracts. Features of no taxonomic value are: stem height, middle-stem leaf length, middle stem length, length of the bracts, achene width.

The high degree of morphometric discreteness between *C. nervosa* and *C. davidovii* correlates with data from the palynological and karyological investigations.

Pollen grains

The pollen grains of both species are of the *Jacea* type (Wagenitz 1955). The light microscopy and SEM studies of pollen morphology showed that the pollen of *C. nervosa* is of the microechinate type, with a spine height of 1–1.5 μm (Fig. 6). The pollen grains of *C. davidovii* are of the echinate type, with highly variable spine size, 3–5 μm (Fig. 7).



Fig. 6. SEM photograph of a pollen grain of *C. nervosa*.

A TEM investigation of *C. nervosa* and *C. davidovii* was carried out. Its results showed that both species have an exine of the cavate type (Figs 8, 9). The endexine is layered and 5–6 times thinner than the ec-

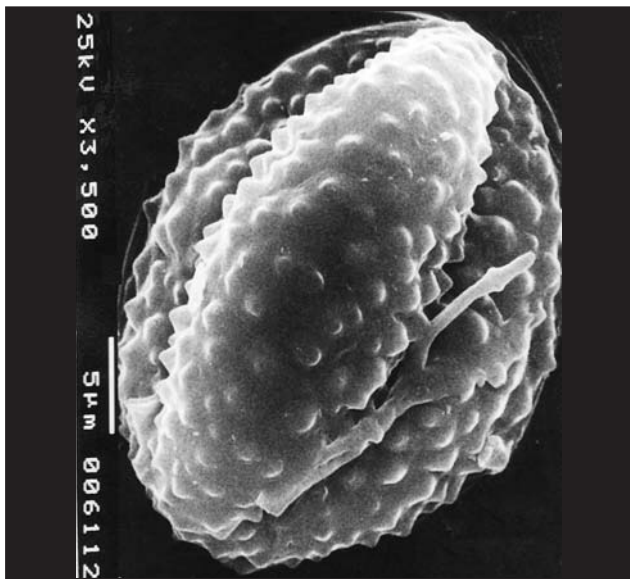


Fig. 7. SEM photograph of a pollen grain of *C. davidovii*.

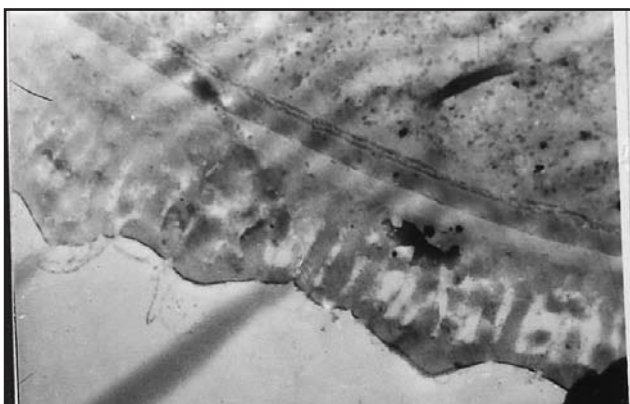


Fig. 8. TEM photograph of a pollen grain of *C. nervosa*.

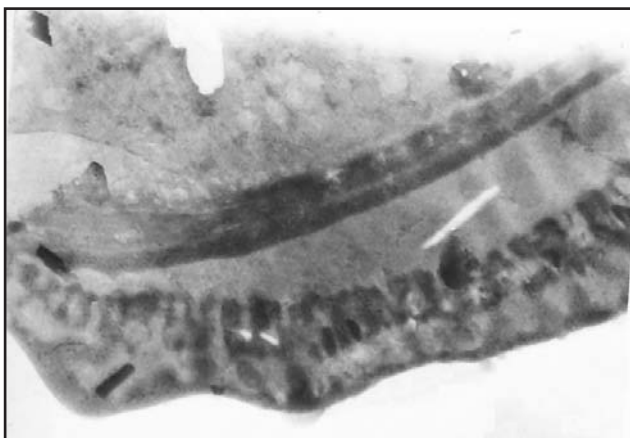


Fig. 9. TEM photograph of a pollen grain of *C. davidovii*.

texine. The foot layer is as thick as the endexine. Some columellae are connected at their sides in the areas between the spines, thus forming an incomplete outer tectum. The tectum is as thick as the exine and is perforated in the areas between spines.

Karyological studies

Karyological studies of two populations of *C. nervosa* and *C. davidovii* have been carried out (Sharkova 1996; Bancheva 1998, 1999; Bancheva & Greilhuber 2006). Two ploidy levels of $2n = 22$ and 44 have been detected. The populations of *C. nervosa* have a diploid chromosome number, whereas the two examined populations of *C. davidovii* have tetraploid chromosome numbers. Chromosomes of the sm-type prevail in the karyotypes of the taxa.

