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Cytogeography of European perennial species of *Cyanus* (Asteraceae)

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To reveal the general cytogeographical pattern of *Cyanus* section *Protocyanus* in Europe, DNA ploidy and/or chromosome numbers were newly examined for 160 populations by flow cytometry (450 plants) and/or chromosome counting (30 plants). Furthermore, previously published karyological data were revised (236 records). Our analyses confirmed chromosome counts of $2n = 22$ for all newly investigated samples of the *C. triumfetti* group (the records for *C. semidecurrens* and *C. ternopoliensis* are new), *C. diospolitanus* and *C. achtarovii*; $2n = 44$ for *C. montanus* and *C. mollis*; and $2n = 20$ for *C. lingulatus*, *C. napulifer*, *C. nissanus*, *C. orbelicus*, *C. thirkei*, *C. tuberosus* and *C. velenovskyi*. The chromosome count of $2n = 20$ is the first report for *C. epirotus*. The cytotype $2n = 40$ was newly recorded for the Crimean endemic *C. fuscomarginatus* and Calabrian and Greek populations of *C. graminifolius*. The cytotypes $2n = 20$ and $2n = 40$ were confirmed for *C. pindicola*. For the first time triploidy ($2n$~$3x$~30) was found in *C. nissanus*, *C. thirkei* and in a newly discovered hybrid, *C. epirotus* × *C. graminifolius*. Two contrasting ecogeographical patterns emerged: cytotypes derived from the base chromosome number $x = 11$ ($2n = 22$, 44) are widespread in northern latitudes and ecologically diverse, whereas cytotypes with $x = 10$ ($2n = 20$, 30, 40) are confined to mountains in southern Europe. In general, tetraploids have smaller ranges than diploids. The new combinations ***Cyanus* section *Protocyanus*** (Dobrocz.) Olšavská **comb. nov.** and ***Cyanus ternopoliensis*** (Dobrocz.) Olšavská **comb. nov.** are provided. © 2013 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2013, **173**, 230–257.

ADDITIONAL KEYWORDS: *Centaurea* – chromosome numbers – Compositae – cytotaxonomy – environmental conditions – flow cytometry – polyploidy.

INTRODUCTION

Cyanus Mill. [sometimes treated as *Centaurea* L. section *Cyanus* (Mill.) DC.] is confined to the Old World and includes 25–50 species depending on taxonomic classification (Dostál, 1969; Hellwig, 2004). The number of species continues to increase as new species have recently been described (Bancheva & Stoyanov, 2009; Kaya & Bancheva, 2009). The genus has been divided into *Cyanus* section *Cyanus* containing five annual species and *Cyanus* section *Protocyanus* (Dobrocz.) Olšavská comb. nov. (see Discussion) composed of 45 perennial species (Table 1). The sections are not only distinguished by pollen morphology (annuals have the '*Cyanus*' pollen type, perennials have the '*Montana*' pollen type), but also differ in important life-history traits (Wagenitz, 1955). In annuals the variability in base chromosome number ($x = 8, 9, 11, 12$; Wagenitz & Hellwig, 1996; Bancheva, 1998; Gömürgen & Adigüzel, 2001; Romaschenko *et al.*, 2004; Ghaffari & Kelich, 2006) reflects their tendency towards increased selfing, rapid gain of reproductive isolation and fixation of karyotypic changes. Perennials have a presumably outcrossing breeding system (Gonnet, 1993; Olšavská & Löser, 2013) and often reproduce vegetatively by stolons or

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Table 1. The taxonomic conception of the genus *Cyanus* adopted in this paper, including the distribution areas of included species, published chromosome numbers (for details see Table S2) and new chromosome counts (for details see Table 2)

List of species included in the genus *Cyanus* Mill. and their taxonomic position			Distribution area	Chromosome counts		
				Published		New
				n	2*n*	2*n*
Cyanus* section *Cyanus		*C. cyanoides* (Wahlenb.) Wagenitz & Greuter	AS: IL, JO, LB, SY		18	
		C. depressus (M.Bieb.) Soják	EU: BU, ES, GR, TR, UA; AS: AM, AZ, GE, KZ, TR		16	
		C. segetum Hill	EU; AF; AS		24	
		C. pinardii (Boiss.) Soják	EU: BU, GR, TR; AS: TR		16	
		C. tchihatcheffii (Fisch. & C.A.Mey.) Wagenitz & Greuter	AS: TR		20	
***Cyanus* section *Protocaynus* (Dobrocz.) Olšavská comb. nov.**	***C. triumfetti* group**	*C. triumfetti s.l.*	EU: AT, BIH, BU, CH, CZ, DE, ES, FR, HU, HR, IT, ME, MK, PO, RO, SI, SK, SRB, UA		20*, 22, 44*	22
		C. triumfetti (All.) Á.Löve & D.Löve†	EU: FR, IT, CH		22	22
		C. adscendens (Bartl.) Soják†	EU: AT, BIH, ME, HR, SI, SRB		22	22
		C. albofimbriatus (Stef. & T.Georgiev) Greuter†	EU: BG		22	
		C. angelescui (Grinţ.) Holub†	EU: MD, RO		–	
		C. axillaris non auct. Greuter 2006–2009, *sensu* Olšavská *et al.* 2011†	EU: CZ, DE, HU, SK, PO		20, 22	22
		C. dominii (Dostál) Holub†	EU: SK, UA		22	
		C. lugdunensis (Jord.) Fourr.†	EU: FR		22	
		C. nanus (Ten.) [valid combination is not available]†	EU: IT		–	
		C. novakii (Dostál) Greuter†	EU: BG		–	
		C. pinnatifidus (Schur) Holub†	EU: RO		22	22
		C. semidecurrens (Jord.) Holub	EU: FR, ES		–	22
		C. strictus (Waldst. & Kit.) Soják†	EU: HU, SK		22	22
		C. ternopoliensis (Dobrocz.) Olšavská comb. nov.	EU: UA			22
	***C. montanus* group**	*C. maramarosiensis* (Jáv.) Dostál‡	EU: RO, SK?, UA		–	
		C. mollis (Waldst. & Kit.) J.Presl & C.Presl‡	EU: CZ, HR, HU, PL, RO, SK, SI, UA		22*, 44	44
		C. montanus (L.) Hill‡	EU: AT, BE, BIH, CH, CZ, DE, ES,FR, HR, LI, SI, SK, SRB		24*, 44	44
	***C. napulifer* group**	*C. eflanensis* Kaya & Bancheva§	AS: TR		–	
		C. napulifer (Rochel) Soják§	EU: BIH, BG, GR, MK, SRB		20	20
		C. nissanus (Petrović) Soják§	EU: SRB		20, 40	20, 30
		C. orbelicus (Velen.) Soják§	EU: BG, GR, MK, SRB		20	20
		C. thirkei (Sch.Bip.) Holub§	EU: BG, MD, RO; AS: TR		20	20
		C. tuberosus (Vis.) Soják§	EU: AL?, BIH, BG, GR, HR, ME?, MK, SRB		20, 22*, 40*	20
		C. velenovskyi (Adamović) Wagenitz & Greuter§	EU: BG, GR, SRB		20	20

Table 1. *Continued*

			Chromosome counts		
			Published		New
List of species included in the genus *Cyanus* Mill. and their taxonomic position		Distribution area	*n*	2*n*	2*n*
uncertain taxonomic position	*C. achtarovii* (Urum.) Holub†	EU: BG		22	22
	C. acmophyllus (Boiss.) Soják	AS: AM, AZ		–	
	C. atratus (Willd.) Holub	AS: AM, GE, IL, LB, SY, TR		–	
	C. baldaccii (Bald.) Holub†	EU: Crete		–	
	C. bourgaei (Boiss.) Wagenitz & Greuter†	AS: TR		–	
	C. diospolitanus Bancheva & S.Stoyanov§	EU: BG		22	22
	C. elbrusensis (Boiss. & Buhse) Wagenitz & Greuter	AS: AM		–	
	C. epirotus (Halácsy) Holub	EU: AL, GR		—	20
	C. germanicopolitanus (Bornm.) Holub	AS: TR			
	C. graminifolius (Lam.) Olšavská†	EU: FR, GR, IT		44*, 40	40
	C. fuscomarginatus (K.Koch) Greuter†	EU: UA		–	40
	C. laniger (DC.) Holub	AS: TR		–	
	C. lingulatus (Lag.) Holub†	EU: ES, PT; AF: MA	10, 11*	22*	20
	C. matthiolifolius (Boiss.) Wagenitz & Greuter†	AS: TR		–	
	C. nigrifimbrius (K.Koch) Soják‡	AS: AM, GE, TR		–	
	C. pindicola (Griseb.) Soják†	EU: AL, GR, MK		20, 40, 44*	20, 40
	C. pichleri (Boiss.) Holub	EU: BG?, TR		44	
	C. pseudaxillaris (Stef. & T.Georgiev) Holub†§	EU: BG		22	
	C. reuterianus (Boiss.) Holub	AS: TR			
	C. cheiranthifolius (Willd.) Soják	AS: AM, AZ, GE, TR		18*, 32*, 40	
	C. tanaiticus (Klokov) Soják	EU: RUe, UA		–	
	C. woronowii (Sosn.) Soják	AS: GE, TR		–	

*Doubtful chromosome counts due to miscounting or misidentification.

†According to Borhidi (1957) assigned to the *C. triumfetti* group.

‡According to Cherepanov (1963) assigned to *Centaurea* section *Protocyanus* subsection *Montanae* (Hayek) Stef. & T.Georgiev.

§According to Bancheva & Raimondo (2003), Bancheva & Stoyanov (2009) and Kaya & Bancheva (2009) assigned to *Cyanus* section *Napuliferi* (Stef. & T.Georgiev) Bancheva & Raimondo.

