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Biosystematic studies of seven Balkan species from genus *Cyanus* (Compositae)

Abstract

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A critical taxonomic revision of seven species from sect. *Napulifera* of genus *Cyanus* – *Cyanus pseudoaxillaris*, *C. orbelicus*, *C. velenovskyi*, *C. napuliferus*, *C. nyssanus*, *C. tuberosus* and *C. thirkei* has been made. For each species descriptions of the overall morphology, pollen grains and karyotype as well as the distribution area are given. *Cyanus tuberosus* is recorded for the first time for Greece. It has been established that *C. nyssanus*, so far considered the most widely distributed species within the group, is in fact restricted to a small area in Eastern Serbia. The type of hair-covering of leaves has taxonomic value for distinguishing between *C. pseudoaxillaris* and *C. thirkei*. The pollen morphology of all species is of Montana-type (Wagenitz 1955). Pollen features support the species taxonomic rank of *C. pseudoaxillaris* and *C. tuberosus*. Two basic chromosome numbers, $x = 11$ and $x = 10$, have been found which show good correlation with the pollen features. *Cyanus tuberosus* is tetraploid. One B-chromosome has been observed in the karyotype of *C. thirkei*. The main evolutionary mechanisms in the group are discussed.

Introduction

Section *Napulifera* (Stef. & Georgiev) Bancheva & Raimondo (the status changed in this article) belongs to the genus *Cyanus* Mill. and comprises seven taxa - *Cyanus pseudoaxillaris*, *C. orbelicus*, *C. velenovskyi*, *C. napuliferus*, *C. nyssanus*, *C. tuberosus* and *C. thirkei*. The group is well distinguished from the other members of the genus *Cyanus* by its fusiform or napiform roots – adaptation to more xerophytic habitats; basal leaves in a rosette; reduced leaf size, often accompanied by deeply cut leaf blade with white-lanate hairing – adaptation for reduction of the evaporating area. The center of origin of the representatives of sect. *Napulifera* is the Balkan Peninsula. Even more - all species, except *C. thirkei*, are restricted to this region. In *Flora Europaea* (Dostal 1976a), and in all regional floras (Gajić 1975; Wagenitz 1975b; Strid & Kit 1991; Kožuharov 1992) the taxa from sect. *Napulifera* are placed in the genus *Centaurea* and have taxonomic rank different from the one mentioned above. Recent molecular studies - ITS sequence analysis of nuclear ribosomal DNA (Susanna & al. 1995; Häffner & Hellwig 1999; Garcia Jacas & al. 2000), ITS and *matK* sequence analysis (Garcia-Jacas & al. 2000) have proved the heterogeneity of the genus *Centaurea*. This requires its splitting into several smaller genera. As basis of the new taxonomic scheme of the *Centaureinae* (Greuter & al. 2001; Wagenitz 2003) serve

Table 1. Origin of the studied materials from Bulgaria.

Taxon	Origin	Coll. date	Collector
<i>Centaurea pseudoaxillaris</i>	Thracian plain, Bessaparski ridove loc., near Sinitevo vill, 490 m alt.	26. 05. 1998	Bancheva Sh9801
<i>C. orbelica</i>	Pirin Mt, above Banderitza hut, 1850 m alt.	22. 07. 1995	Bancheva Sh9562
	Pirin Mt, Sveta Elena peak, 1800 m alt.	10. 07. 1997	Bancheva Sh9761
<i>C. orbelica</i>	West frontier mountains, Osogovska planina Mt, above Gramadite loc.	11. 06. 1998	Valchev Sh9802
	Rila Mt, near Suhotozero leak, 1950 m alt.	12. 07. 1995	Bancheva Sh9524
	Rila Mt, above Grancher hut, 2350 m alt.	11. 07. 1996	Bancheva Sh9627
	Rila Mt, above Ivan Vazov hut	15. 08. 1997	Bancheva Sh9707
	Rila Mt, above Kirilova poljana loc., 1600 m alt.	14. 07. 1996	Bancheva Sh9662
	Rila Mt, above Skakavitza water fall.	09. 09. 1997	Bancheva Sh9719
	Rhodope Mts, Mursalitza loc., below Chaeva chuka peak, 1800 m alt.	08. 08. 1998	Vladimirov Sh9830
<i>C. napulifera</i>	Stara planina Mt (Central), near Planinski izvori hut, 1700 m alt.	04. 08. 1995	Bancheva Sh9605
	Stara planina Mt (Central), below Vezhen peak, 1800 m alt.	13. 07. 1996	Bancheva Sh9635
	Stara planina Mt (Central), near Vasil Levski hut, 1600 m alt.	03. 08. 1997	Bancheva Sh9724
	Stara planina Mt (Central), on path from Vasil Levski hut to Rai hut.	23. 08. 1997	Bancheva Sh9711
	Stara planina Mt (Central), open grassy places above Vasil Levski hut.	23. 08. 1997	Bancheva Sh9737
	Stara planina Mt (Central), near Rai hut.	24. 08. 1997	Bancheva Sh9712
	Stara planina Mt (Central), above Vezhen hut.	24. 07. 1998	Bancheva Sh9827
<i>C. napulifera</i> subsp. <i>velenovskii</i>	Stara planina Mt (Central), Djuzata loc	24. 08. 1997	Bancheva Sh9733
<i>C. tuberosa</i>	Belasitza Mt, above Kongor peak, in meadows at c. 1800-2000 m alt.	08. 06. 1997	Bancheva Sh9726
	Pirin Mt, below the peak Vihren, 2000—2300 m alt.	07. 07. 1995	Bancheva Sh9521
	Slavjanka Mt., open grassy places above village of Paril, 1350-1800 m alt.	18. 06. 1995	Bancheva Sh9514
	Slavjanka Mt., Kojnarite loc., open grassy places, 1700 m alt.	28. 08. 1996	Bancheva Sh9660
	Slavjanka Mt., open grassy places from Vapata loc. and Kojnara peak, c. 1400-1800 m alt.	10. 07. 1998	Bancheva Sh9821
<i>C. thirkei</i>	Black Sea coast (Northern), near Balgarevo village, c. 70 m alt.	17. 05. 1996	Bancheva Sh9604
	Black Sea coast (Northern), Kaliakra promontory.	21. 05. 1996	Dimitrov Sh9606
	Stara planina Mt (Eastern), Kachula loc., c. 900 m alt.	18. 06. 1996	Bancheva Sh9616
	Rhodopes Mts (Eastern)	15. 05. 1997	Petrova Sh9760

the Wagenitz's pollen-type classification and the base chromosome numbers (Wagenitz & Hellwig 1996) and the newly accumulated molecular data. The new taxonomic scheme confirms the genus rank of *Cyanus*. The aims of the present study are clarification of the distribution and current status of the taxa of the sect. *Napulifera* and discussion of the main evolutionary trends within the group.

Material and methods

The research is based on material collected from different floristic regions of Bulgaria, where the studied taxa are naturally distributed. Material from 27 localities has been gathered (Table 1). Altogether 721 samples have been studied, vouchers of which are kept in the Bulgarian herbaria - SO, SOM, SOA, Herbarium of the Agrarian University in Plovdiv [HAU(Pl)] and Herbarium of National History Museum in Plovdiv [NHU(Pl)], as well as in the Herbarium of the Goulandris Natural History Museum (ATH) in Athens, Greece, the Herbarium of National History Museum (BEO) in Belgrade, Yugoslavia, the Herbarium of Institute of Botany in Belgrade (BU) and in the personal collection of the first author. Also comparative material from the herbaria: B, BP, FI has been used.

Comparative morphological approach, including investigation on overall morphology, morphology of leaves and pollen grains together with karyological, ecological and geographical studies, as well as some observations on the population status has been applied.

A key for the accepted taxa in sect. *Napulifera* is included.

For the preparation of the gross-morphological descriptions quantitative and qualitative features have been chosen. The selection is a result of the estimation of the taxonomic value of these features in some taxonomic works on *Centaurea* s.l. (Routsi & Georgiadis 1994), *Centaureinae* (Wagenitz & Hellwig 1996), *Cardueae* (Häffner 2000), as well as on personal judgment.

The pollen morphology has been studied by light and scanning electronic microscopes (SEM). The material for the light microscopy has been prepared after Faegri & Iversen (1975). The feature matrix is conformed to the matrix suggested by Leonardis & al. (1984). The preparation of the pollen grains and the leaves for observation in SEM has been accomplished according to Huttunen & Laine's (1983). Observations were carried out with a SEM Leica S420 at 15 kV. The morphological terminology of Skvarla & Turner (1966), Skvarla et al., Walker (1974) and Faegri & Iversen (1975) have been used.

The studies of the karyotypes have been made on metaphase plates of primary meristem of young root tips by the method recommended by Vogt & Oberprieler (1993). As a stain haematoxylin after Gomory (Melander & Wingstrand 1953) has been used. The karyotypes have been determined by the scheme of Levan & al. (1964) on the basis of 3-5 metaphase plates. The karyological vouchers have been deposited in SOM. The abbreviations of the authors follow Brummitt & Powell (1992). The chorological data of the taxa are a summary of the literature information, the revised herbarium specimens and the personal collections and observations.

Results

In the present investigation the following taxa are accepted:

Genus *Cyanus* Mill.

Sect. *Napulifera* (Stef. & Georgiev) Banchева & Raimondo, stat. nov.

Basionym: subsect. *Napuliferae* Stef. & Georgiev, Rev. Acad. Bul. Sci., XLIV (1931) 146.