Distribution patterns

The general distribution of *C. nervosa* includes the Alps, the Apennines (*North*), the Carpathians (*South*) and the Balkan mountain systems (*North & Central*). In Bulgaria, the species is distributed in grassy places, meadows, pastures, and stony places in the sub-alpine belt, and seldom at the tree line in the Balkan Range (*Western*), Vitosha, Pirin, Rila, and Rhodopi Mts, at altitudes of (1500–) 1700–2700 m. It is reported incorrectly for Mt Osogovska (West Frontier Mts) and Mt Sredna Gora floristic regions, instead of *C. stenolepis*, and for Central Balkan Range, instead of *C. davidovii*.

Centaurea davidovii is a Bulgarian endemic distributed in meadows and pastures only in the floristic region of Balkan Range (*Western & Central*), at 1600–1800 m a.s.l.

Population and conservation status of *C. davidovii*

According to the *IUCN Criteria* (2001), *C. davidovii* should be classified as critically endangered: **CR B1ab(iii)+2ab(iii); C2a(ii)**.

Centaurea davidovii is a perennial entomophilous plant, currently represented by seven or eight very fragmented subpopulations, each with an area of occupancy of about 1–2 decares. The total number of individuals does not exceed 1000. During the field observations (1996–2008) we have noticed a significant reduction of the population size. For example, the population at Kozyata Stena locality at the beginning of our observations numbered about 200 individuals, whereas in 2008 only ten individuals have been found.

The main threat for distribution of the species is the succession changes. Cessation of grazing is a serious threat for a number of grassland species. This threat has usually been underestimated in Bulgaria. It leads to overgrowing of open grasslands with shrubs and trees and ultimately changes the habitat conditions and interactions among species.

Conclusions

The results obtained from the present comparative biosystematic study lead to the conclusion that *C. davidovii* is a very well differentiated species. We strongly recommend *C. davidovii* to be included in the Bulgarian Biological Diversity Act and some *in situ* and *ex situ* conservation actions to be taken.

Specimens seen:

C. davidovii

Morphological study:

SO. Balkan Range (Central): Botev peak (Yumrukchal, *V. Stribrny* – 78423); Triglav peak (Kademiya, *B. Barzakov* – 7841; *D. Jordanov* & *B. Achtarov* – 78413).

SOM. Balkan Range (Western): Murgash (*I. Urumov* – 84968, 84984, 84982, 84983, 84981, 84979, 84985); **Balkan Range (Central):** Troyanski Balkan (*I. Urumov* – 84975, 84977, 84978, 84980); Karlovski Balkan (*I. Urumov* – 84976, 84974, 84973; *A. Drenovski* – 84967, 84969); Mazalat (*B. Achtarov* – 90553); Gabrovski Balkan (*A. Yurkov* – 84875); Tikiyski Balkan (*I. Urumov* – 84972); Klisurski Balkan (*I. Urumov* – 84971); Botev peak (Yumrukchal, *I. Urumov* – 84970, *B. Achtarov* – 84986, 84988, 84987).

SOA. Balkan Range (Central): Karlovski Balkan (*B. Stoyanov* & *T. Georgiev* – 12323, 12333); **Balkan Range (Western):** Murgash (*V. Stribrny* – 18309).

Collection of the first author. Balkan Range (Central): near Kozyata Stena chalet (*S. Bancheva* – Sh9538); near Eho chalet (*S. Bancheva* – Sh9653); above Levski chalet (*S. Bancheva* – Sh9713); below Botev peak (*S. Bancheva* – Sh9714).

Pollen-morphological study. Balkan Range (Central): near Kozyata Stena chalet (Sh9538, *S. Bancheva*).

Karyological study. Balkan Range (Central): near Kozyata Stena chalet (Sh9538, *S. Bancheva*); near Eho chalet (Sh9653, *S. Bancheva*).

Comparative exsiccates. W: Bu, Kalofer Balkan, Jumrukchal (leg. *Schneider* & *Bergmann* – 20678, sub *C. gheorghieffii*); Bu, Balkan Range, Jumrukchal (leg. *Mandel* – 104, sub *C. gheorghieffii*).

C. nervosa

Morphological study:

SO. Balkan Range (Central): Rusalka peak (Mara Gidik, *I. Urumov* – 84288); **Mt Vitosha Region:**

(*S. Georgiev* – 78412; *D. Jordanov* – 78422); Cherni Vruh peak (*B. Kitanov* – 78409, 4876; *I. Nedev* & *D. Stoyanov* – 98984); **Pirin Mts:** Razlozhki Suhodol (*N. Stojanov* – 78415); **Rila Mts:** Smradlivoto Ezero (*D. Stoyanov* – 95304); Marichini Ezera (*V. Stribrny* – 78424); Mussala (*D. Jordanov* – 78421); Mermera (*M. Simova* – 78420); Urdina river (*Y. Koeva* – 78418); Kanarata (*S. Georgiev* – 78411).