AF, Africa; AL, Albania; AM, Armenia; AS, Asia; AT, Austria; AZ, Azerbaijan; BE, Belgium; BG, Bulgaria; BIH, Bosnia and Herzegovina; CH, Switzerland; CZ, Czech Republic; DE, Germany; ES, Spain; EU, Europe; FR, France; GE, Georgia; GR, Greece; HR, Croatia; HU, Hungary; IL, Israel; IT, Italy; JO, Jordan; KZ, Kazakhstan; LB, Lebanon; LI, Liechtenstein; MA, Morocco; MD, Moldova; ME, Montenegro; MK, Macedonia; PO, Poland; PT, Portugal; RO, Romania; RUe, European part of Russian Federation; SI, Slovenia; SK, Slovakia; SRB, Serbia; SY, Syria; TR, Turkey; UA, Ukraine.

creeping rhizomes. They have one of the two base chromosome numbers, $x = 10$ or 11, and both diploids ($2n = 20, 22$) and tetraploids ($2n = 40, 44$) are known (Table 1). A phylogenetic study based on nuclear ribosomal DNA (Boršić *et al*., 2011) suggested a monophyletic origin of both sections. In *C.* section *Protocyanus*, phylogenetic relationships remain elusive due to extensive reticulate evolution or still-incomplete evolutionary differentiation (Boršić *et al*., 2011; C. J. Löser, unpubl. data).

Perennials of *C.* section *Protocyanus* are distributed in north-western Africa, Europe (except northern parts) and western Asia. The highest diversity of perennial taxa is found in the Balkan Peninsula (Bancheva & Raimondo, 2003; Boršić *et al*., 2011) where 18 species occur (Table 1). *Cyanus* section *Protocyanus* includes narrow endemics and widely distributed taxa occupying a broad range of environmental conditions. They grow in various types of grasslands and forests, and rocky and eroded slopes, including extremely dry biotopes and also wet places close to water sources, from sea level to the sub-alpine belt.

European representatives of *C.* section *Protocyanus* form three aggregates of closely related taxa (Table 1) including taxa which have close relationships to *C. triumfetti* (All.) Dostál ex Á.Löve & D.Löve *s.s.*, *C. montanus* (L.) Hill *s.s.* or *C. napulifer* (Rochel) Soják *s.s.* (see Table 1). These relationships have been inferred mainly from shared morphological characters of roots and involucra. All available taxonomic concepts of the three aggregates are controversial with regard to the variable number of species and the taxonomic position of included taxa (see Supporting Information Table S1). Therefore, we prefer to use an informal classification for the groups: the *C. triumfetti* group, the *C. montanus* group and the *C. napulifer* group. The taxonomic position of several European species and their relationships to the three groups remain unclear (Table 1).

Chromosome numbers have been published for a number of European taxa (Table 1, Table S2), but knowledge of the karyology of Asian and African perennial representatives of *Cyanus* is still poor; only four chromosome counts have been reported outside Europe (Fedorov, 1969). Until 2006 all published karyological data for perennials of *Cyanus* were based on chromosome counting. In recent years, the more efficient approaches of flow cytometry (cf. Olšavská *et al*., 2011, 2012) and Feulgen DNA image densitometry (cf. Bancheva & Greilhuber, 2006) have led to a large increase in knowledge of cytogenetics in these plants.

Recent molecular investigations of *Cyanus* have provided conflicting information because phylogenetic trees obtained from different molecular markers are incongruent (Boršić *et al*., 2011; C. J. Löser, unpubl. data). Information on chromosome numbers and ploidy might help to elucidate taxonomic affiliations and aspects of their evolution such as crossing barriers or historical and contemporary gene flow (Levin, 2002; Stuessy, 2009; Sánchez-Jiménez *et al*., 2012). The importance of karyology in the systematics of various genera of Centaureinae has been verified by establishing connections among karyological, morphological and molecular data (Wagenitz & Hellwig, 1996; Hellwig, 2004; Romaschenko *et al*., 2004). However, until now no such attempt in *Cyanus* has been carried out with the exception of a study examining the relationships between reproductive behaviour (allogamy or autogamy) and the occurrence of dysploidy in this genus (Hellwig, 2004).

In the present study we examined the geographical distribution of cytotypes in European representatives of *C.* section *Protocyanus*. Our aims were to: (1) complete karyological records for the section; (2) reveal general cytogeographical patterns in Europe; (3) evaluate correlations between the distribution of particular cytotypes and current ecological conditions; and (4) evaluate karyology of the section in a biogeographical and phylogenetic context.

MATERIAL AND METHODS

Plant material

During 2006–2011 plants were collected throughout western, southern, central and eastern Europe (Table 1) and transferred to experimental sites at the Jena Botanical Garden, Germany, or the Institute of Botany of the Slovak Academy of Sciences in Banská Bystrica or Bratislava, Slovakia, for cultivation. Voucher herbarium specimens were deposited in herbaria JE or SAV.

Chromosome counts

Chromosomes were counted in mitotic root tip meristem cells of cultivated plants as described by Olšavská *et al*. (2009), with modifications as follows. Root tips were macerated for 10 min in a 1:1 mixture of concentrated hydrochloric acid and ethanol at 24 °C or in a 1 M HCl solution at 60 °C. We made either temporary slides prepared by compressing with a glass cover and staining in lacto-propein orcein or permanent slides prepared by compressing with a cellophane square and staining in a 10% solution of Giemsa stock dye in Sörensen phosphate buffer (45 min).

Flow cytometric analysis

Ploidy was estimated using propidium iodide (PI) flow cytometry and a Becton Dickinson FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with an argon-ion laser exciting at 488 nm in the Laboratory of Flow Cytometry, Institute of Biology and Ecology, P. J. Šafárik University in Košice, Slovakia, or 4′,6-diamidino-2-phenylindole (DAPI) flow cytometry with a Partec Cyflow ML instrument (Partec GmbH, Munster, Germany) equipped with an HBO-100 mercury arc lamp in the Laboratory of Flow Cytometry, Institute of Botany, Slovak Academy of Sciences in Bratislava, Slovakia. Samples were prepared from young leaves of the cultivated plants (1–3 plants per population in one sample) and an internal standard in a two-step procedure (Doležel, Greilhuber & Suda, 2007). The detailed preparation of samples is given in Olšavská *et al*. 2009, 2011). *Solanum lycopersicum* L. 'Stupnické polní rané' (2C = 1.96 pg; Doležel, Sgorbati & Lucretti, 1992) or *Bellis perennis* L. (2C = 3.38 pg; Schönswetter *et al*., 2007b) were used as internal standards in flow cytometric analyses. Fluorescence of at least 5000 particles was recorded, and only histograms with symmetrical peaks and a coefficient of variance (CV) of the standard and sample G1 peaks < 3% for DAPI and < 6.5% for PI were considered. Estimation of the level of ploidy of samples was based on the ratio of the mean positions of the G1 peak of the tested plant and the G1 peak of the standard in the flow histogram. The relationship between chromosome number and ploidy was verified using chromosome counts presented in this paper or published previously (Olšavská *et al.*, 2009, 2011). The Tukey–Kramer test was used to test differences in DNA content (2C values) between particular cytotypes and the Mann–Whitney *U*-test was used to check differences in DNA content (Cx values) between diploids and tetraploids.

MAP PREPARATION

Distribution maps of cytotypes/ploidy in Europe are based on field GPS positioning (160 localities; Table 2) and on coordinates of published data (198 localities; Table S2). For references lacking geographical coordinates but having an exact description of collecting sites, coordinates were retrieved from Google Earth 6.1 (Google Inc.). Records which were doubtful due to miscounting or misclassification were not included (Table S2). Distribution maps were prepared using Quantum GIS 1.7.3 (Open Source Geospatial Foundation).

GEOGRAPHICAL AND ENVIRONMENTAL DATA

A matrix of 358 localities was prepared to test for associations of cytotypes with environmental conditions at their localities. The matrix included all samples used for map preparation. The following data are tabulated for each locality: altitude, environmental conditions [19 bioclimatic variables (BioClim) obtained from http://www.worldclim.org (Hijmans *et al.*, 2005): annual mean temperature, mean diurnal range, isothermality, temperature seasonality, maximum temperature of warmest month, minimum temperature of coldest month, temperature annual range, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of wettest month, precipitation of driest month, precipitation seasonality, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, precipitation of coldest quarter] and type of bedrock (1, calcareous; 2, alkaline; basic; 3, intermediate or mixed; 4, acidic – coded as binary characters). The type of bedrock was recorded during the field research or completed from available geological maps for published localities (Gabaldón, 1994; Dadlez, Marek & Pokorski, 2000; Compagnoni & Galluzo, 2004; Swisstopo, 2005). One variable from each pair of highly correlated variables (altogether six characters from a total of 24 characters; Pearson/Spearman correlation coefficient > 0.95) was excluded. Thus, principal component analysis (PCA) of 17 environmental variables and one geographical variable was used to provide insights into the overall distribution pattern of particular cytotypes, to reveal their ecological preferences and to form hypotheses about cytotype distribution/migration in the past. CANOCO 4.5 for Windows package (ter Braak & Šmilauer, 2002) was used for PCA.