Cyanus pseudoaxillaris (Stef. & Georgiev) Holub

Cyanus orbelicus (Velen.) Soják

Cyanus velenovskyi (Adamović) Wagenitz & Greuter

Cyanus napuliferus (Rochel) Soják

Cyanus nyssanus (Petrovič) Bancheva & Raimondo, comb. nov., Basionym: *Centaurea nyssana* Petrovič, Addit. Fl. Agr. Nyss. (1885) 110.

Cyanus tuberosus (Vis.) Bancheva & Raimondo, comb. nov., Basionym: *Centaurea napulifera* Vis., Fl. Dalm., II (1847) 33.

Cyanus thirkei (Sch. Bip.) Holub

Key

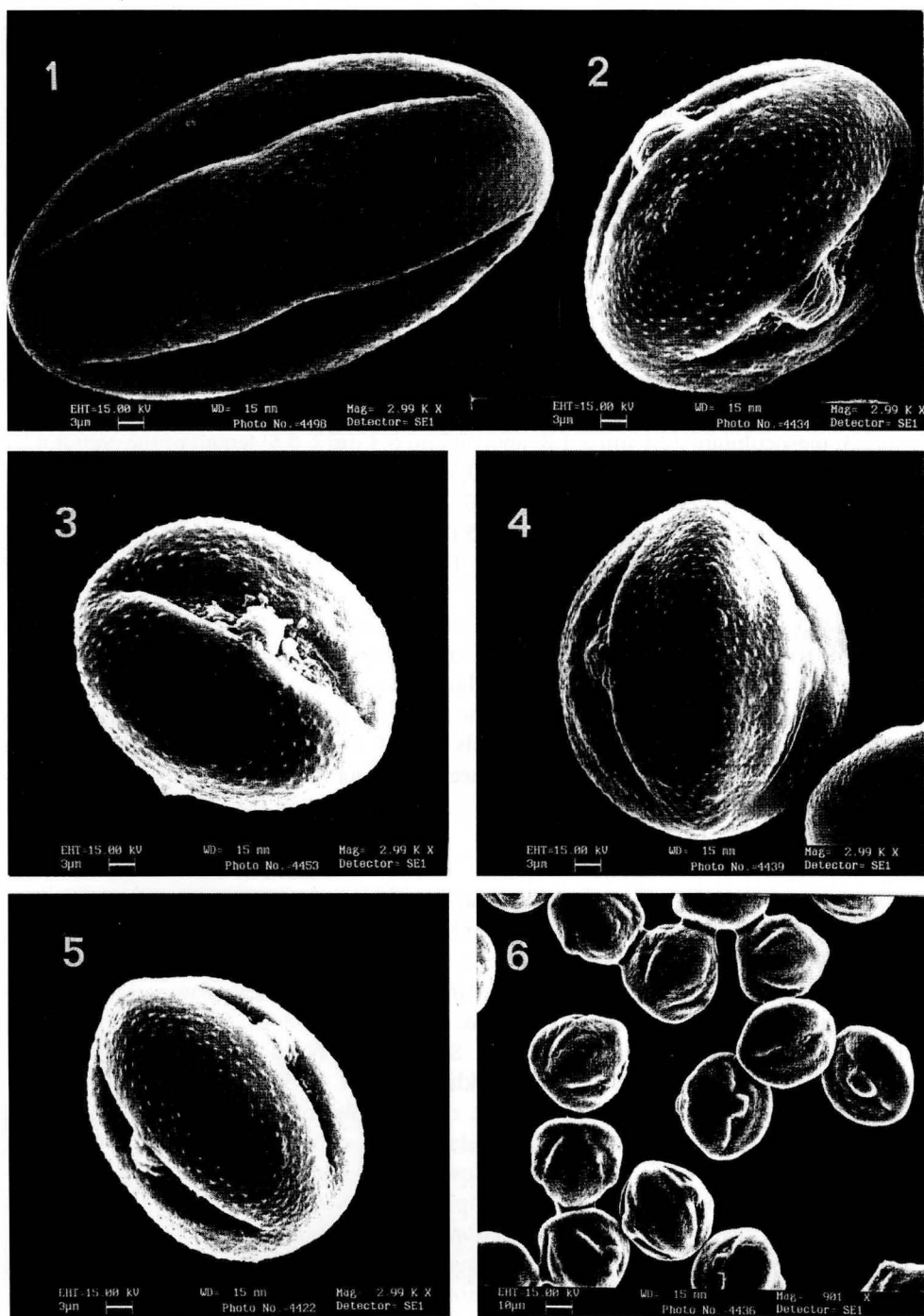
- 1 Outer florets cornflower-blue or purple-violet 2.
- 2 Outer florets cornflower-blue *C. velenovskyi*
- 2* Outer florets purple-violet 3.
- 3 Roots up to 20 cm, narrowly fusiform with very long, slender apex; basal leaf rosette absent in anthesis *C. pseudoaxillaris*
- 3* Roots up to 5 cm, fusiform with a short apex; basal leaf rosette present in anthesis *C. tuberosus*
- 1* Outer florets pink or pale cream 4.
- 4 Rhizome short, without stolones, roots crowded, fusiform or napiform 5.
- 5 Roots fusiform gradually narrowing in a short apex; flowering VI-VII *C. tuberosus*
- 5* Roots napiform; abruptly narrowing in a short apex; flowering III-V *C. thirkei*
- 4* Rhizome long-creeping, sometimes with stolons; roots spreading, fusiform 6.
- 6 Outer florets pink *C. napuliferus*
- 6* Outer florets pale cream 7.
- 7 Leaves 1-2 mm wide, glabrous and shining on the upper surface, white-arachnoid on the lower surface *C. nyssanus*
- 7* Leaves (3) 5-11 mm wide, ± densely tomentose on both surface *C. orbelicus*

(1) *Cyanus pseudoaxillaris* (Stef. & Georgiev) Holub, Preslia, 45 (1973) 145. Basionym: *Centaurea pseudoaxillaris* Stef. & Georgiev, Rev. Acad. Bul. Sci., XLIV (1931) 170; Stoj. & Stef. Fl. Bulg., ed 2 (1933) 1036; *Centaurea napulifera* subsp. *pseudoaxillaris* (Stef. & Georgiev) Stoj. & Acht., Cent. Bulg. (1935) 21; Dostal, Fl. Eur., IV (1976) 300.

Lectotypus: (designated here). In graminosis ad Nova Zagora, leg. V. Stribrni sub *Centaurea variegata* Lam. (SOA12097!). Isolectotypi: (SOA12098!, SOA12101!). Paralectotypi: Ad Sotira prope Tatar Pazardjik, leg. V. Stribrni sub *Centaurea napulifera* Rochel in Friv (SOA12100!); Ad Haskovo, in declivibus siccis prope pagum Vakav ad Kavakli, leg. N. Stojanoff, B. Stefanoff et T. Georgieff (SOA12099!).

Centaurea napulifera sensu Velen., Fl. Bulg., Suppl. I (1898) 171, non Rochel.

Hemicryptophyte. Rhizome short; roots up to 20 cm, narrowly fusiform with very long, slender apex. Stem simple, rarely little branched, erect, 15-25 (-30) cm high, white



Figs 1-6. Pollen grains of: 1. *Centaurea pseudoaxillaris*; 2. *C. orbelica*; 3. *C. napulifera* subsp. *napulifera*; 4, 6. *C. tuberosa*; 5. *C. thirkei*.

arachnoideus or lanate. Lower leaves elliptic or lyrate, undivided to pinnatifid not decurrent; the middle and upper leaves lanceolate or linear-lanceolate, entire; the most upper leaves longer, rarely shorter than the capitula. Leaves white-lanato-tomentose. Hair-covering is presented by 15- 16-celled hairs, strongly widen at their basis (about 10 times wider than in their middle part) (Fig. 7). Capitula single, rarely 2-3; wide (2,5-) 3-3,5 (-4) cm.

Involucre 13-20 mm long and 9-13 mm wide. Phyllaries light green, wide-triangle to semi-elliptical. Appendages glabrate to spread hairy with decurrent dark brown margin; fimbriae with brown basis and pale yellow apex. The inner florets purple to dark pink, with purple stamens. The outer florets radiance, purple-violet, separated in 4-6 triangle lobes. Achenes 4-5 mm long, with a lateral elaiosome. Pappus about 2,5 mm long, about twice shorter than the achene, with 2 rows of biserate bristles.

The pollen grains are of Wagenitz's Montana-type (1955), prolate, with correlation polar axis/ equatorial diameter 1,9. The exine sculpture is scabrate, with density of the sculpture elements 3-4 to 9 square μm . Equatorial diameter $35,1 \mu\text{m} \pm 1,5$; polar axis $65,4 \mu\text{m} \pm 3$; mesocolpium $22,1 \mu\text{m} \pm 1,8$; apocolpium $6,3 \mu\text{m} \pm 0,8$; length of the colpus $43,1 \mu\text{m} \pm 3,4$; breadth of the colpus $2,3 \mu\text{m} \pm 0,6$; length of the pore $5,9 \mu\text{m} \pm 1,2$; height of the exine in the equatorial area $3,3 \mu\text{m} \pm 0,7$; height of the exine in the polar area $2,1 \mu\text{m} \pm 1,2$; height of the coste colpi $2,4 \mu\text{m} \pm 1,5$ (Fig. 1).

Karyotype: $2n = 2x = 8m + 14sm = 22$. The chromosomes are with small size (1,5-2 μm) (Bancheva 1999).