SOM. Balkan Range: (*I. Neichev* – 84956, 84959); (*Western*): Petrohan (*I. Mrkvička* – 84865); Berkovski Balkan (*B. Achtarov* – 84881; ? – 84953); above Kom chalet (*B. Kuzmanov* – 132934); Kom (*I. Stamboliev* – 84950; *I. Drenovski* & *I. Urumov* – 84951); Midzhur (*I. Urumov* – 84957); **Mt Vitosha Region:** (*N. Stojanov* – 84874; *N. Stojanov* & *T. Georgiev* – 84930; *B. Davidov* – 84870; *I. Urumov* – 84954; *B. Kitanov* & *al.* – 84955); Chernata Skala (*V. Stribrny* & *al.* – 84872, 84869); Reznovovete (*N. Stojanov* & *B. Achtarov* – 84871); Kamen Del (*B. Achtarov* – 84967); Platoto (*A. Drenovski* – 84882); Aleko (*P. Stoyanov* – 144599); Skoparnika peak (*N. Andreev* – 136047); Chuipetlovo (*B. Kitanov* – 84952); Kominite (*B. Davidov* – 84941); Kumata (*B. Achtarov* – 84949; *N. Andreev* – 135466); **Pirin Mts:** (*D. Ilchev* & *al.* – 84948; *I. Urumov* – 84966, 84945); Spano Pole (*A. Drenovski* & *al.* – 84873); Yavorov chalet (*B. Kuzmanov* – 132472); Banderishki Ezera (*B. Davidov* – 84935); Vihren chalet (*N. Andreev* – 134112); Demyanitsa chalet (*V. Velchev* – 104442); Demirkapiya (*B. Davidov* – 84946, 84939); Bayuvi Dupki (*N. Andreev* – 138132); **Rila Mts:** (*N. Fenenko* – 84932, 84231; *V. Stribrny* & *al.* – 84943; *B. Davidov* – 84942, 84940, 84937, 84232, 84227, 84226, 84225); Belata Voda (*B. Davidov* & *al.* 84863, 84864); Levi Ibar (*B. Davidov* – 84868); Cherni Bor (*I. Mrkvička* – 84866); Mussala (*I. Bondev* – 108971; *B. Davidov* – 84958, 84960, 84961, 84944; ? – 117994); Parangalitsa (*N. Andreev* – 132121, 132096; *N. Fenenko* – 84965, 84962, 84964, 84931, 84929, 84933, 84934, 84228); Urdina river (*B. Davidov* – 84963); Sedemte Ezera (*B. Davidov* – 84230); Dupnishka Bistritsa (*B. Davidov* – 84947); Karabunar (*B. Davidov* – 84224); Ibar river (*B. Achtarov* – 84229).

HAU(PI). Mt Vitosha Region: Aleko chalet (*N. Vihodtsevski* – 03500). **Rila Mts:** Ivan Vazov chalet (*S. Stanev* – 09195); Sedemte Ezera (*S. Stanev* – 03497, 03498).

Collection of the first author. Balkan Range (Western): Midzhur (*S. Bancheva* – Sh9529); **Balkan Range (Central):** above Ribaritsa (*D. Peev* – Sh9624); **Mt Vitosha Region:** Ushite area (*S. Bancheva* – Sh9528); below Cherni Vruh (*C. Denchev* – Sh9667); **Pirin Mts:** Todorka peak (*D. Uzunov* – Sh9561); **Rila Mts:** Beli Iskar valley (*I. Kozuharova* – Sh9543); above Beli Iskar dam (*S. Bancheva* – Sh9629); Granchar chalet (*S. Bancheva* – Sh9628); above Malyovitsa chalet (*S. Bancheva* – Sh9631); Marichini Ezera (*S. Bancheva* – Sh9645); above Mussala chalet (*S. Bancheva* – Sh9646); below Ravni Chal peak (*S. Bancheva* – Sh9650); above Suhoto Ezero (*D. Stoyanov* – Sh9669); above Sedemte Ezera chalet (*S. Bancheva* – Sh9715); above Rilski Ezera chalet (*S. Bancheva* – Sh9721); above Lovna chalet (*S. Bancheva* – Sh9730).

For pollen-morphological study. Balkan Range (Central): above Ribaritsa (leg. *D. Peev* – Sh9624).

For karyological study. Balkan Range (Central): above Ribaritsa (leg. *D. Peev* – Sh9624); **Mt Vitosha Region:** Ushite area (*S. Bancheva* – Sh9528).

Comparative exsiccates. B: Bu, Rila Mts: Mussala (leg. *Degen* & *Wagner*, sub *C. plumosa* Lam.).

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