RESULTS

CHROMOSOME NUMBERS AND PLOIDY

Chromosome numbers and/or ploidy are newly reported for 160 populations in Europe using chromosome counting (30 plants; Fig. 1) and/or flow cytometry (450 plants; Fig. 2) (Table 2), and 236 previously published karyological records have been revised (Table S2). Our analyses confirm chromosome counts of $2n = 22$ for all newly investigated samples of the *C. triumfetti* group [Fig. 1L; the records for *C. semidecurrens* (Jord.) Holub and *C. ternopoliensis* (Dobrocz.) Olšavská comb. nov. are new], *C. diospolitanus* Bancheva & S.Stoyanov and *C. achtarovii* (Urum.) Holub, $2n = 44$ for *C. mollis* (Waldst. & Kit.) J.Presl & C.Presl and *C. montanus*, and $2n = 20$ for *C. napulifer* (Rochel) Soják, *C. orbelicus* (Velen.) Soják, *C. thirkei* (Sch.Bip.) Holub, *C. tuberosus* (Vis.) Soják (Fig. 1H) and *C. velenovskyi* (Adamović) Wagenitz & Greuter (Fig. 1F). As the delimitation of some taxa of the *C. triumfetti* group in southern and eastern Europe is still under study, we prefer not to refer to particular taxa for some chromosome counts (Fig. S1; Table 1, Table S2). Chromosome counts of $2n = 20$ are the first reports for *C. epirotus* (Halácsy) Holub (Fig. 1G) and *C. nissanus* (Petrović) Soják (Fig. 1I). Previous chromosome records assigned to *C. nissanus* from the Pirin Mts, Rila Mts, Rodope Mts and Slavjanka Mts (Bulgaria, Greece; Kuzmanov & Georgieva, 1987; Sharkova, 1996; Sharkova & Peev, 1997; Bancheva, 1999) belong to *C. orbelicus* or *C. tuberosus*, which are currently reported from these areas (Bancheva & Greilhuber, 2006; Petrova & Vladimirov, 2010) (Table S2). Moreover, based on our field observations, plants fitting the circumscription of *C. nissanus* (Petrovič, 1885) are endemics to the Svrljiska Planina Mts in eastern Serbia. Our results for *C. lingulatus* (Lag.) Holub (Fig. 1E) agree with a report by Morales (1974) as we confirmed only $2n = 20$ for this species. A new cytotype $2n = 40$ was recorded for Calabrian and Greek populations stated previously as *C. triumfetti s.s.*/*C. triumfetti* subsp. *variegata* (Lam.) Dostál/*C. triumfetti* subsp. *seusana* (Chaix) Hayek or *C. pichleri* (Boiss.) Holub (Zángheri, 1976; Pignatti, 1982; Gamal-Eldin & Wagenitz, 1991); their correct name is *C. graminifolius* (Lam.) Olšavská (Fig. 1A, B). The cytotype $2n = {\sim}40$ found for *C. fuscomarginatus* (K.Koch) Greuter is the first documented report for the taxon. The cytotype $2n = 20$ was found for *C. pindicola* (Griseb.) Soják from the type locality ['copiose

Table 2. Locality details, including geographical coordinates, altitude, date, collector/collectors and voucher information of the plants of *Cyanus* section *Protocyanus* from Europe investigated for chromosome number (number of investigated plants/number of plants used also in FCM analyses) or by flow cytometry (DAPI or PI staining; standard: BP – *Bellis perennis* or SL – *Solanum lycopersicum*, number of plants/number of FCM analyses; 2C-value of DNA content in pg ± standard deviation)

	Locality details	Chromosome counts	Ploidy (FCM)
	Cyanus lingulatus (Lag.) Holub		
‡	Spain; Sierra de Javalambre, ski resort Javalambre, 40°06′55″N, 01°01′00″W; 1853 m; 3.vi.2010, *SŠ, JZ & KM* (SAV: JAV)		$2n \sim 20$ (DAPI, BP, 5/5; 2.00 ± 0.01)
‡	Spain; Mt Penyagolosa, S of San Joan de Penyagolosa; 40°13′31″N, 00°20′56″W; 1695 m; 4.vi.2010, *SŠ*, *JZ & KM* (SAV: PEN)		$2n \sim 20$ (DAPI, BP, 5/5; 2.03 ± 0.02)
‡	Spain; Sierra del Moncayo, N of Purujosa; 41°42′47″N, 1°44′54″W; 1280 m; 8.v.2011, *SŠ* (SAV: PUR)	$2n = 20$ (1/1)	$2n \sim 20$ (DAPI, BP, 5/5; 2.04 ± 0.01)
	Cyanus graminifolius (Lam.) Olšavská		
	France; Hautes-Alpes, Montagne de Céüse, Pic de Céüse; 44°30′20″N, 05°57′25″E; 1900 m; 10.viii.2006, *CL* (JE: SEU)	$2n = 40$ (1/0)	
‡	Italy; Pollino Mts, E from Colle Impiso, banks of the river Frido; 39°55′50″N, 16°10′12″E; 1447 m; vi.2011, *KO* (SAV: TRI 195)		$2n \sim 40$ (DAPI, BP, 4/4; 3.76 ± 0.03)
‡	Italy; Pollino Mts, Colle del Dragone; 39°54′46.4″N, 16°07′30.8″E; 1531 m; 14.vi.2011, *KO* (SAV: TRI 196)		$2n \sim 40$ (DAPI, BP, 3/3; 3.77 ± 0.03)
‡	Italy; Calabria, La Sila Mts, SE from Fermata S. Ianni; 39°19′50.3″N, 16°22′36.6″E; 1515 m; 14.vi.2011, *KO* (SAV: TRI 197)		$2n \sim 40$ (DAPI, BP, 2/2; 3.75 ± 0.01)
‡	Italy; Calabria, La Sila Mts, 4 km SW from Moccone; 39°20′05.7″N, 16°23′56.5″E; 1613 m; 14.vi.2011, *KO* (SAV: TRI 198)		$2n \sim 40$ (DAPI, BP, 5/5; 3.74 ± 0.02)
‡	Italy; Calabria, La Sila Mts, N from Lorica; 39°15′21.4″N, 16°30′53.6″E; 1356 m; 14.vi.2011, *KO* (SAV: TRI 200)		$2n \sim 40$ (DAPI, BP, 2/2; 3.74 ± 0.02)
‡	Italy; Cosenza, La Sila Mts, Mt Botte Donato; 39°18′11″N, 16°24′47″E; 1671 m; 11.v.2008, *CL* (JE: SIL 1)	$2n = 40 + 4B$ (1/0)	$2n \sim 40$ (DAPI, SL, 2/2; 3.75 ± 0.00)
‡	Greece; Imathia, Vourinos Mts, NE from Paliokastro; 40°13′20.9″N, 21°38′34.0″E; 1094 m; 28.v.2011, *CL & KO* (SAV: TRI 167)		$2n \sim 40$ (DAPI, SL, 3/3; 3.67 ± 0.04)
‡	Greece; Grevena, Agh. Georgios; 39°52′56.8″N, 21° 45′ 06.9″E; 706 m; 29.v.2011, *CL & KO* (SAV: TRI 171)		$2n \sim 40$ (DAPI, SL, 3/3; 3.63 ± 0.02)
‡	Greece; Larissa, SE part of Ossa Mts; 39°48′32.0″N, 22°43′57.2″E; 1341 m; 29.v.2011, *CL & KO* (SAV: 176)	$2n = 40$ (2/1)	$2n \sim 40$ (DAPI, SL, 3/3; 3.69 ± 0.08)
‡	Greece; Larissa, Ossa Mts, meadow below Ossa peak; 39°46′59.0″N, 22°41′46.2″E; 1272 m; 30.v.2011, *CL & KO* (SAV: TRI 177)		$2n \sim 40$ (DAPI, SL, 1/1; 3.68)
‡	Greece; Peloponnese, Mt Panachiko; 38°15′52″N, 21°51′06″E; 1146 m; 31.v.2011, *CL & KO* (SAV: TRI 179)		$2n \sim 40$ (DAPI, SL, 2/2; 3.68 ± 0.03)
‡	Greece; Peloponnese, Ziria Mts, Mt Killini; 38°02′02.3″N, 22°24′10.6″E; 1306 m; 1.vi.2011, *CL & KO* (SAV: TRI 180)		$2n \sim 40$ (DAPI, SL, 2/2; 3.60 ± 0.00)
‡	Greece; Peloponnese, Ziria Mts, Trikala; 37°57′34.4″N, 22°24′57.4″E; 1552 m; 1.vi.2011, *CL & KO* (SAV: TRI 181)		$2n \sim 40$ (DAPI, SL, 1/1; 3.57)