At grassy places and at the periphery of stony meadows in oak and mixed forests and brushwood, in plains and in front of the mountains. Flowering V-VI. Thracian plain, Tundza hilly region from 100 to 450 m. Bulgarian endemic.

The species is exclusively rare. In fact, the species is presently known only from 2 sites and consists of not more than 150 individuals in total. In a result of personal observations, it has been determined that one of the main mechanisms for the seed dispersal is the myrmecochory.

Specimina visa:

For morphological description: Bulgaria: - *Tundza hilly region*: SO-78381, SO-78946, SO-78945, Voynishkia Bakadzik, Jambol distr., leg. et det. D. Jordanov; SO-78394, SO-78384, Manastirskite vazvishenia hills, leg. et det. D. Jordanov; SO-78383, Sv. Iliyskite visochini hills, leg. et det. D. Jordanov; SO-91272, Sacar planina Mt., near the Matochina village, leg. et det. D. Jordanov & A. Janev; SO-78927, SO-78928, Derventski visochini hills leg. et det. D. Jordanov, SOA-18227 Nova mahala, leg. ed det. I. Markvichka; SOA-12099, Vakav and Kavakli leg. et det. N. Stojanov et al.; SOA-12097, SOA-12098, SOA-12101, Nova Zagora, leg. et det. V. Stribrni, SOM-84927, Nova mahala /?/; SOM-84921, SOM-84924, Nova mahala, leg. ed det. I. Markvichka; SOM-84922, SOM-84928, SOM-84926, SOM-84925, Nova mahala leg. et det. V. Stribrni, HAU(PI)-29608, Sakar planina mt., leg. et det. Delipavlov, FI-Nova Mahala loc., leg. et det. V. Stribrni, 21. 05. 1898, sub *Centaurea napulifera*; FI-Ad graminosis ad Nova Mahala, leg. et det. V. Stribrni, 10. 06. 1897, sub *Centaurea napulifera*; FI-Ad campi ad Nova Mahala, leg. et det. V. Stribrni, may 1900, sub *Centaurea napulifera*; *Thracian plain*: SOM-137982, Ovchi halmove hills, leg et det. S. Stanev; SOM-84919, Plovdiv distr., leg et det. I. Urumov; SOM-84923, Haskovo distr., /?/; SOM-84920, Stara Zagora distr., leg. et det. B.

Achtarov, SOM-Sh9801, Besaparski ridove hills, leg. et det. S. Bancheva, SOA-12100, Tatar Pazardzik, leg. et det. V. Stribni, NHM(PI)-08025, NHM(PI)-08027, Besaparski ridove hills, leg. et det. St. Stanev; NHM(PI)-09495, NHM(PI)-09497, NHM(PI)-09496, Ovchi halmove hills, leg. et det. St. Stanev, HAU(PI)-40573, near the Parvenetz village, Plovdiv distr., leg. et det. Delipavlov.

For palinomorphological description: SOM - 137982, *Thracian plain*, Ovchi halmove hills leg. et det. St. Stanev.

For karyological investigation: SOM - Sh9801, *Thracian plain*, Besaparski ridove hills, leg. et det. S. Bancheva.

(2) *Cyanus orbelicus* (Velen.) Soják. Čas. Nár. Muz., Odd. Přír. 140 (1972) 131.

Basionym: *Centaurea orbelica* Velen., Sitzungsber. Böhm. Ges. Wiss. Prag. 1 (1890) 51; Velen., Fl. Bulg., Suppl. I (1898) 170; *Centaurea nyssana* subsp. *orbelica* (Velen.) Hayek in Stoj. & Stef., Fl. Bulg. 2 (1925) 1181; *Centaurea napulifera* subf. *orbelica* (Velen.) Stoj. & Acht., Cent. Bulg. (1935) 20.; *Centaurea cheiranthifolia* subsp. *borisii* Turill & Stoj., Mittel. König. Nat. Wiss. Inst., IV (1931) 154; Holotypus: "In Persenk, mt. Rhodope, VI.1930, leg. S.M. Boris III" (SOA 12 119 !).

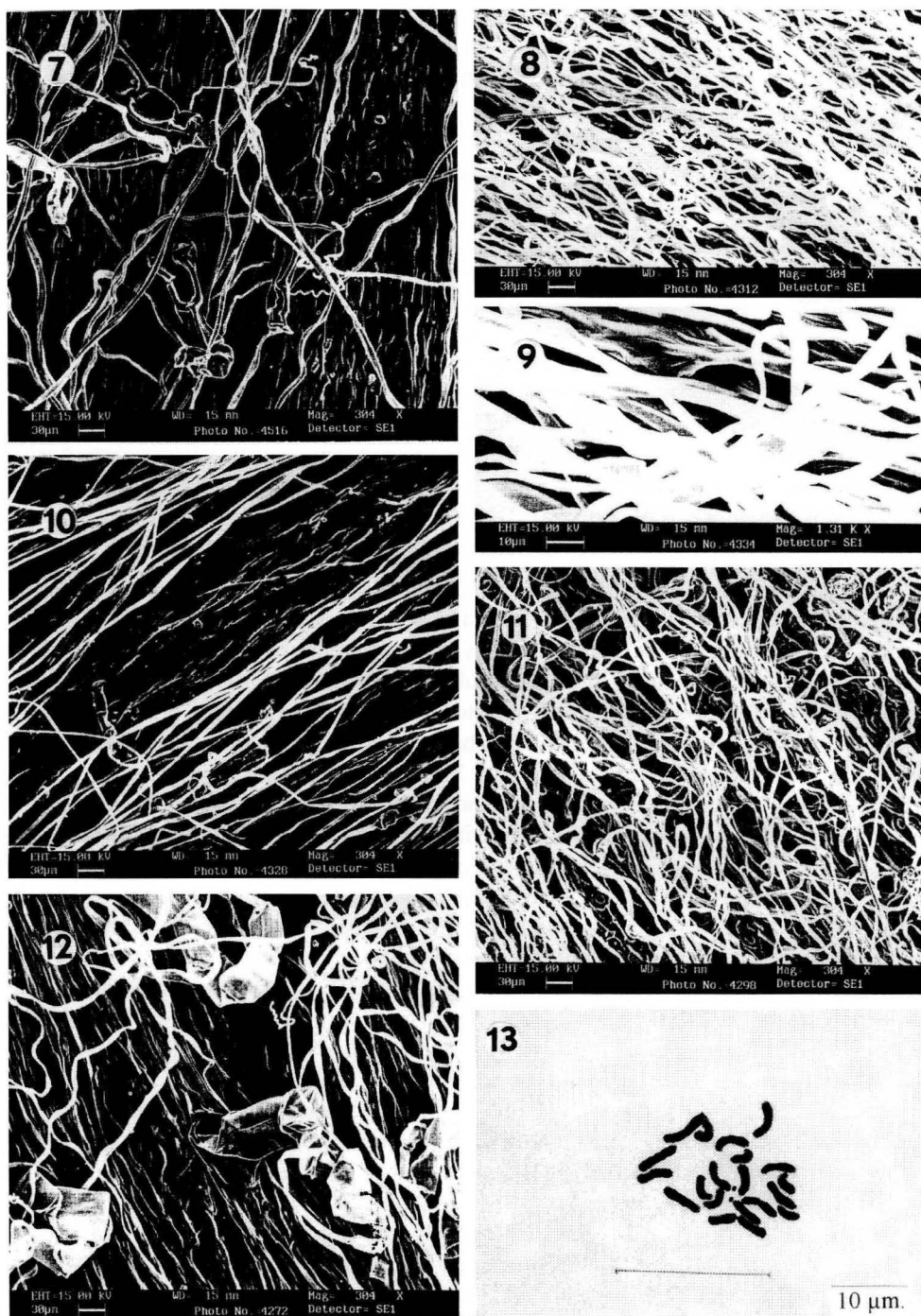
Centaurea nyssana auct. bulg., non Petrovic.

Hemicryptophyte. Rhizome long 10-30 cm, creeping, with or without stolons, roots up to 5 cm, fusiform. Stem simple, erect, 10-40 cm high, gray-arachnoideus. Leaves grey tomentose; lower linear-lanceolate or lyrate lobed, entire or remotely dentate, forming or not basal rosette. The middle and upper cauline leaves lineal, 35-90 mm long and 4-11-mm wide, entire or remotely dentate, grey-tomentose. Hair covering is presented by 3-4 celled hairs, which is approximately with the same width along their length (Figs 8, 9). Capitula 35-48 mm wide. Involucre ovoid, 17-20 mm long, 10-15 mm wide. Phyllaries triangle to ovoid, glabrate or with single short hairs, with 5-7 clearly noticeable nerves. Appendages glabrate or with spread hairy with decurrent, light to dark brown, 1 mm wide margin, the fimbriae with brown basis and pale yellow or silver apex. Inner florets pale cream with purple-violet stamens. Outer florets radiant, pale cream, divided into 4-7 narrow triangle lobes. Achenes 4-5 mm long and 2-3 mm wide, with lateral elaiosome; pappus well developed, 2-2,5 mm long, with 2 rows of biserate, bristles.

The pollen grains are of Wagenitz's Montana-type (1955), with correlation polar axis/equatorial diameter – 1,4. The exine sculpture is scabrate with density of the sculpture elements 4-6 to 9 square μm . Equatorial diameter $29,8 \mu\text{m} \pm 2,4$; polar axis $40,7 \mu\text{m} \pm 2,4$; mesocolpium $20,3 \mu\text{m} \pm 1,7$; apocolpium $6,9 \mu\text{m} \pm 0,8$; length of the colpus $32,9 \mu\text{m} \pm 2,1$; breadth of the colpus $2,7 \mu\text{m} \pm 0,8$; length of the pore $5 \mu\text{m} \pm 0,8$; height of the exine in the equatorial area $4 \mu\text{m} \pm 0,5$; height of the exine in the polar area $2,8 \mu\text{m} \pm 0,5$; height of the coste colpi $2,6 \mu\text{m} \pm 0,7$ (Fig. 2).

Karyotype: $2n = 2x = 4m + 12sm + 4sm\text{-SAT} = 20$ (Sharkova 1996) as *Centaurea napulifera* subsp. *nyssana*. The same chromosome number has been announced also by Kuzmanov & Georgieva (1987, 1990) on materials from Pirin mountain.

At meadows and pastures, in the mountains and in front of the mountains. Stara Planina Mt., Vitosha Mt., West frontier Mts, Pirin Mt., Rila Mt., Rhodope Mts (West), Belasitza Mt. from 800 to 2100 m s.m. Flowering VI-VII. For Sredna Gora Mt. it has been pointed



Figs 7-12. SEM of hair covering of: 7. *Centaurea pseudoaxillaris*; 8, 9. *C. orbelica*; 10. *C. napulifera*; 11. *C. tuberosa*; 12. *C. thirkei*. Fig. 13. Karyotype of *C. napulifera* subsp. *napulifera*.

out as *Centaurea napulifera* subforma *orbetica* (Stojanov & Achtarov 1935). It has been wrongly pointed out for Slavjanka Mt. (Stojanov 1921), instead of *Cyanus tuberosus* and for the Black Sea coast (North) (Velenovsky 1902) and the Thracian plain (Toshev 1895), instead of *C. thirkei*.

General distribution. Balkan Peninsula (Bulgaria, N. Greece, Macedonia, Yugoslavia).

Specimina visa

For morphological description: Bulgaria: - *Vitosha region*: SO-84290, Lyulin planina, leg. et det. B. Achtarov; Vitosha Mt. /?/; SOM-84852, SOM-82854, Ljulin Mt, leg. et det. Zollikofer, SOM-84819, Ljulin Mt, leg. et det. N. Stojanov; SOM-84858 Ljulin Mt, leg. et det. Toshev; SOM-84816, Ljulin Mt, leg. et det. Achtarov; SO-91110, *West frontier Mts region*, Vlahina planina, leg. et det. B. Kitanov; *Pirin Mt. Region*: SO-78703, Pirin Mt., leg. et det. N. Stojanov; SO-78390, Pirin, leg. et det. N. Stojanov; SO-78389, Pirin, leg. et det. D. Jordanov et B. Achtarov, SOM-Sh9562, Pirin, above Banderitza hut, leg. et det. S. Bancheva; SOM-Sh9761, Pirin Mt, Sveti Elena peak, 1800 m alt., leg. et det. S. Bancheva; SOM-84821, SOM-84824, Pirin, Banderitza, leg. et det. Davidov; SOM-138092, Pirin, Bajuvi dupki Reserve, leg. et det. N. Andreev; SOM-84861, Pirin, leg. et det. B. Achtarov; SOM-84851, Pirin, peak Baba, leg. et det. A. Toshev; SOM-84839, SOA-12138, Pirin, leg. et det. V. Stribrni; SOA-84849, *Rila Mt. region*: SO-84706, Rila Mt., leg. et det. N. Andreev et T. Meshinev; SO-78393, Rila, leg. et det. St. Georgiev; SO-78387, Rila, leg. et det. N. Vihodzevski; SO-78391, Rila, leg. et det. I. Neichev; SO-78392, Rila, leg. et det. V. Stribrni; SO-84291, Rila leg. et det. leg. et det. V. Stribrni, Rila, leg. et det. B. Davidov; SOM-84846, Rila, leg. et det. B. Kitanov; SOM-84820, SOM-84818, SOM-84841, Rila, leg. et det. B. Achtarov; SOM-134264, Rila, Parangalitza, leg. et det. Andreev, Meshinev sub *Centaurea napulifera*; SOM-84827, Rila, Maritza /?/; SOM-84832, Rila, Ribni ezera lakes leg. et det. Toshev; SOM-84779, Rila, leg. et det. B. Davidov; SOM-84823, Rila, above Mussala peak, leg. et det. I. Mrkvicka, SOM-84847 Rila, leg. et det. B. Davidov; SOM-84844, Rila, Belmeken, /?/; SOM-84826, Rila /?/; SOM-84840, SOM-84817, SOM-84825, SOM-84776, SOM-84828, SOM-84829, SOM-84838, SOM-84843, SOM-84836, SOM-84837, SOM-84835, SOM-84845, SOM-84775, SOM-84830, SOM-84834, SOM-84842, Rila, leg. et det. B. Davidov; SOM-Sh9524, Rila, Suhoto ezero, leg. et det. S. Bancheva; SOM-Sh9627, Rila, above Granchar hut, leg. et det. S. Bancheva; SOM-Sh9662, Rila, above loc. "Kirilova polyana", leg. et det. S. Bancheva; SOM-Sh9707, Rila, above Ivan Vazov hut, leg. et det. S. Bancheva; SOM-Sh9719, Rila, above Skakavitza hut, leg. et det. S. Bancheva, NHM(PI)-9329, Rila Mt., leg. et det. St. Stanev; NHM(PI)-3496, Rila, leg. et det. St. Stanev, BU-Rila Mt., In m. Mussala, leg. et det. Stribrny; *Rhodope region*: SO-78385, Rhodope (West) leg. et det. D. Jordanov et B. Achtarov, SOM-84788, ? /?/; SOM-84765, SOM-84766, SOM-84767, SOM-84769, SOM-84768, SOM-84774, SOM-84772, SOM-84773, SOM-84771, SOM-84770, SOM-84833, Rhodope Mts, leg. et det. I. Urumov, sub *Centaurea velenovskyi*, SOM-84849, Rhodope, leg. et det. Drenovsky, SOM-102785, Rhodope, Kurtovo, leg. et det. Kitanov; SOM-Sh9830, Rhodope, Moursalitza, leg. V. Vladimirov, Rhodope, Drenovski et I. Urumov; SOA-102783, SOA-102782, SOA-102785, SOA-102784, Rhodope (West), leg. et det. B. Kitanov; SOA-12119, Rhodope, peak Persenk, leg. et det. Tzar Boris III, BP-572999, BP-486913, sub *C. velenovskyi*, Bulgaria, in montibus Rhodopae ad Filibilibiskata poljana, leg.

et det. I. Urumov, 1907; BP-482917, sub *C. velenovskyi*; *Stara planina Mts region*: SOM-84784, Ambaritza, leg. et det. I. Neichev; SOM-84850, Mourgash, leg. et det. I. Urumov; SOM-153409 Stara planina Mt, near Dobrila hut, leg. et det. Meshinev & Apostolova; *Znepole region*: SOM-84822, SOM-84859 Milevska planina, leg. et det. Achtarov; *West frontier Mts region*: SOM-84848, Osogovska planina Mt, leg. et det. I. Urumov; SOM-Sh9802, Ossogovska planina Mt., leg. et det. V. Valchev; SOM-139538, SOM-139533, Osogovska planina, leg. et det. D. Jorsanov. **Greece**: ATH-Rhodopi Mts, N of Zagradenia forest station, near the Bulgarian border, leg. et det. Strid sub *C. napulifera* subsp. *nyssana*. **Macedonia**: BEO-26257, Crna Reka, Vrbsko-Vitaliste; BEO-26260, Korab Mt., Crna cuka; BEO-26271, Korab Mt., Vraca; BEO-26262, BEO-26263, BEO-26267, Perister Mt., Bitolja; BEO-26266, Perister, supra Magarevo; BEO-26273, Perister, Begova Cesma; Porece, Breznica kula, FI-Skopje: In pratis montanis in monte Horne Vodno supra urbem, 900 m s.m., leg. et det. Fr. Cernoch, 19. 05. 1969, sub *Centaurea napulifera* subsp. *napulifera* var. *orbelica* (Vel.) Stoj. et Acht. **Serbia**: BEO-26256, Kopaonik Mt., Karaman; BEO-26261, BEO-26272, Kopaonik Mt., Panchichev peak; BEO-32143, BEO-32144, Kopaonik Mt., Suvo Rudiste, BP-Monte Midzor (Stara planina), 2000 m alt., VII, leg. et det. Adamovic, BU-Kopaonik Mt., Mali Karman, leg. et det. Lacusic, 4 sheets; BU-Kopaonik, Suvo Rudishte, leg. et det. Kosanin, 2 sheets.

For palinomorphological description: SOM - Sh9627, Rila, above Granchar hut, leg. et det. S Bancheva

For karyological investigation: SOM-Sh9524, Rila, Suhoto ezero, leg. et det. S. Bancheva; SOM-Sh9830, Rhodope, Moursalitza, leg. V. Vladimirov.

(3) *Cyanus napuliferus* (Rochel) Soják, Čas. Nár. Muz., Odd. Přír., 140 (1972) 131. Basionym: *Centaurea napulifera* Rochel in Friv., Magyar. Tud. Társ. Évkon. II (1835) 250; Velen., Fl. Bulg. (1891) 310; Hayek in Stoj. & Stef., Fl. Bulg., ed. 1, II (1925) 1181; Hayek, Prodr. Fl. Penins. Balc., II (1931) 741; Stef. & Georgiev., Rev. Acad. Bul. Sci., XLIV (1931) 171; Stoj. & Acht., Cent. Bulg. (1935) 18; Dostal, Fl. Eur., IV (1976) 300; *Centaurea karlovensis* Friv. ex Hampe, Flora, XX (1837) 228; *Centaurea adamovicii* Velen., Fl. Bulg., Suppl. I (1898) 173.