Table 2. *Continued*

	Locality details	Chromosome counts	Ploidy (FCM)
‡	Greece; Ioannina, Mitsikeli Mts; 39°43′09.4″N, 20°53′15.2″E; 1478 m; 3.vi.2011, *CL & KO* (SAV: TRI 184)	$2n = 40$ (1/1)	$2n \sim 40$ (DAPI, SL, 3/3; 3.68 ± 0.03)
‡	Greece; Ioannina, Timfi Mts, Vradeto; 39°53′53.2″N, 20°46′23.8″E; 1339 m; 3.vi.2011, *CL & KO* (SAV: TRI 186)		$2n \sim 40$ (DAPI, SL, 4/4; 3.70 ± 0.04)
‡	Greece; Kastoria, between Nea Kotyli and Kato Nestorio; 40°22′16.6″N, 21°02′41.4″E; 1268 m; 4.vi.2011, *CL & KO* (SAV: TRI 188)		$2n \sim 40$ (DAPI, SL, 2/2; 3.67 ± 0.03)
‡	Greece; Mt Chortiatis, above the village of Chortiatis; 40°35′57.8″N, 23°06′01.2″E; 826 m; 20.v.2012, *MO & KO* (SAV: TRI 239)		$2n \sim 40$ (DAPI, SL, 4/4; 3.67 ± 0.03)
‡	Bulgaria; Strandzha Mts, Golyamo Bukovo, above the road E 79; 42°14′28.3″N, 27°06′36.6″E; 340 m; 17.v.2012, *MO & KO* (SAV: TRI 231)		$2n \sim 40$ (DAPI, SL, 1/1; 3.70)
	Cyanus fuscomarginatus (K.Koch) Greuter		
*	Ukraine; Crimea, Dolgorukovskaja Jajla, SE of Simferopol; 44°53′08″N, 34°20′16″E; 821 m; 6.vii.2012, *KO* (SAV: TRI 277)		$2n \sim 40$ (DAPI, SL, 7/7; 3.20 ± 0.02)
*	Ukraine; Crimea, Aj-Petrinskaja Jajla, Mt Aj-Petri; 44°27′29″N, 34°03′37″E; 1178 m; 7.vii.2012, *KO* (SAV: TRI 278)		$2n \sim 40$ (DAPI, SL, 4/4; 3.21 ± 0.00)
*	Ukraine; Crimea, Aj-Petrinskaja Jajla; 44°29′06″N, 34°02′39″E; 1200 m; 7.vii.2012, *KO* (SAV: TRI 280)		$2n \sim 40$ (DAPI, SL, 3/3; 3.20 ± 0.02)
*	Ukraine; Crimea, Čatyr-Dag Jajla, near Emine-Bair-Koba cave; 44°47′51″N, 34°18′12″E; 1012 m; 9.vii.2012, *KO* (SAV: TRI 281)		$2n \sim 40$ (DAPI, SL, 3/3; 3.21 ± 0.03)
*	Ukraine; Crimea, Čatyr-Dag Jalja, near Marmornaja cave; 44°47′25″N, 34°16′48″E; 973 m; 9.vii.2012, *KO* (SAV: TRI 282)		$2n \sim 40$ (DAPI, SL, 3/3; 3.20 ± 0.01)
	Cyanus epirotus × *Cyanus graminifolius*		
*	Greece; Ioannina, Mitsikeli Mts; 39°43′09.4″N, 20°53′15.2″E; 1478 m; 3.vi.2011, 20.v.2012, *CL & KO* (SAV: TRI x184)		$2n \sim 30$ (3/3; 2.96 ± 0.00)
	Cyanus epirotus (Halácsy) Holub		
*	Greece; Ioannina, Timfi Mts, Astraka peak; 39°57′56″N, 20°45′02″; 1896 m; 16.vii.2007, *CL* (JE: PAP 2)	$2n = 20$ (1/0) $2n = 20 + 3B$ (1/0)	
*	Greece; Ioannina, Tzoumerka Mts, Kryopigi; 39°27′09″N, 21°07′39″E; 1443 m; 2.vi.2011, *CL & KO* (SAV: TRI 182)		$2n \sim 20$ (DAPI, SL, 3/3; 2.21 ± 0.01)
*	Greece; Ioannina, Tzoumerka Mts, close to Mparos pass; 39°36′36.6″N, 21°08′32.9″E; 1676 m; 2.vi.2011, *CL & KO* (SAV: TRI 183)		$2n \sim 20$ (DAPI, SL, 3/3; 2.21 ± 0.02)
*	Greece; Ioannina, Mitsikeli Mts, above Ioannina; 39°43′01.6″N, 20°53′0.7″E; 1669 m; 3.vi.2011, *CL & KO* (SAV: TRI 185)		$2n \sim 20$ (DAPI, SL, 5/5; 2.22 ± 0.01)
*	Greece; Ioannina, Timfi Mts, above the road to Vradeto; 39°55′03″N, 20°48′21″E; 1738 m; 3.vi.2011, *CL & KO* (SAV: TRI 187)		$2n \sim 20$ (DAPI, SL, 3/3; 2.23 ± 0.00)

Table 2. *Continued*

	Locality details	Chromosome counts	Ploidy (FCM)
	Cyanus pindicola (Griseb.) Soják		
‡	Greece; Pella, NE slope of Mt Piperitsa; 40°51′38″N, 21°44′32″E; 1812 m; 14.vi.2010, *MO & KO* (SAV: TRI 145)	2*n* = 20 (1/1)	2*n*~20 (DAPI, SL, 3/3; 2.29 ± 0.02)
‡	Greece; Pieria, Oros Olympos Mts, below Zolotas cottage; 40°04′56″N, 22°22′50″E; 1709 m; 15.vi.2010, *MO & KO* (SAV: TRI 148)	2*n* = 40 (1/0)	
‡	Greece; Larisa, 2 km SW from Loutro; 39°57′19.8″N, 21°55′03.9″E; 726 m; 29.v.2011, *CL & KO* (SAV: TRI 172)	2*n* = 40 (1/0)	
‡	Greece; Larisa, Kato Olympos Mts, 2 km NE from Kallipefki; 39°58′25″N, 22°28′46″E; 1141 m; 29.v.2011, *CL & KO* (SAV: TRI 175)	2*n* = 40 (1/0)	
‡	Greece; Larisa, S slope below the peak of Mt Ossa; 39°47′32.3″N, 22°41′47.9″E; 1650 m; 30.v.2011, *CL & KO* (SAV: TRI 178)	2*n* = 40 (1/0)	
	Cyanus napulifer (Rochel) Soják		
	Bulgaria, Stara Planina Mts, above Pascal hut, Zlatica; 42°45′48″N, 24°12′43″E; 1983 m; 13.vii.2009, *CL* (JE: ZLA)		2*n*~20 (DAPI, SL, 8/8; 2.22 ± 0.01)
	Bulgaria; Stara Planina Mts, Karlovo, above Ravnec cottage; 42°40′53″N, 24°50′50″E; 1843 m; 8.viii.2007, *CL* (JE: KAR 4)		2*n*~20 (DAPI, SL, 1/1; 2.20)
	Bulgaria; Stara Planina Mts, Korduna peak; 42°45′19″N, 24°04′36″E; 1605 m; 2.viii.2011, *KO* (SAV: TRI 214)		2*n*~20 (DAPI, SL, 3/3; 2.22 ± 0.02)
	Bulgaria; Stara Planina Mts, 2 km SE from the Vezhen cottage; 42°45′47.5″N, 24°24′10.8″E; 1869 m; 2.viii.2011, *KO* (SAV: TRI 215)		2*n*~20 (DAPI, SL, 3/3; 2.23 ± 0.01)
	Bulgaria; Stara Planina Mts, Troyanski prokhod, Beclemento; 42°46′37″N, 24°36′52″E; 1601 m; 3 August 2011, *KO* (SAV: TRI 216)		2*n*~20 (DAPI, SL, 3/3; 2.22 ± 0.01)
	Bulgaria; Stara Planina Mts, Troyanski prokhod, Mt Viloto; 42°46′47″N, 24°39′56″E; 1603 m; 3.viii.2011, *KO* (SAV: TRI 217)		2*n*~20 (DAPI, SL, 3/3; 2.23 ± 0.01)
	Cyanus nissanus (Petrović) Soják		
†	Serbia; Svrljiska Planina Mts, S slope of Mt Zeleni Vrh; 43°20′13″N, 22°13′26″E; 1140 m; 30.vi.2009, *CL* (JE: SIC 1)	2*n* = 20 (1/0) 2*n* = 30 (1/0)	2*n*~20 (DAPI, SL, 3/3; 2.20 ± 0.00) 2*n*~30 (DAPI, SL, 2/2; 3.30 ± 0.01)
†	Serbia; Svrljiska Planina Mts, Mt Popova glava; 43°21′23.4″N, 22°05′30.2″E; 1008 m; 14.vi.2012, *CL & KO* (SAV: TRI 256)		2*n*~20 (DAPI, SL, 2/2; 2.18 ± 0.01) 2*n*~30 (DAPI, SL, 2/2; 3.24 ± 0.02)
	Cyanus thirkei (Sch.Bip.) Holub		
‡	Bulgaria; Stara Planina Mts, Varbishki prokhod, above Sadovo, 42°53′17.6″N, 26°39′05.8″E; 686 m; 15.v.2012, *MO & KO* (SAV: TRI 228)		2*n*~30 (DAPI, BP, 6/7; 2.92 ± 0.01)
‡	Bulgaria; Balgarova near Burgas; 42°37′58.3″N, 27°16′48.2″E; 99 m; 16.v.2012, *KO* (SAV: TRI 229)		2*n*~20 (DAPI, BP, 4/4; 1.93 ± 0.00)
‡	Bulgaria; Otmanli near Burgas; 42°25′49.1″N, 27°32′59.1″E; 52 m; 16.v.2012, *MO & KO* (SAV: TRI 230)		2*n*~20 (DAPI, BP, 4/4; 1.93 ± 0.00)

Table 2. *Continued*

	Locality details	Chromosome counts	Ploidy (FCM)
	Cyanus orbelicus (Velen.) Soják		
‡	Serbia; Mt Besna Kobila; 42°32′57.9″N, 22°13′56.2″E; 1662 m; 15.vi.2012, *CL & KO* (SAV: TRI 260)		2*n*~20 (DAPI, SL, 1/1; 2.11)
‡	Serbia; Mt Besna Kobila, N of the peak; 42°33′29.9″N, 22°14′09.4″E; 1559 m; *CL & KO* (SAV: TRI 261)		2*n*~20 (DAPI, SL, 3/3; 2.12 ± 0.00)
‡	Bulgaria; Velingrad, Golyama Syutkya; 41°54′24″N, 24°01′41″E; 1800 m; 8.vii.2009, *CL* (JE: VGR 3)		2*n*~20 (DAPI, SL, 3/3; 2.16 ± 0.01)
‡	Bulgaria; Rila Mts, 2.5 km NE from Granchar Hut; 42°08′08″N, 23°36′54″E; 2294 m; 19.vi.2010, *KO* (SAV: TRI 153)		2*n*~20 (DAPI, SL, 3/3; 2.18 ± 0.00)
‡	Bulgaria; Rila Mts, 1.2 km N from Granchar Hut; 42°07′51″N, 23°35′45″E; 2277 m; 20.vi.2010, *KO* (SAV: TRI 154)		2*n*~20 (DAPI, SL, 3/3; 2.17 ± 0.00)
‡	Bulgaria; Rila Mts, Belmeken dam; 42°09′51″N, 23°46′57″E; 1963 m; 9.viii.2011, *KO* (SAV: TRI 222)		2*n*~20 (DAPI, SL, 4/4; 2.20 ± 0.04)
	Bulgaria; Rila Mts, Cyrilova Polana, Sohoto Ozero; 42°10′02″N, 23°25′07″E; 1875 m, 21.vi.2010, *KO* (SAV: TRI 155)		2*n*~20 (DAPI, SL, 5/5; 2.20 ± 0.01)
	Bulgaria; Pirin Mts, Mt Vihren, above Vihren cottage; 41°45′27″N, 23°24′17″E; 2440 m; 17.vi.2010, *KO* (SAV: TRI 151)		2*n*~20 (DAPI, SL, 4/4; 2.25 ± 0.01)
	Bulgaria; Pirin Mts, Bakushevata Mura; 41°45′54″N, 23°24′44″E; 2217 m; 10.viii.2011, *KO* (SAV: TRI 223)		2*n*~20 (DAPI, SL, 3/3; 2.26 ± 0.03)
	Cyanus tuberosus (Vis.) Soják (blue florets)		
‡	Croatia; Lika, Mt Poštak, along a road to Rastičevo; 44°13′28.4″N, 16°05′48.8″E; 752 m; 5.vi.2012, *MO, JO & KO* (SAV: TRI 247)		2*n*~20 (DAPI, SL, 4/4; 2.48 ± 0.01)
‡	Croatia; Split-Dalmatia County, between Sinj and Sutina; 43°42′04.0″N, 16°36′52.5″E; 402 m; 7.vi.2012, *MO, JO & KO* (SAV: TRI 251)		2*n*~20 (DAPI, SL, 4/4; 2.52 ± 0.05)
‡	Croatia; Split-Dalmatia County, above Sinj, near Zelovo; 43°45′04.4″N, 16°33′12.2″E; 800 m; 7.vi.2012, *MO, JO & KO* (SAV: TRI 252)		2*n*~20 (DAPI, SL, 4/4; 2.49 ± 0.02)
‡	Croatia; Mosor Mts, near Rašca; 43°33′48.8′N, 16°42′33.8′E312 m; 8.vi.2012; *MO, JO & KO* (SAV: TRI 253)		2*n*~20 (DAPI, SL, 4/4; 2.50 ± 0.03)
‡	Macedonia; Babuna Mts, Prisad pass; 41°26′54.0″N, 21°37′50.2″E; 1100 m; 19.vi.2012, *CL & KO* (SAV: TRI 266)		2*n*~20 (DAPI, SL, 3/3; 2.25 ± 0.01)
‡	Macedonia; Dren Mts, pass above Štavica; 41°15′03.5″N, 21°35′41.1″E; 1132 m; 21.vi.2012, *CL & KO* (SAV: TRI 270)		2*n*~20 (DAPI, SL, 2/2; 2.24 ± 0.01)
	Cyanus cf. *tuberosus* (Vis.) Soják (white florets)		
	Macedonia; Ramno Mts, above Brodec; 42°08′35.8″N, 21°26′57.5″E; 1100 m; 15.vi.2012, *CL & KO* (SAV: TRI 262)		2*n*~20 (DAPI, SL, 4/4; 2.29 ± 0.02)
‡	Macedonia; SW from Raduša; 42°04′36.6″N, 21°11′48.2″E; 798 m; 16.vi.2012, *CL* (SAV: TRI 263)		2*n*~20 (DAPI, SL, 2/2; 2.27 ± 0.00)

Table 2. *Continued*

	Locality details	Chromosome counts	Ploidy (FCM)
‡	Macedonia; Vodno Mts, Skopje, near Millenium cross; 41°58′11.7″N, 21°23′54.6″E; 922 m; 16.vi.2012, *CL* (SAV: TRI 264)		2*n*~20 (DAPI, SL, 1/1, 2.29)
‡	Macedonia; Dren Mts, near Veprchani and Dunje; 41°16′11.9″N, 21°45′24.4″E; 832 m; 22.vi.2012, *KO* (SAV: TRI 271)		2*n*~20 (DAPI, SL, 2/2; 2.29 ± 0.01)
‡	Macedonia; Dren Mts, between Dunje and Belovodica; 41°27′27.2″N, 21°46′07.4″E; 1479 m; 22.vi.2012, *CL* (SAV: TRI 272)		2*n*~20 (DAPI, SL, 2/2; 2.26 ± 0.01)
‡	Bulgaria; Slavyanka Mts, S slope of Mt Carev Vrah, 41°23′55″N, 23°38′27″E, 2199 m; 17.vii.2009, *CL* (JE: SLA 3)	2*n* = 20 + 2B (1/0)	
	Bulgaria; Slavyanka Mts, Koynara: 41°24′37″N, 23°33′57″E; 1641 m; 17.vii.2009, *CL* (JE: SLA 1)		2*n*~20 (DAPI, SL, 2/2; 2.33 ± 0.07)
‡	Greece; Serres, Pangaion Mts, Mt Moni Ikosifinissis; 40°56′03″N, 24°05′40″E; 1714 m; 21.vii.2007, *CL* (JE: PAN)	2*n* = 20 (1/0)	2*n*~20 (DAPI, SL, 2/2; 2.24 ± 0.02)
‡	Greece; Drama, Falakro ski center; 41°17′55″N, 24°04′19″E; 1755 m; 21.vii.2007, *CL* (JE: FAL)		2*n*~20 (DAPI, SL, 1/1; 2.27)
‡	Greece; Imanthia, Vermio Mts, from Naousa to 3–5 Pigadia ski center, 40°39′29″N, 21°59′31″E, 1183 m, 14.vi.2010, *KO* (SAV: TRI 146)		2*n*~20 (DAPI, SL, 4/4; 2.28 ± 0.01)
‡	Greece; Kozani, Pieria Mts, SE of Velvendo; 40°13′52″N, 22°06′39″E; 1278 m; 16.vi.2010, *KO* (SAV: TRI 149)		2*n*~20 (DAPI, SL, 4/4; 2.26 ± 0.02)
‡	Greece; Kilkis, SW of Kastaneri; 40°58′49″N, 22°22′29″E; 980 m; 26.v.2011, *KO* (SAV: TRI 161)		2*n*~20 (DAPI, SL, 3/3; 2.28 ± 0.03)
‡	Greece; Kilkis, Paiko Mts, S of Livadhia; 40°59′40″N, 22°17′46″E; 1160 m; 26.v.2011, *KO* (SAV: TRI 162)		2*n*~20 (DAPI, SL, 5/5; 2.26 ± 0.01)
‡	Greece; Kilkis, Paiko Mts, NW of Livadhia; 41°01′″N, 22°16′30″E; 1180 m; 26.v.2011, *KO* (SAV: TRI 163)		2*n*~20 (DAPI, SL, 6/6; 2.27 ± 0.01)
‡	Greece; Imathia, Vermio Mts, road from Naousa to Kato Vermio; 40°36′38″N, 22°03′25″E; 550 m; 27.v.2011, *KO* (SAV: TRI 165)		2*n*~20 (DAPI, SL, 1/1; 2.23)
‡	Greece; Grevena, Vourinos Mts, S from Exarchos, 40°09′02″N, 21°37′30″E, 684 m, 28.v.2011, *KO* (SAV: TRI 170)		2*n*~20 (DAPI, SL, 4/4; 2.29 ± 0.03)
	Cyanus velenovskyi (Adamović) Wagenitz et Greuter		
‡	Serbia; Stara Planina Mts, S slope of Mt Midžor; 43°22′44″N, 22°40′48″E; 1662 m; 2.vii.2009, *CL* (JE: TOP 1)	2*n* = 20 (1/0)	2*n*~20 (DAPI, SL, 2/2; 2.10 ± 0.01)
‡	Serbia; Stara Planina Mts, Mt Midžor, near the ski resort Babin zub; 43°22′45.6″N, 22°37′39″E; 1592 m; 15.vi.2012, *KO* (SAV: TRI 258)		2*n*~20 (DAPI, SL, 4/4; 2.12 ± 0.01)
‡	Macedonia; Babuna Mts, above Nezhilovo; 41°40′11.8″N, 21°29′29″E; 2064 m; 17.vi.2012, *CL* (SAV: TRI 265)		2*n*~20 (DAPI, SL, 4/4; 2.17 ± 0.04)
‡	Macedonia; Baba Planina Mts, Mt Pelister, ridge Porta above Brajčino; 40°56′00.4″N, 21°12′03.5″E; 1843 m; 21.vi.2012, *CL* (SAV: TRI 269)		2*n*~20 (DAPI, SL, 4/4; 2.19 ± 0.02)
‡	Macedonia; Baba Planina Mts, Mt Pelister, tourist road to Golemo Ezero; 40°57′10″N, 21°11′22″E; 2113 m; 20.vii.2009, *CL* (SAV: PEL 2)		2*n*~20 (DAPI, SL, 1/1; 2.17)