Lectotypus: *C. napulifera* Rochel, "Bulgaria, Balkan mt., leg. Frivaldsky" (BP 466 100!) (designated by E. Gamal-Eldin).

Hemicryptophyte. Rhizome 10-30 cm long, creeping, with or without stolons, roots up to 5 cm, fusiform. Stem simple, erect, 3-15 cm high, sometimes almost absent, gray-arachnoideus. Lower leaves at basal rosette, linear-lanceolate, usually entire, rarely remote-dentate. Middle and upper cauline leaves linear, 35-60 mm long and 3-11 mm wide, entire, usually slightly revolute outside, grey-tomentose. Hair covering is presented by 3-celled hairs which are 2 times wider at their basis (Fig. 10). Capitula 35-45 mm wide.

Involucre cylindric to narrowly ovoid, 17-20 mm long, 7-10 mm wide. Phyllaries narrowly triangle, glabrate or with single short hairs, with 5-7 clearly noticeable nerves. Appendages glabrate or with single short hairs, with decurrent, dark brown margin, 0.5-1 mm wide; fimbriae with brown basis and silver apex. Inner florets pink, with purple-violet stamens. Outer florets radiate, pink, divided into 4-7 narrowly triangle lobes. Achenes 4-5

mm long and 2-3 mm wide, with lateral elaiosome; pappus well developed, 2-2.5 mm long, with 2 rows of biserate bristles.

The pollen grains are of Wagenitz's Montana-type (1955), subprolate, with correlation polar axis/ equatorial diameter 1,3. The exine sculpture is of a scabrate type with density of the sculpture elements 3-4 to 9 square μm . Equatorial diameter $30,8 \mu\text{m} \pm 2,3$; polar axis $39,9 \mu\text{m} \pm 2,4$; mesocolpium $21,2 \mu\text{m} \pm 2,3$; apocolpium $7,1 \mu\text{m} \pm 0,3$; length of the colpus $28,3 \mu\text{m} \pm 2,1$; breadth of the colpus $3,5 \mu\text{m} \pm 1,3$; length of the pore $5,7 \mu\text{m} \pm 1$; height of the exine in the equatorial area $4,3 \mu\text{m} \pm 0,5$; height of the exine in the polar area $2,8 \mu\text{m} \pm 0,6$; height of the coste colpi $2,5 \mu\text{m} \pm 0,2$ (Fig. 3).

Karyotype: $2n = 2x = 20$. The chromosomes of sm-type predominate. Also a SAT-chromosome pair can be observed (Fig. 13). The results correspond to the data for studied population from Macedonia (Šopova & Sekovski 1982).

At meadows, pastures, rocky slopes in the subalpine belt, 1700-2400 m. Flowering VII-VIII. Stara planina Mts (West, Central), Rila Mt. It has been pointed out for Rhodope Mts (Boissier 1875) and West frontier mountains (Urumov 1935). Wrongly it has been announced for Tundza hilly region (Velenovsky 1898) and Thracian plain (Podpera 1902), instead of *Cyanus pseudoaxillaris*, as well as for Black Sea coast (Urumov 1913), instead of *Cyanus thyrekei*.

General distribution. Balkan Peninsula (Bulgaria, Greece, Macedonia, Yugoslavia).

Specimina visa:

For morphological description: Bulgaria: — *Stara planina region*: SO-78388, Stara planina Mts (Central), leg. et det. D. Jordanov; SO-95828, Stara planina Mts (West), leg. et det. B. Mihov; SO-95829, Stara planina Mts (West), Vrachanska planina, leg. et det. V. Shulev; SO-78315, Stara planina Mts (Central), leg. et det. B. Kitanov et I. Penev; SO-78386, Stara planina Mts (Central), leg. et det. N. Atanasov et N. Vihodzevski; SO-95998, SO-146814, Stara planina Mts (Central), leg. et det. B. Kitanov, SOM-84814, Stara planina Mts (Central), Kozyata stena, leg. et det. I. Urumov; SOM-84783, SOM-84786, SOM-84802, SOM-84797, Stara planina Mts (Central), Karlovski balkan, leg. et det. I. Urumov; SOM-84781, SOM-84791, Stara planina Mts (Central), Kaloferski balkan /?/; SOM-84813, Stara planina Mts (Central), Kozyata stena, leg. et det. I. Urumov; SOM-84856, SOM-85024, Stara planina Mts (Central), Gabrovski balkan, leg. et det. A. Yurkovski; SOM-84796, ? /?/; SOM-84795, SOM-84794, SOM-84793, SOM-84801, Stara planina Mts (Central), Troyanski balkan, leg. et det. I. Urumov; SOM-84804, SOM-84803, Stara planina Mts, leg. et det. I. Neichev; SOM-84805, SOM-84807, SOM-84806, Stara planina Mts (Central), Koru dere. /?/; SOM-84798, SOM-84808, Stara planina Mts (Central), Kaloferski balkan, leg. et det. Drenovski; SOM-84800, Stara planina Mts (Central), Trojanski balkan, leg. et det. A. Toshev; SOM-84808; SOM-90858, SOM-90861, SOM-90860, SOM-90549, SOM-90552, SOM-90551, SOM-90550, Stara planina Mts (Central), leg. et det. B. Achtarov; SOM-152509 Stara planina, Bezhen hut, leg. et det. Apostolova sub *Centaurea triumphettii*; SOM-Sh9605, Stara planina Mts (Central), below Vezhen peak, leg. et det. S. Bancheva; SOM-Sh9635, Stara planina Mts (Central), below Planinski izvori hut, leg. et det. S. Bancheva; SOM-Sh9644, Stara planina Mts (Central), above Anton village, leg. et det. S. Bancheva; SOM-Sh9724, Stara planina Mts (Central), below Botev peak, leg. et det. S. Bancheva; SOM-Sh9711, Stara planina Mts (Central), above V. Levski

hut, leg. et det. S. Bancheva; SOM-Sh9712, Stara planina Mts (Central), "Dyuzata" loc., leg. et det. S. Bancheva; SOM-Sh9737, Stara planina Mts (Central), "Kupenite" loc., leg. et det. S. Bancheva; SOM-Sh9827, Stara planina Mts (Central), above Vezhen hut, leg. et det. S. Bancheva, SOA-12105, Stara planina Mts (Central), Karlovski balkan, leg. et det. I. Urumov; SOA-12107, Stara planina Mts (Central), Kaloferski balkan, leg. et det. B. Stefanov et T. Georgiev; SOA-18228, Stara planina Mts (Central), Yumrukchal /?/; SOA-12106, ?, leg. et det. T. Georgiev, NHM(PI)-8611, Stara planina Mts (Central), Troyanski balkan, leg. et det. N. Vihodzevski, HAU(PI)-26879, Stara planina Mts (Central), Kozyata stena, leg. et det. Delipavlov; HAU(PI)-20098, Stara planina Mts (Central), Rai hut, leg. et det. I. Cheshmetzhiev, BP-285344, Stara planina, VII, 1903 /leg. I. Neiceff/, rev. C. *napulifera* subsp. *napulifera*, det. Gamall – Eldin & Wagenitz, 01. 04 1984. **Macedonia:** FI-Bezirk, Bitoly, alpine hatten auf dem Kaimakcalan, 2000 m.

For palinomorphological description: SOM - Sh9644, Stara planina Mts (Central), above Anton village, leg. et det. S. Bancheva.

For karyological investigation: SOM - Sh9644, Stara planina Mts (Central), above Anton village, leg. et det. S. Bancheva.

(4) *Cyanus velenovskyi* (Adamovič) Wagenitz & Greuter in Greuter, Willdenowia (2003) 33:57.

Basionym: *Centaurea velenovskyi* Adamovič, Österr. Bot. Z., XLIII (1893) 172; *Centaurea nyssana* subsp. *velenovskyi* (Adamovič) Hayek in Stoj. & Stef., Fl. Bulg., ed. 1 (1925) 1181; Hayek, Prodr. Fl. Penins. Balc., II (1931) 740; *Centaurea napulifera* subsp. *velenovskyi* (Adamovič) Wagenitz & Gamal-Eldin in Strid & Tan, Mount. Fl. Greece (1991) 3: 523.

Lectotypus: *C. velenovskyi* Adamovič - "In pascuis alpinis monti Midzur (Stara Planina), ad alt., c. 2000 m s.m., VII.1892, leg. Adamovič" (BP 285 375!). Isolectotypus: (BP 482917!). The type material is keeping also in PRC (n.v.).

Hemicryptophyte. Rhizome 10-30 cm long, creeping, with or without stolons, roots up to 5 cm, fusiform. Stem simple, erect, (15)-20-40 cm high, gray-arachnoideus. Lower leaves usually not forming basal rosette, linear-lanceolate, usually entire, rarely remote-dentate. Middle and upper cauline leaves linear, 35-60 mm long and 3-11 mm wide, entire, usually slightly revolute outside, grey-tomentose. Hair covering is presented by 3-celled hairs which are 2 times wider at their basis. Capitula 35-45 mm wide. Involucre ovoid, 17-20 mm long, 10-15 mm wide. Phyllaries narrowly triangle, glabrate or with single short hairs, with 5-7 clearly noticeable nerves. Appendages glabrate or with single short hairs, with decurrent, dark brown margin, 0.5-1 mm wide; fimbriae with brown basis and silver apex. Inner florets cornflower-blue, with purple-violet stamens. Outer florets radiate, cornflower-blue, divided into 4-7 narrowly triangle lobes. Achenes 4-5 mm long and 2-3 mm wide, with lateral elaiosome; pappus well developed, 2-2,5 mm long, with 2 rows of biserate bristles.