Table 2. *Continued*

	Locality details	Chromosome counts	Ploidy (FCM)
‡	Greece; Pella, E slope of Mt Kaimaktsalan; 40°55′03″N, 21°48′41″E; 2160 m; 13.vi.2010, *MO & KO* (SAV: TRI 144)	2*n* = 20 (1/1)	2*n*~20 (DAPI, SL, 4/4; 2.17 ± 0.00)
‡	Greece; Florina, Pissoderi, Kalo Nero; 40°48′48″N, 21°15′21″E; 1937 m; 17.vii.2007, *CL* (JE: PIS 1)		2*n*~20 (DAPI, SL, 2/2; 2.15 ± 0.00)
	Greece; Florina, Vernon Mts, W slope of Mt Vitsi; 40°38′53.0″N, 21°22′47.1″E; 1921 m; 22.v.2012, *MO & KO* (SAV: TRI 241)		2*n*~20 (DAPI, SL, 4/4; 2.16 ± 0.02)
	Cyanus mollis (Waldst. & Kit.) J.Presl et C.Presl		
‡	Slovakia; Belianske Tatry Mts, Kopské sedlo; 49°13′54″N, 20°12′56″E; 1709 m; 3.x.2007, *CL* (JE: TAT 2)	2*n* = 44 (1/1)	2*n*~44 (DAPI, SL, 1/1; 4.91)
‡	Slovakia; Chočské vrchy Mts, Holica peak; 49°10′10″N, 19°21′23″E; 981 m; 1.x.2007, *CL* (JE: LES 1)	2*n* = 44 (1/1)	2*n*~44 (DAPI, SL, 1/1; 5.16)
‡	Slovakia; Malá Fatra Mts, Mt Poludňový Grúň, Hromové; 49°12′35″N, 19°03′47″E; 1456 m; 29.ix.2007, *CL* (JE: TEC 1)	2*n* = 44 (1/1)	2*n*~44 (DAPI, SL, 1/1; 4.96)
‡	Poland; Bieszczady Mts, near Tarnica peak; 49°04′33″N, 22°43′07″E; 1150 m; 4.x.2007, *CL* (JE: UST 2)	2*n* = 44 (1/0)	
‡	Romania; Bucegi Mts, Poiana Stani; 45°22′10″N, 25°31′06″E; 1290 m; 19.vi.2009; *KO* (SAV: MOL 6)		2*n*~44 (DAPI, SL, 2/2; 4.72 ± 0.07)
‡	Romania; Alba Iulia, Gârda de Sus, north of Gheţari; 46°30′12.1″ N 22°49′50″E; 1217 m; 16.vi.2011, *CL* (JE: GHE)		2*n*~44 (DAPI, SL, 1/1; 4.89)
	Cyanus montanus (L.) Hill		
‡	Austria; Semmering, between Hirschenkogel and Sonnwendstein peaks; 47°38′15″N, 15°50′36″E; 1334 m; 6.x.2007, *CL* (JE: SEM 1)	2*n* = 44 (1/1)	2*n*~44 (DAPI, SL, 1/1; 5.41)
‡	Switzerland; Jura Mts, La Dôle observatory; 46°25′29″N, 06°05′56″E; 1670 m; 21.v.2010, *SŠ, JZ & KM* (JE: DOL)		2*n*~44 (DAPI, SL, 3/3; 5.42 ± 0.04)
	Cyanus achtarovii (Urum.) Holub		
	Bulgaria, Pirin Mts, Popovi Livadi settlement, Mt Orelyak, 41°33′50″N, 23°36′56″E, 1970 m, 17.v.2010, *MO & KO* (SAV: TRI 150)		2*n*~22 (DAPI, SL, 3/3; 2.74 ± 0.04)
	Bulgaria, Pirin Mts, Bansko, Banderica valley, 41°46′13″N, 23°24′46″E, 2336 m, 18.v.2010, *MO & KO* (SAV: TRI 152)		2*n*~22 (DAPI, SL, 5/5; 2.76 ± 0.03)
	Bulgaria, Pirin Mts, Bansko, Bakushevata Mura, 41°46′05″N, 23°24′48″E, 2344 m, 10.viii.2011, *MO & KO* (SAV: TRI 224)		2*n*~22 (DAPI, SL, 3/3; 2.75 ± 0.03)
	Cyanus diospolitanus Bancheva & S. Stoyanov		
	Bulgaria; Sakar Planina Mts, S of Mramor; 42°02′04.8″N, 26°24′40.6″E; 200 m; 17.v.2012; *MO & KO* (SAV: TRI 233)		2*n*~22 (DAPI, SL, 1/1; 2.77)

Table 2. *Continued*

	Locality details	Chromosome counts	Ploidy (FCM)
	Cyanus triumfetti group – *Cyanus adscendens* (Bartl.) Soják		
‡	Slovenia; Nanos Mts, pine forest above Podnanos; 45°47′48″N, 14°00′39″E; 803 m; 24.vi.2007, *IH, MP & KO* (SAV: TRI 71)		2*n*~22 (PI, SL, 6/3; 2.72 ± 0.05)
‡	Slovenia; Nanos Mts, summit Plesa; 45°46′21″N, 14°03′06″E; 1230 m; 24.vi.2007, *IH, MP & KO* (SAV: TRI 72)		2*n*~22 (DAPI, SL, 2/1; 2.77)
‡	Slovenia; NW of Kostelec, karst pastures above the cemetery; 45°35′01″N, 13°51′41″E; 357 m; 27.v.2007, *VK, MP & SŠ* (SAV: P07/24)		2*n*~22 (DAPI, SL, 3/1; 2.62)
‡	Croatia; Samoborska Gora, Smerovišče; 45°46′29.2″N, 15°38′40.2″E; 317 m; 3.vi.2012, *MO, JO & KO* (SAV: TRI 242)		2*n*~22 (DAPI, SL, 1/1; 2.56)
	Cyanus triumfetti group – *C. axillaris*, non auct. Greuter 2006–2009, *sensu* Olšavská *et al*. 2011		
	Czech Republic; Stredočeská pahorkatina, rocks close to Zvíkov Castle; 49°26′13″N, 14°11′37″E; 397 m; 23.v.2008, *KO* (SAV: TRI 91)		2n~22 (PI, SL, 2/1; 2.52)
‡	Czech Republic; Český kras, slope close to České Srbsko; 49°55′52″N, 14°08′25″E, 236 m; 10.v.2008, *IH & MP* (SAV: TRI 86)		2*n*~22 (PI, SL, 2/1; 2.51)
	Cyanus triumfetti group – *Cyanus pinnatifidus* (Schur) Holub		
	Romania; Munţii Ceahlău, Toaca peak; 46°58′36″N, 25°57′05″E; 1800 m; 25.viii.2006, *CL* (JE: CEA 1)	2*n* = 22 (1/0)	
‡	Romania; Munţii Rarău-Giumalău, Pietrele Doamnei peak; 47°26′51″*N*, a; Munţii Bucegi, Valea Jepilor; 45°24′27″N, 25°29′37″E; 1800 m; 28.viii.2006, *CL* (JE: BUS 2)	2*n* = 22 (1/1)	2*n*~22 (DAPI, SL, 1/1; 2.83)
	Romania; Munţii Poiana Braşov, Postavarul peak; 45°33′57″N, 25°33′39″E; 1750 m; 27.viii.2006, *CL* (JE: BRA 5)	2*n* = 22 (1/0)	2*n*~22 (DAPI, SL, 1/1; 2.70)
‡	Romania; Munţii Rarău-Giumalău, Pietrele Doamnei peak; 47°26′51″ N, 25°33′50″E; 1638 m; 13.vii.2008, *MP & KO* (SAV: TRI 114)		2*n*~22 (DAPI, SL, 4/2; 2.55 ± 0.00)
‡	Romania; Munţii Bucegi, Cheile Tatarului; 45°21′42″N, 25°25′52″E; 1460 m; 17.vii.2008, *MP & KO* (SAV: TRI 118)		2*n*~22 (DAPI, SL, 4/2; 2.59 ± 0.00)
‡	Romania; Munţii Poiana Braşov, Mt Tâmpa; 45°38′03″N, 25°35′32″E, 955 m; 27.vi.2007; *IH*, *MP & KO* (SAV: TRI 73)		2*n*~22 (PI, SL, 7/4; 2.74 ± 0.04)
	Cyanus triumfetti group – *Cyanus semidecurens* (Jord.) Holub		
*	Spain; Rasos de Peguera, Mt Roca d′Auró; 42°07′34″N, 01°48′22″E; 1480 m; 21.v.2008, *CL* (JE: BER 2)		2*n*~22 (DAPI, SL, 3/3; 2.88 ± 0.08)
*	Spain; Serra de Cabrerra, Mt Cabrerra; 42°04′20″N, 02°24′30″E, 1276 m, 20.v.2008, *CL* (JE: RUP 1)		2*n*~22 (DAPI, SL, 2/2; 2.89 ± 0.04)
	Cyanus triumfetti group – *Cyanus strictus* (Waldst. & Kit.) Soják		
‡	Hungary; Bükk Mts, Kis-Kimázsa Hill; 48°03′23″N, 20°42′31″E; 339 m; 17.v.2008, *MP & KO* (SAV: TRI 85)		2*n*~22 (PI, SL, 4/2; 2.36 ± 0.00)

Table 2. *Continued*

	Locality details	Chromosome counts	Ploidy (FCM)
	Cyanus ternopoliensis (Dobrocz.)		
*	Ukraine; Ternopilska oblasť, Berezhany; 49°28′32.7″N, 24°53′36.8″E; 331 m; 12.vii.2012, *KO* (SAV: TRI 283)		$2n \sim 22$ (DAPI, SL, 4/4; 2.52 ± 0.01)
*	Ukraine; Ternopilska oblasť, NE of Jablunivka; 49°13′59.9″N, 25°05′05.0″E; 351 m; 12.vii.2012, *KO* (SAV: TRI 284)		$2n \sim 22$ (DAPI, SL, 4/4; 2.57 ± 0.01)
	Cyanus triumfetti group – *Cyanus triumfetti* (All.) Á.Löve & D.Löve		
‡	Italy; Gran Paradiso Mts, above Lillaz; 45°35′45,9″N, 07°23′31,2″E; 1644 m; 10.vi.2008, *MP, MO & KO* (SAV: TRI 104)		$2n \sim 22$ (DAPI, SL, 1/1; 2.62)
‡	Italy; Valle del Gran Sanbernardo, close to Saint-Oyen; 45°49′18,5″N, 07°13′40,1″E; 1299 m; 11.vi.2008, *MP, MO & KO* (SAV: TRI 105)		$2n \sim 22$ (DAPI, SL, 3/1; 2.62)
‡	Switzerland; Wallis, meadow close to Blattern; 46°21′40″N, 07°58′49,4″E; 1469 m; 12.vi.2008, *MP, MO & KO* (SAV: TRI 106)		$2n \sim 22$/ (DAPI, SL, 5/2; 2.61 ± 0.01)
‡	Switzerland; Wallis, above road to Ried bei Mörel; 46°20′47,1″N, 08°01′10,9″E; 1103 m; 12.vi.2008, *MP, MO & KO* (SAV: TRI 107)		$2n \sim 22$/ (DAPI, SL, 4/2; 2.60 ± 0.00)
‡	France; Alpes Maritimes Mts, close to Colle di Tende saddle; 44°08′22″N, 07°33′00″E; 1851 m; 17.vi.2007, *MP & KO* (SAV: TRI 68)		$2n \sim 22$ (PI, SL, 4/3; 2.72 ± 0.07)
	Cyanus triumfetti group – uncertain taxonomy		
‡	Italy; Mt Cimone, Passo di San Boldo; 46°01′09″N, 12°09′54″E; 656 m; 12.vi.2007, *MP & KO* (SAV: TRI 58)		$2n \sim 22$ (PI, SL, 6/3; 2.77 ± 0.07)
	Italy, Gran Sasso d' Italia, Campo Imperatore; 42°26′35″N, 13°33′00″E; 2170 m; 17.v.2008, *CL* (JE: LAQ 2)	$2n = 22$ (1/1)	$2n \sim 22$ (DAPI, SL, 1/1; 2.63)
‡	Italy; Umbria, Mt Catria above Cantiano; 43°27′58″N, 12°42′19″E; 1487 m; 21.vii.2006, *MP & KO* (SAV: TRI 28)		$2n \sim 22$ (PI, SL, 6/4; 2.78 ± 0.04)
‡	Italy; Umbria, Mt Acuto above Cantiano; 43°28′31″N, 12°41′03″E; 1589 m; 21.vii.2006, *MP & KO* (SAV: TRI 29)		$2n \sim 22$ (PI, SL, 5/3; 2.81 ± 0.02)
‡	Italy; Umbria, road from Caprile to Mt Acuto; 43°28′23″N, 12°34′59″E; 1127 m; 21.vii.2006, *MP & KO* (SAV: TRI 30)		$2n \sim 22$ (PI, SL, 5/3; 2.83 ± 0.07)
	Italy; Appennino Ligure Mts, road to Piani di Lesima; 44°40′48.1″N, 09°16′45.2″E; 1296 m; 5.vi.2008, *VK, MP & KO* (SAV: TRI 98)		$2n \sim 22$ (DAPI, SL, 2/1; 2.64)
‡	Italy; Appennino Ligure Mts, close to Codorso; 44°26′10.3″N, 09°32′14.1″E; 898 m; 6.vi.2008, *VK, MP & KO* (SAV: TRI 99)		$2n \sim 22$ (DAPI, SL, 2/2; 2.66 ± 0.01)
‡	Italy; Appennino Ligure Mts, 1 km S of Passo del Bocco; 44°25′0.9″N, 09°27′29.8″E; 883 m; 6.vi.2008, *VK, MP & KO* (SAV: TRI 100)		$2n \sim 22$ (DAPI, SL, 2/1; 2.60)
‡	Italy; Dolomiti Mts, meadow close to Moena; 46°23′01″N, 11°40′01″E; 1229 m; 12.vi.2007, *MP & KO* (SAV: TRI 59)		$2n \sim 22$ (PI, SL, 6/3; 2.72 ± 0.07)
‡	Italy; Sasso Malascarpa Mts, Corni di Canzo peak; 45°51′45″N, 09°19′31″E; 1270 m; 13.2008, *MP & MO* (SAV: TRI 108)		$2n \sim 22$ (DAPI, SL, 3/1; 2.59)

Table 2. *Continued*

	Locality details	Chromosome counts	Ploidy (FCM)
	Italy; Gargano, Foresta Umbra, road to Casa Forestale; 41°43′53.5″N, 15°57′13.0″E; 758 m; 13.vi.2011, *MO, JO & KO* (SAV: TRI 190)		$2n$~22 (DAPI, SL, 2/2; 2.57 ± 0.00)
	Italy; Gargano, Foresta Umbra, close to Casa Forestale; 41°46′14.4″N, 15°59′08.8″E; 596 m; 13.vi.2011, *MO, JO & KO* (SAV: TRI 191)		$2n$~22 (DAPI, SL, 3/3; 2.56 ± 0.00)
‡	Italy; Mt Vulture, road W from Barile; 40°57′39.0″N, 15°59′00.4″E; 773 m; 13.vi.2011, *JO & KO* (SAV: TRI 192)		$2n$~22 (DAPI, SL, 1/1; 2.60)
‡	Italy; Mt Vulture, Rionero in Vulture; 40°56′48.1″N, 15°38′05.2″E; 1220 m; 13.vi.2011, *JO & KO* (SAV: TRI 193)		$2n$~22 (DAPI, SL, 2/2; 2.59 ± 0.01)
‡	Italy; Basilicata, Mt Serranetta; 40°33′26.1″N, 15°49′04.5″E; 1277 m; 13.vi.2011, *MO, JO & KO* (SAV: TRI 194)		$2n$~22 (DAPI, SL, 1/1; 2.60)
‡	Italy; Dolomiti Lucane Mts, Mt Impiso; 40°28′42″N, 16°05′45″E; 1106 m; 13.vi.2011, *JO & KO* (SAV: TRI 201)		$2n$~22 (DAPI, SL, 3/3; 2.60 ± 0.01)
‡	Italy; Sirente Mts, Mt Revecena; 41°16′32″N, 13°33′32″E; 1815 m; 17.vi.2011, *MO, JO & KO* (SAV: TRI 203)		$2n$~22 (DAPI, SL, 1/1; 2.61)
‡	Italy; Sibillini Mts, Mt Prata; 42°52′26.6″N, 13°12′22.7″E; 1785 m; 17.vi.2011, *KO* (SAV: TRI 206)		$2n$~22 (DAPI, SL, 2/2; 2.60 ± 0.01)
‡	Italy; Sibillini Mts, Forca Canapine, 42°45′07.5″N, 13°11′51.3″E; 1519 m; 28.v.2007, *VK, MP & SŠ* (SAV: P07/47)		$2n$~22 (DAPI, SL, 2/1; 2.64)
‡	Italy; Pratomagno Mts, Vallambrosa; 43°42′58.9″N, 11°32′29.1″E; 845 m; 17.vi.2011, *JO & KO* (SAV: TRI 207)		$2n$~22 (DAPI, SL, 4/4; 2.63 ± 0.01)
‡	Italy; Monti Berici Mts, Sossano; 42°22′49″N, 11°55′48.7″E; 265 m; 18.vi.2011; *MO, JO & KO* (SAV: TRI 208)		$2n$~22 (DAPI, SL, 3/3; 2.62 ± 0.02)
‡	Croatia; Velebit Mts, Mt Tulove Grede; 44°16′18.7″N, 15°38′37.9″E; 948 m; 4.vi.2012, *MO, JO & KO* (SAV: TRI 244)		$2n$~22 (DAPI, SL, 1/1; 2.57)
‡	Croatia; N of Zelengrad; 44°09′10.3″N, 15°43′10.0″E; 393 m; 4.vi.2012, *MO, JO & KO* (SAV: TRI 245)		$2n$~22 (DAPI, SL, 4/4; 2.55 ± 0.01)
‡	Croatia; Mt Poštak, near Oton; 44°06′12.3″N, 16°09′18.3″E; 527 m; 5.vi.2012, *MO, JO & KO* (SAV: TRI 248)		$2n$~22 (DAPI, SL, 2/2; 2.54 ± 0.01)
‡	Croatia; Mt Promina (Čavnovka), N of Drniš; 43°54′35.5″N, 16°10′19.5″E; 851 m; 5.vi.2012, *MO, JO & KO* (SAV: TRI 249)		$2n$~22 (DAPI, SL, 1/1; 2.52)
‡	Serbia; Svrljig Mts, Mt Popova glava, W part of the peak area; 43°21′40.8″N, 22°05′24.3″E; 962 m; 13.vi.2012, *CL & KO* (SAV: TRI 255)		$2n$~22 (DAPI, SL, 1/1; 2.48 ± 0.01)
‡	Montenegro; Lovćen Mts, Štirovnik peak; 42°23′55″N, 18°49′″E; 1698 m; 15.viii.2007, *CL* (JE: LOV 1)	$2n = 22$ (1/0)	
‡	Bulgaria; Stara Planina Mts, E part of Mt Chepun; 42°57′10″N, 22°57′35″E; 1172 m; 30.vi.2011, *KO* (SAV: TRI 211)		$2n$~22 (DAPI, SL, 3/3; 2.53 ± 0.01)
‡	Bulgaria; Stara Planina Mts, Sliven, Sinite Kameni; 42°42′54.3″N, 26°23′58.4″E; 469 m; 14.v.2012, *MO & KO* (SAV: TRI 227)		$2n$~22 (DAPI, SL, 3/3; 2.65 ± 0.04)
‡	Bulgaria; Vitosha Mts, Kapitoto; 42°38′17″N, 23°14′13″E; 1234 m; 1.viii.2011, *KO* (SAV: TRI 213)		$2n$~22 (DAPI, SL, 3/3; 2.53 ± 0.01)
‡	Bulgaria; Rhodope Mts, S from Malevo; 41°57′03″N, 24°21′44″E; 900 m; 8.viii.2011, *KO* (SAV: TRI 218)		$2n$~22 (DAPI, SL, 2/2; 2.65 ± 0.01)