Chromosome number: $2n = 2x = 20$. The data has been obtained from mountain Vitsi, Greece (Strid & Andersson).

Alpine meadows, 1800-2000 m, on non calcareous substrates.

General distribution. Balkan Peninsula (Bulgaria, Greece, Yugoslavia).

Specimina visa:

For morphological description: Bulgaria: - *Stara planina region* SOM-84782, Stara planina Mts, leg. et det. I. Neichev; SOM-84799, Stara planina Mts (Central), Kademlia peak, /?/; SOM-153105 - Stara planina (Central), Maragidik loc., leg. et det. Kachaunova & Bondev; SOM-90548 - Stara planina (Central), Kaloferski balkan, leg. et det. Achtarov; SOM-84764 - Stara planina (Central), Kaloferski balkan, leg. et det. /?/; SOM-84790 - Stara planina (Central), Koru dere, leg. et det. Neichev; SOM-84784, SOM-84785 - Stara planina Ambaritza, leg. et det. Neichev; SOM-84778 /?/; SOM-84777 - Rila Mt, Samokov distr., leg. et det. Davidoff; SOM-84792 - Rila Mt, Gorni Ibar, leg. et det. Davidoff; SOM-Sh9733, Stara planina Mts (Central), above Rai hut, leg. et det. S. Bancheva; **Serbia:** BEO-26251, Stara planina Mt., Mizhur peak, leg. et det. Grebenscikov; BEO-32137, BEO-32138, Stara planina Mt., Drugo bilo.

(5) *Cyanus nyssanus* (Petrovič) Bancheva & Raimondo, comb. nov.

Basionym: *Centaurea nyssana* Petrovič, Addit. Fl. Agr. Nyss. (1885) 110; Hayek, Prodr. Fl. Penins. Balc., II (1931) 740; Stef. & Georgiev, Rev. Acad. Bul. Sci., XLIV (1931) 173; *Centaurea napulifera* subf. *nyssana* (Petrovič) Stoj. & Acht., Cent. Bulg. (1935) 20; *C. napulifera* subsp. *nyssana* (Petrovič) Dostal, Bot. Journ. Linn. Soc. LXXI (1976) 209; Dostal, Fl. Eur., IV (1976) 300.

Hemicryptophyte. Rhizome long creeping, with or without stolons, roots up to 5 cm, fusiform. Stem simple, erect, 3-10 (-15) cm high, sometimes almost absent. Lower leaves at basal rosette, linear-lanceolate, usually entire. Middle and upper cauline leaves linear, 30-55 mm long and 1-2 mm wide, entire, usually revolute outside, glabrate and bright on the upper surface, white-arachnoid on the lower surface. Capitula 35-45 mm wide.

Involucre cylindric to narrowly ovoid, 17-20 mm long, 7-10 mm wide. Phyllaries narrowly triangle, glabrate or with single short hair, with 5-7 clearly noticeable nerves.

Appendages glabrate or with single short hair, with decurrent, dark brown margin, 0,5 mm wide; fimbriae with brown basis and silver apex. Inner florets pale cream, with purple-violet stamens. Outer florets radiant, pale cream, divided into 4-6 narrowly triangle lobes. Achenes 4-5 mm long and 2-3 mm wide, with lateral elaiosome; pappus well developed, 2-2,5 mm long, with 2 rows biserate bristles.

At meadows, pastures, rocky slopes, 1100-1400 m. Flowering V-VII. Suva planina Mt., Plas Mt., Svrljiska planina Mt., Besna kobila Mt., Vardenik Mt.

Serbian endemic.

Specimina visa:

For morphological description: SOM – Serbia: 93175, Plas Mt., 31. 05. 1931; BU - Plas Mt., 31.05. 1911; near Palanka, 05. 1884 sub *C. stachinaefolia* (*C. napulifera* x *orbilica*); BU-Suva planina Mt. (Golas: Divna gorica), 1120-1300 m, leg. et det. M. Niketic – 5 sheets, BNHM-32128, BNHM-32139, BNHM-32130, Besna kobila Mt., led. et det. Diklic & Nicolice; BNHM-32131, Vardenik, Veliki Strester, led. et det. Diklic & Nicolice; BNHM-32132 Svrljiske planine Mt., Tumba peak, led. et det. Diklic & Nicolice; BNHM-32133, Svrljiske planine Mt., Zeleni Vrh, leg. et det. V. Nicolice; BNHM-32134, BNHM-

32136, Svrljiske planine Mt., Goli Vrh, leg. et det. V. Nicolice; BNHM-32135, Svrljiske planine Mt., Tumba-Pernatiza.

(6) *Cyanus tuberosus* (Vis.) Bancheva & Raimondo, comb. nov.

Basionym: *Centaurea tuberosa* Vis., Fl. Dalm., II (1847) 33; Hayek in Stoj. & Stef., Fl. Bulg., ed. 1 (1925) 1181; Hayek, Prodr. Fl. Penins. Balc., II (1931) 741; Stef. & Georgiev., Rev. Acad. Bul. Sci., XLIV (1931) 177; *Centaurea napulifera* subsp. *tuberosa* (Vis.) Stoj. & Acht., Cent. Bulg. (1935) 22; Dostal, Fl. Eur., IV (1976) 300.

Hemicryptophyte. Rhizome short, truncate, without stolons; roots up to 5 cm, fusiform, gradually narrowing in short apex. Stem simple, erect, 8-30 (40) cm high, gray-archnoideus. Leaves grey-tomentose; the lower lanceolate, remotely dentate or lyrate-pinnate, in basal rosette; cauline linear lanceolate, remotely dentate or entire 35-70 mm long and 4-8 mm wide. The hair-covering of hairs which are approximately with the same width along their whole length (Fig. 11). Capitula 35-45 mm wide. Involucre ovoid, 17-20 mm long, 10-15 mm wide. Phyllaries triangle to ovoid, glabrate or with single short hairs. Appendages glabrate or with single short hairs, with decurrent, light to dark brown, 1 mm wide margin; the fimbrie with brown basis and pale yellow or silver apex. Inner florets purple-violet or pale cream. Outer florets radiant, purple-violet or pale cream, divided into 4-6 narrowly triangle lobes. Achenes 4-5 mm long and 2-3 mm wide, with lateral situated elaiosome; pappus 2-2,5 mm long, with two rows of biserate bristles.

The pollen grains are of Wagenitz's Montana-type (1955), subprolate with correlation polar axis/ equatorial diameter 1.16. In polaris view it can be observed very specific knob in the area of the mesocolpium, only for that species from the group. The exine sculpture is of a scabrate type with density of the sculpture elements 5-6 to 9 square μm . Equatorial diameter $33,1 \mu\text{m} \pm 2,6$; polar axis $37,9 \mu\text{m} \pm 2,6$; mesocolpium $26,2 \mu\text{m} \pm 2,3$; apocolpium $7,2 \mu\text{m} \pm 1,4$; length of the colpus $27,3 \mu\text{m} \pm 2,1$; breadth of the colpus $2,5 \mu\text{m} \pm 0,8$; length of the pore $4,5 \mu\text{m} \pm 1$; height of the exine in the equatorial area $4,3 \mu\text{m} \pm 0,6$; height of the exine in the polar area $3,4 \mu\text{m} \pm 0,8$; height of the coste colpi $2,6 \mu\text{m} \pm 0,3$ (Figs 4, 6).

Karyotype $2n = 4x = 40$. The chromosomes are with small size, up to $1 \mu\text{m}$ (Sharkova 1996) sub *Centaurea napulifera* subsp. *nyssana*. There are literary data for diploid chromosome number $2n = 2x = 20$ for materials from Bosnia (Siljak-Yakovlev 1985).

At meadows, pastures and rocky meadows in the mountains. Znepole region (Milevska planina Mt.), West frontier mountains (Osogovo Mt.) Belasitza Mt., Slavjanka Mt., Pirin Mt., from 1000 to 2200 m. Flowering VI-VII.

The present investigation has established that *Cyanus tuberosus* is distributed in Greece as well.

General distribution. Balkan Peninsula (Bulgaria, Greece, Macedonia, Yugoslavia).

Specimina visa:

For morphological description: Bulgaria: – *Belasitza region*: SOM-Sh9726, Belasitza Mt., leg. et det. D. Stojanov, SOA-12086, Belasitza Mt., leg. et det. N. Stojanov; *Pirin region*: SOA-12137, SOA-12128; Pirin Mt. leg. et det. E. Bozhilova; SO-91111, Pirin Mt. leg. et det. D. Stojanov; SOM-84855; SOM-84857; SOM-Pirin Mt, leg. et det. I. Urumov; SOM-Sh9521, Pirin, below the peak Vihren, leg. et det. S. Bancheva; SOM-

84864, Pirin Mt., leg. et det. B. Achtarov, SOA-18231, Pirin Mt., leg. et det. T. Georgiev; SOA-12096, Pirin Mt., leg. et det. Kalerrer; *Slavjanka region*: SOM-84981, SOM-84888, Slavjanka Mt., leg. et det. Drenovski; SOM-84884, SOM-84885, SOM-84889, Slavjanka Mt., leg. et det. B. Achtarov; SOM-105946, Slavjanka Mt. ?/; SOM-132122, Slavjanka Mt., leg. et det. N. Andreev; SOM-104590, Slavjanka Mt., leg. et det. V. Velchev et C. Petrov; SOM-84810, SOM-84812; SOM-Sh9514, SOM-Sh9660, SOM-Sh9821, Slavjanka Mt., leg. et det. S. Bancheva, SO-78704, Slavjanka Mt. ?/, SOA-12100, SOA-12104, Slavjanka Mt., leg. et det. N. Stojanov; SO-82663, SO-82664, SOA-12100, SOA-12104, Slavjanka Mt., leg. et det. N. Stojanov; *Znepole region*: SOA-12135, Milevska planina Mt., ?/; *West frontier Mts region*: SOA-12134, (Osogovo Mt.), leg. et det. N. Stojanov. **Greece**: ATH-40175 – Mt. Orvilos (Slavjanka Mt.), Greek-Bulgarian frontier, near Katafito village, leg. E. Stamatiadou det. Strid, sub. *C. napulifera* subsp. *napulifera*; ATH-3339 – Mts Vrontous, Serres distr., leg. E. Stamatiadou. **Macedonia**: BEO-26246, Nerezi village; BEO-26252, Treska river; BEO-26270, Skopska Crna Gora; BEO-26274, Belasitza Mt.; BU-Karadagh, supra Belanovce, leg. et det. Gradojenic; BU-1 sheet from Kosanini; BU-Porec, ad pagum Slatina, leg. et det. Simonoriol; **Yugoslavia**: BU-Dalmatia; FI-Flora fon Dalmatien, Clifsa, Marchesina greda, leg. et det. K. Studnicka, 06. 1875.

For palinomorphological description: SOM - Sh9514, Slavjanka Mt., leg. et det. S. Bancheva.

For karyological investigation: SOM - Sh9514, Slavjanka Mt., leg. et det. S. Bancheva.

(6) *Cyanus thirkei* (Sch. Bip.) Holub, Preslia, 45 (1973) 145.

Basionym: *Centaurea thirkei* Sch. Bip., Linnaea, XIX (1846) 314; Velenovsky, Fl. Bulg. Suppl. I (1898) 311; Stef. & Georgiev, Rev. Acad. Bul.Sci., XLIV (1931) 178; Hayek, Prodr. Fl. Penins. Balc., II (1931) 741; *Centaurea napulifera* subsp. *thirkei* (Sch. Bip.) Stoj. & Acht., Cent. Bulg. (1935) 22; Dostal, Fl. Eur., IV (1976) 300.

Typus: [N. Turkey] Nordkuste Kleinasien oder bithynischer Olymp (?), leg. Thirke (B, n.v.).

Hemicryptophyte. Rhizome short, truncate, without stolons, roots up to 1-2 cm long, about 1 cm wide, napiform, at abruptly narrowing in short apex, usually coming out from one point. Stem single, erect, up to 10 (-15) cm high, with dense, white-lanate hair covering. Leaves white or grey-tomentose or lanate; lower broadly obovate to broadly oblong, elliptical, remotely dentate or lyrate-pinnate, in basal rosette; cauline linear lanceolate, remotely dentate or entire 35-70 mm long and 4-8- mm wide. The hair covering is presented by multi-celled hairs, with 12 celled-base which is about 10 times as wide as the middle part (Fig. 12). Capitula single, 2,5-3,5 (-4) cm wide. Involucre 11-20 mm high and 9-15 mm wide. Phyllaries lanceolate or prolong-triangle, glabrate, with well seen 5-7 nerves. Appendages glabrate or with single short hair with decurrent, light to dark brown, 1-2 mm wide margin; fimbries with brown basis and pale yellow or silver apex. Inner florets pale cream, rarely pink with purple-violet stamens. Outer florets radiant, pale cream, rarely pink, divided into 4-6 narrowly triangle lobes. Achenes 3-4 (-4,5) mm long, 1,8-2,2 (-2,5) mm wide, with lateral elaiosome; pappus well developed, about three times shorter than the achene, with two rows biserate bristles.

The pollen grains are of Wagenitz's Montana-type (1955), subprolate, with correlation

polar axis/ equatorial diameter 1,3. The exine sculpture is of a scabrate type with density of the sculpture elements 4-6 to 9 square μm . Equatorial diameter $27,8 \mu\text{m} \pm 3,4$; polar axis $37,3 \mu\text{m} \pm 4,4$; mesocolpium $21,2 \mu\text{m} \pm 1,9$; apocolpium $6,6 \pm 1,3 \mu\text{m}$; length of the colpus $28,5 \mu\text{m} \pm 3$; breadth of the colpus $2,7 \mu\text{m} \pm 1,3$; length of the pore $4,9 \mu\text{m} \pm 1,3$; height of the exine in the equatorial area $4,4 \mu\text{m} \pm 0,7$; height of the exine in the polar area $2,9 \mu\text{m} \pm 0,9$; height of the coste colpi $3,1 \mu\text{m} \pm 0,6$ (Fig. 5).

Karyotype: $2n = 2x = 6m + 10sm + 2st + 2st-SAT + 1B = 20 + 1B$ (Bancheva, 1998) as *Centaurea napulifera* subsp. *thirkei*.

At dry grassy and rocky places in the plains and in front of the mountains, from 0 to 800 m alt. Flowering III-V. Black Sea coast, North-Eastern Bulgaria, Balkan foothill region (East), Stara Planina (East), Sredna Gora mountain (East), Thracian plain, Tundza hilly region, Rhodope Mts (East). Wrongly it has been pointed out for Slavjanka Mt. (Dimitrov & al. 1967) instead of *Cyanus tuberosus*.

General distribution. Bulgaria, Moldavia, European Turkey, Asia Minor.

Specimina visa:

For morphological description: SO – Bulgaria: - *Black Sea coast*: SO-78847, near Beloslav village, leg. et det. D. Jordanov; SO-78904, near Novoseltzi village, leg. et det. D. Jordanov; SO-93460, Kaliakra promontory, leg. et det. B. Kitanov; SO-78840, Burgas distr., leg. et det. D. Jordanov; SO-78848, near Gorno Ezerovo village, leg. et det. D. Jordanov; SOM-84914, near town of Varna, leg. et det. J. Velenovsky; SOM-Sh9604, Kaliakra promontory, leg. et det. S. Bancheva; SOM-Sh9606, near Balgarevo village, leg. et det. D. Stojanov; SOM-78904, near Novoseltzi village, leg. et det. D. Jordanov; SOM-93460, Kaliakra promontory, leg. et det. B. Kitanov; SOM-78871, near Beloslav village, leg. et det. D. Jordanov; HAU(PI)-20100, near town of Aitos, leg. et det. I. Chesmedzhiev; *North-Eastern Bulgaria*: SOM-12117, Provadia distr., leg. et det. I. Urumov; SO-84666, SO-78849, SO-78850, North-Eastern Bulgaria, near town of Preslav, leg. et det. D. Jordanov; SO-93214, North-Eastern Bulgaria, Tolbuhin distr., leg. et det. N. Georgiev et D. Stojanov; SO-78838, North-Eastern Bulgaria, near town of Aitos, leg. et det. D. Jordanov; SO-78854, North-Eastern Bulgaria, near town of Provadia, leg. et det. N. Stojanov, SOM-84903, SOM-84904, North-Eastern Bulgaria, Shoumen distr., leg. et det. B. Davidov; SOM-93214, North-Eastern Bulgaria, Tolbuhin distr., leg. et det. N. Georgiev et D. Stojanov; SOM-78854, North-Eastern Bulgaria, Provadia distr., leg. et det. N. Stojanov; SOM-84666, North-Eastern Bulgaria, near town of Preslav, leg. et det. D. Jordanov; SOM-84909, North-Eastern Bulgaria, Provadia distr., leg. et det. B. Davidov; SOA-12144, North-Eastern Bulgaria, Razgrad distr., leg. et det. I. Urumov; *Stara planina (East) region*: SO-78851, near town of Karnobat, leg. et det. D. Jordanov; SOM-131142, Sliven distr., leg. et det. N. Andreev; SOM-Sh9616, Sadovo village, leg. et det. S. Bancheva, SOA-12116, near town of Sliven, leg. et det. N. Stojanov et B. Stefanov, NHM(PI)-9991, NHM(PI)-9691, Stara planina Mts (East), leg. et det. St. Stanev; *Rhodope region (East)*: SO-78845, Rhodope Mts (East), leg. et det. B. Kitanov et B. Achtarov; SO-78843, Rhodope Mts (East), leg. et det. S. Vassilev; *Sredna gora region*: NHM(PI)-9686, Sredna gora Mt. (East), leg. et det. St. Stanev; *Thracian plain region*: SO-85859, near

Table 2. Comparative investigations on *Centaurea napulifera* gr.

Taxa	<i>Cyanus pseudoaxillaris</i>	<i>Cyanus orbeticus</i>	<i>Cyanus napuliferus</i>
Rhizome and roots	Roots up to 20 cm, narrowly fusiform, with very long slender apex	Rhizome long-creeping, sometimes with stolons, roots spreading, fusiform	Rhizome long-creeping, sometimes with stolons, roots spreading, fusiform
Colour of the outer florets	Purple-violet	Pale cream	Pink, cornflower-blue
Basal leaf rosette	No	No, yes	Yes, no
Hair-covering	15-16-celled, 10 times thicker at the basis	3-4 celled, not thicker at the basis	3-celled, 2 times thicker at the basis
Stomata	Anomocytous	Anomocytous	Anomocytous
Pollen grains (Montana-type (Wagenitz, 1955))	Equatorial dia - meter: 35,1 µm ; Polar axis: 65,4 µm; Ed/Pa ratio: 1,9	Equatorial dia - meter: 29,8 µm ; Polar axis: 40,7 µm; Ed/Pa ratio: 1,4	Equatorial dia - meter: 30,8 µm ; Polar axis: 39,9 µm; Ed/Pa ratio: 1,3
Chromosome number	x = 11 2n = 2x = 22	x = 10 2n = 2x = 20	x = 10 2n = 2x = 20
Biological type	Hemicryptophyte	Hemicryptophyte	Hemicryptophyte
Altitude	100-450 m alt,	800-2100 m alt,	900-2400 m alt,
Florescens	V-VI	VI-VII	VII-VIII

Taxa	<i>Cyanus nyssanus</i>	<i>Cyanus tuberosus</i>	<i>Cyanus thirkei</i>
Rhizome and roots	Rhizome long-creeping, sometimes with stolons, roots spreading, fusiform	Rhizome short, without stolons, roots fusiform	Rhizome short, without stolons, roots napiform
Colour of the outer florets	Pale cream	Purple-violet, pale cream	Pale cream
Basal leaf rosette	Yes	Yes	Yes
Hair-covering	Absence on the upper side	Hairs are not thicker at the basis	12-celled, 10 times thicker at the basis
Stomata	Not know	Diacytous	Anisocytous
Pollen grains (Montana-type (Wagenitz, 1955))	Not know	Equatorial dia - meter: 33,1 µm ; Polar axis: 37,9 µm; Ed/Pa ratio: 1,16	Equatorial dia - meter: 27,8 µm ; Polar axis: 37,3 µm; Ed/Pa ratio: 1,3
Chromosome number	Not know	x = 10 2n = 4x = 40	x = 10 2n = 2x = 20 + 1 B
Biological type	Hemicryptophyte	Hemicryptophyte	Geophyte
Altitude	1100-1400 m alt,	1000-2200 m alt,	0-800 m alt,
Florescens	V-VII	VI-VII	III-V

Leshnikovo village, leg. et det. V. Velchev et P. Vassilev; SO-85860, near Mezek village, leg. et det. V. Velchev et P. Vassilev; SO-78856, SO-78844, Haskovo distr., leg. et det. V. Stribrni SOM-84789, Thracian plain /?/; SOM-78841, near Mezek village, leg. et det. D. Jordanov; NHM(PI)-10481, Harmanli distr., leg. et det. St. Stanev; B-Ad Haskovo, leg. V. Stribrni; *Tundza hilly region*: SO-84705, SO-84704, Jambolski Bakadzic loc., leg. et det. M. Markova; SO-78855, near Dervishkata mogila loc., leg. et det. D. Jordanov; SO-78853, SO-78842, Jambol distr., leg. et det. D. Jordanov; SO-78852, Golyam Dervent loc., leg. et det. D. Jordanov; SO-78846, Voinishki Bakadzic loc., leg. et det. D. Jordanov; SO-78839, Sveti Iliiskite vazvishenia loc., leg. et det. D. Jordanov; SO-78841, Svilengrad distr., leg. et det. D. Jordanov, SOA-20531, Sveti Iliiskite vazvishenia loc., leg. et det. N. Stojanov; SOA-19857, SOA-18230, Jambol distr., leg. et det. V. Stribrni; SOA-12112, Jambol distr., leg. et det. B. Davidov; HAU(PI)-42579, HAU(PI)-20102, Jambol distr., leg. et det. Delipavlov et I. Chesmedzhiev; HAU(PI)-34368, Jambol distr., leg. et det. E. Doncheva et I. Chesmedzhiev; HAU(PI)-29104, Bakadzitite loc., leg. et det. I. Chesmedzhiev. **Macedonia**: BP-1634115 In petrosis alpinis montis Kaimakcalan, 05. VII, leg. Dorfler,

sub *C. cana* Sibth. & Sm.; BP-482897, Kaimakcalan, 05. VII, 1893, leg. et det. Dorfler sub *C. cana* var. *mathielloefolia*.

For palinomorphological description: SOM-Sh9604, Black Sea coast, Kaliakra promontory, leg. et det. S. Bancheva.

For karyological investigation: SOM-Sh9604, Black Sea coast, Kaliakra promontory, leg. et det. S. Bancheva.

Discussion

The results of the current research into genus *Cyanus*, sect. *Napulifera* (Table 2), interpreted in the light of the already published data about *Centaurea* s.l., give reason for the following conclusions to be made, concerning the evolutionary factors and trends. According to data by Wagenitz (1975a), Wagenitz & Hellwig (1996), Meusel, & Jager (1992) the origin center of the genus *Centaurea* s.l. is in East Anatolia and Trans Caucasus. From there the distribution of the species had been realized westwards to the Mediterranean area where a secondary speciation center for the genus was formed.

According to Wagenitz & Hellwig (1996) following those migrational processes, the relatives of *Cyanus tuberosus* form a derivative group on the Balkan Peninsula.

The evolution of the species from that group is in direction of adapting to more xerophytic habitats (Steffanov & Georgiev 1931). Survival in such conditions is related to development of a number of xeromorphic characters. Essential role have the change of the biological type (chemicryptophytes – geophyte); shortening of the creeping rhizomes; the appearance of basal leaf rosette; the decrease of the number and width of the cauline leaves; the division of leaf blade, by which the place of the evaporating surface decreases; presence of very dense stem and leaf hair-covering.

The research on the leaf hair-covering shows that it is equally well presented on both leaf surfaces (excluding *C. nyssanus*). Several types of hairs have been determined: multi-celled (2-3-cells or 12-15 cells); not thicker or up to 10 times thicker at their basis (Table 2). In that respect, we can point out that the type of the hair has taxonomic value and it can be used as an additional character for the differentiation of the taxa in the studied group.

The stomata for all species are situated also on both leaf surfaces. For most of the species they are from anomocytous type. Sometimes, together with that type, stomata from anisocytous type can be observed as well (Ninova 1973). In *C. tuberosus*, however, stomata from diacytous type have been determined (Fig. 11, Table 2). That character is a strong argument in support of species status of *C. tuberosus*.

Depending on the extent of development of xeromorphic characters, the species from the studied group are ordered as follows: *Cyanus pseudoaxillaris* - *C. orbelicus* - *C. velenovskyi* - *C. napuliferus* - *C. nyssanus* - *C. tuberosus* - *C. thirkei*.

The observations on the pollen morphology show the affiliations of all studied taxa to Montana-pollen type of Wagenitz (1955). The pollen grains of *C. pseudoaxillaris* can be very clearly differentiated by their biggest size (65 µm length and 35 µm height) and prolate form. The pollen grains of the rest of the taxa are smaller, 31-40 µm long and 28-33 µm high (Table 2). The pollen grains of *C. tuberosus* can also be very clearly distinguished - in polar position knobs in the area of the three mesocolpia can be observed (Figs 4, 6).

Main evolutionary mechanism in subtribe *Centaureinae* is the change of the basic chromosome number. Wagenitz & Hellwig (1996) have been determined the dispoloid order $x = 8, 9, 10, 11, 12, 13, 15, 16$. In sect. *Napulifera* there have been determined two basic chromosome numbers: $x = 11$ for *C. pseudoaxillaris* and $x = 10$ for all other species. Fernández Casas & Fernández Morales (1979) and Fernández Casas & Susanna (1986) argue that for the genus *Centaurea* s.l. there has been a trend towards reduction of the basic chromosome number and increasing of the karyotype asymmetry. According to García Jacas & Susanna (1992) in sect. *Acrocentron* the species with $x = 11$ are more primitive species than these with $x = 10$. Wagenitz & Hellwig (1996) claim that this trend seems to be the same in the perennials of *Cyanus*.

As a whole, the basic chromosome numbers show good correlations to other characters due to which they are considered a reliable key for phylogenetic reconstructions (Wagenitz & Hellwig 1996). In the studied group correlation between the basic chromosome number and the size and form of the pollen grains can be observed. The pollen grains of *C. pseudoaxillaris* ($x = 11$) are 50% bigger than the rest of the species which have basic chromosome number $x = 10$ (Table 2).

Polyploidy is also an important evolutionary mechanism for the genus. In the studied group a polyploid has been determined - *C. tuberosus* ($2n = 4x = 40$). García Jacas & Susanna (l. c.) consider that in the East Mediterranean area polyploids with $x = 10$ are always neopolyploids.

One B-chromosome has been found only in *C. thirkei*. It is supposed that the evolutionary significance of the B-chromosomes is that they can influence the expression of quantitative characters and sometimes they show correlation to the ecological conditions (Stuessy 1990).

The population structure depends on the combination of the biological form, the type of pollination, the genetic system and the way of dispersal of the diaspores.

The studied species are perennial rhizomatous plants, where the cross-pollination predominates. The individuals, forming the populations, are usually with mosaic distribution, as a result of the type of dispersal of the achenes. The latter big, with short or strongly reduced pappus and therefore, with weak flying capabilities. Usually, they remain close to the mother individual. According to literary data (Wagenitz & Hellwig 1996) and personal observations, it has been determined the importance of the myrmekochory syndrome for the dispersal of the diaspores. The achenes have well developed lateral elaiosomes, rich of fat oil used for food by the ants. It has been determined in *C. pseudoaxillaris* and *C. napuliferus*.

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