Table 2. *Continued*

	Locality details	Chromosome counts	Ploidy (FCM)
‡	Bulgaria; Rhodope Mts, meadow close to Ravnogor; 41°57′03″N, 24°21′44″E; 1311 m; 8.viii.2011, *KO* (JE: RAV1; SAV: TRI 219)	$2n = 22 + 1\mathrm{B}$ (1/0)	$2n\sim22$ (DAPI, SL, 2/2; 2.57 ± 0.01)
‡	Bulgaria; Rhodope Mts, road to Peshterska Snezhanka; 42°00′40″N, 24°16′41″E; 600 m; 8.viii.2011, *KO* (SAV: TRI 220)		$2n\sim22$ (DAPI, SL, 4/4; 2.20 ± 0.02)
‡	Bulgaria; Rhodope Mts, tourist path to Golyama Syutkya; 41°58′14″N, 23°58′17″E; 1309 m; 9.viii.2011, *KO* (SAV: TRI 221)		$2n\sim22$ (DAPI, SL, 2/2; 2.52 ± 0.02)
‡	Bulgaria; Pirin Mts, rocks near to Bansko; 41°48′35.1″N, 23°27′42.2″E; 1157 m; 11.viii.2011, *KO* (JE: BAN; SAV: TRI 225)		$2n\sim22$ (DAPI, SL, 3/3; 2.66 ± 0.02)
‡	Romania; Cluj, Boj-Catun railway station; 46°42′27″N, 23°49′06″E; 496 m; 19.vii.2011, *CL* (JE: BOJ)		$2n\sim22$ (DAPI, SL, 3/1; 2.51)
‡	Romania; Retezat Mts, Retezatul Mic, Piule peak; 45° 17′ 56″N, 22° 55′ 26″E; 1875 m; 26.vii.2011, *CL* (JE: PIU)		$2n\sim22$ (DAPI, SL, 3/1; 2.60)
‡	Ukraine; Vinogradiv, Čorno Gora Hill; 48°08′15″N, 23°04′26″E; 257 m; 2.vii.2012, *KO* (SAV: TRI 276)		$2n\sim22$ (DAPI, SL, 5/5; 2.52 ± 0.01)

*First chromosome count.
†New ploidy level.
‡New record.
Collector abbreviations: IH, I. Hodálová; VK, V. Kolarčik; CL, C. Löser; KM, K. Marhold; JO, J. Olšavská; MO, M. Olšavský; KO, K. Olšavská; MP, M. Perný; SŠ, S. Španiel; JZ, J. Zozomová.

in m. Nidgé regione oxycedri alt. 2650′–3000′ (substr. marm.)'; Grisebach, 1846; = Mt. Piperitsa] (Fig. 1D); the same cytotype was reported by Baltisberger (2012) from the Vourinous Mts. Besides, we recorded $2n = 40$ for the remaining investigated Greek accessions of *C. pindicola* (Fig. 1C), which agrees with the observation by Baltisberger (1991). For the first time triploids were found in *C.* section *Protocyanus*, namely in both investigated populations of *C. nissanus* from the Svrljiska Planina Mts ($2n = 3x = 30$, eastern Serbia; Figs 1J, 2) and for all studied plants of *C. thirkei* ($2n\sim3x\sim30$) from a population above the village of Sadovo (Bulgaria). Triploidy ($2n\sim3x\sim30$) was also confirmed for a newly discovered hybrid, *C. epirotus* × *C. graminifolius*, in the Mitsikeli Mts and Timfi Mts (Greece) (Fig. 2). The presence of one to four B-chromosomes was observed for *C. epirotus*, *C. graminifolius*, *C. triumfetti* s.l. and *C. tuberosus* (Table 2; Fig. 1A, L).

GENOME SIZE DATA

The 2C-value of DNA content across the whole dataset varied by 183.4% and ranged from 1.93 pg for *C. thirkei* ($2n = 20$) to 5.47 pg for *C. montanus* ($2n = 44$). In particular cytotypes 2C-values varied by 33.9% for plants with $2n = 20$ (1.93–2.58 pg), by 12.1% for plants with $2n = 30$ (2.91–3.31 pg), by 19% for plants with $2n = 40$ (3.18–3.78 pg), by 13% for plants with $2n = 22$ (2.36–2.98 pg) and by 17% for plants with $2n = 44$ (4.67–5.47 pg) (Fig. 3). The differences in 2C-values between any pair of cytotypes were significant (Tukey–Kramer test, $P < 0.001$). Differences in Cx-values between diploids and corresponding tetraploids were significant in the case of plants with $2n = 20$ and $2n = 40$ (Mann–Whitney *U*-test; $P < 0.001$), whereas differences between plants with $2n = 22$ and $2n = 44$ were not significant (Mann–Whitney *U*-test, $P = 0.062$). The CV values of G1 peaks of *Cyanus* samples were 1.71 ± 0.36 and 5.49 ± 0.75% for DAPI and PI staining, respectively. The mean CV values of the G1 peaks of internal standards were 1.79 ± 0.44% for *Bellis perennis* with DAPI staining and 1.78 ± 0.36 and 5.91 ± 0.73% for *Solanum lycopersicum* with DAPI and PI staining, respectively.

GEOGRAPHICAL DISTRIBUTION OF CYTOTYPES

Cytotype variation is geographically structured with overlaps among the distribution areas of particular cytotypes (Fig. 4, Fig. S1). The cytotype $2n = 22$ has the widest distribution covering a substantial part of Europe between 40° and 51° N (dominated by populations of the *C. triumfetti* group). Plants with $2n = 44$ are concentrated mainly in the Alps (*C. montanus*)

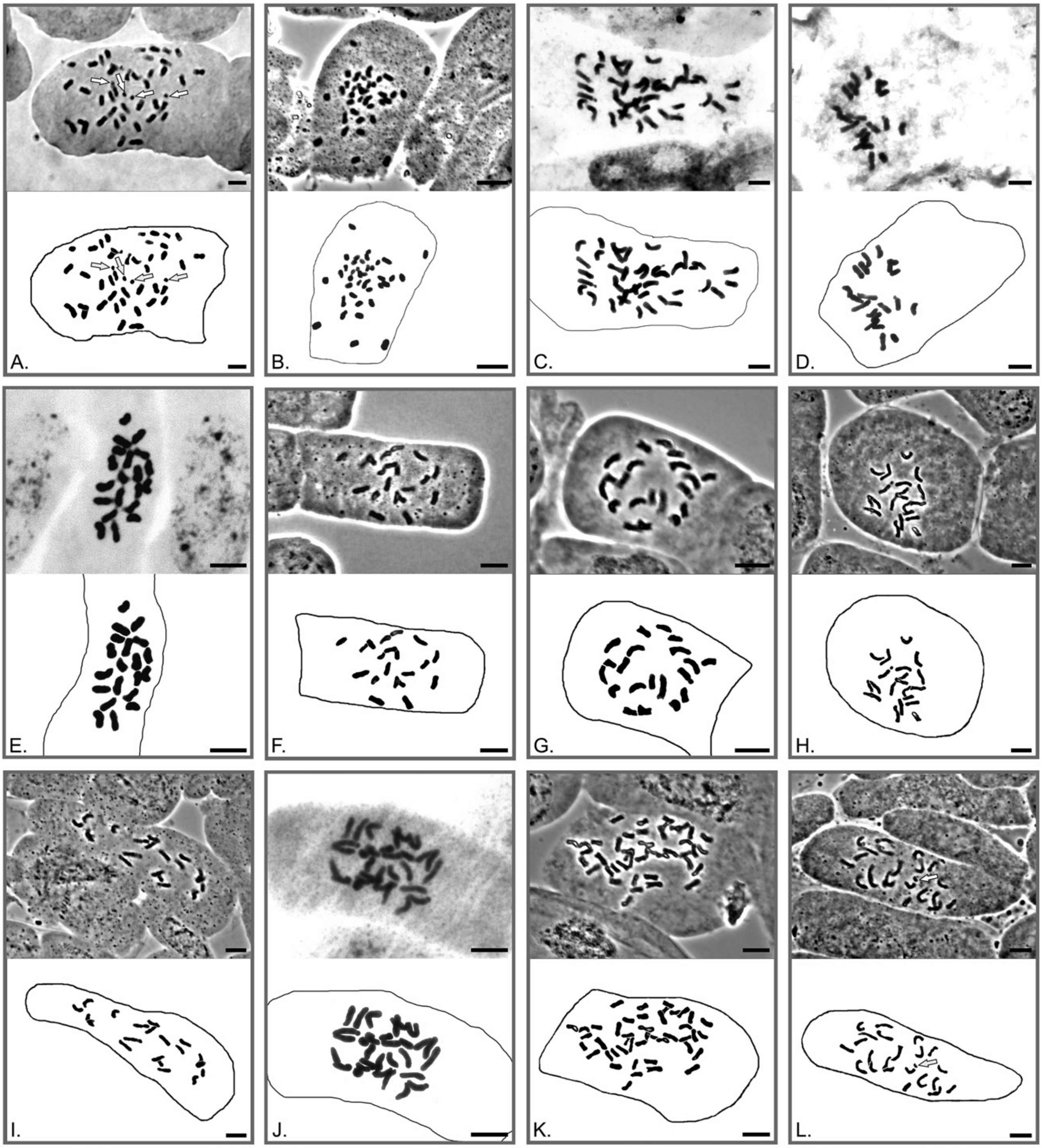

Figure 1 Somatic metaphases of: A, *Cyanus graminifolius* (SIC1/I, 2*n* = 40 + 4B); B, *C. graminifolius* (172/6, 2*n* = 40); C, *C. pindicola* (178/6, 2*n* = 40); D, *C. pindicola* (145/6, 2*n* = 20); E, *C. lingulatus* (PUR313/10, 2*n* = 20); F, *C. velenovskyi* (TOP1/I, 2*n* = 20); G, *C. epirotus* (PAP2/I, 2*n* = 20); H, *C. tuberosus* (PAN1/I, 2*n* = 20); I, *C. nissanus* (SIC1/I, 2*n* = 20); J, *C. nissanus* (SIC1/1, 2*n* = 30); K, *C. mollis* (TAT2/I, 2*n* = 44); L, *C. pinnatifidus* (BRA5/I, 2*n* = 22 + 1B). Scale bars = 5 µm; B-chromosomes are marked by arrows.

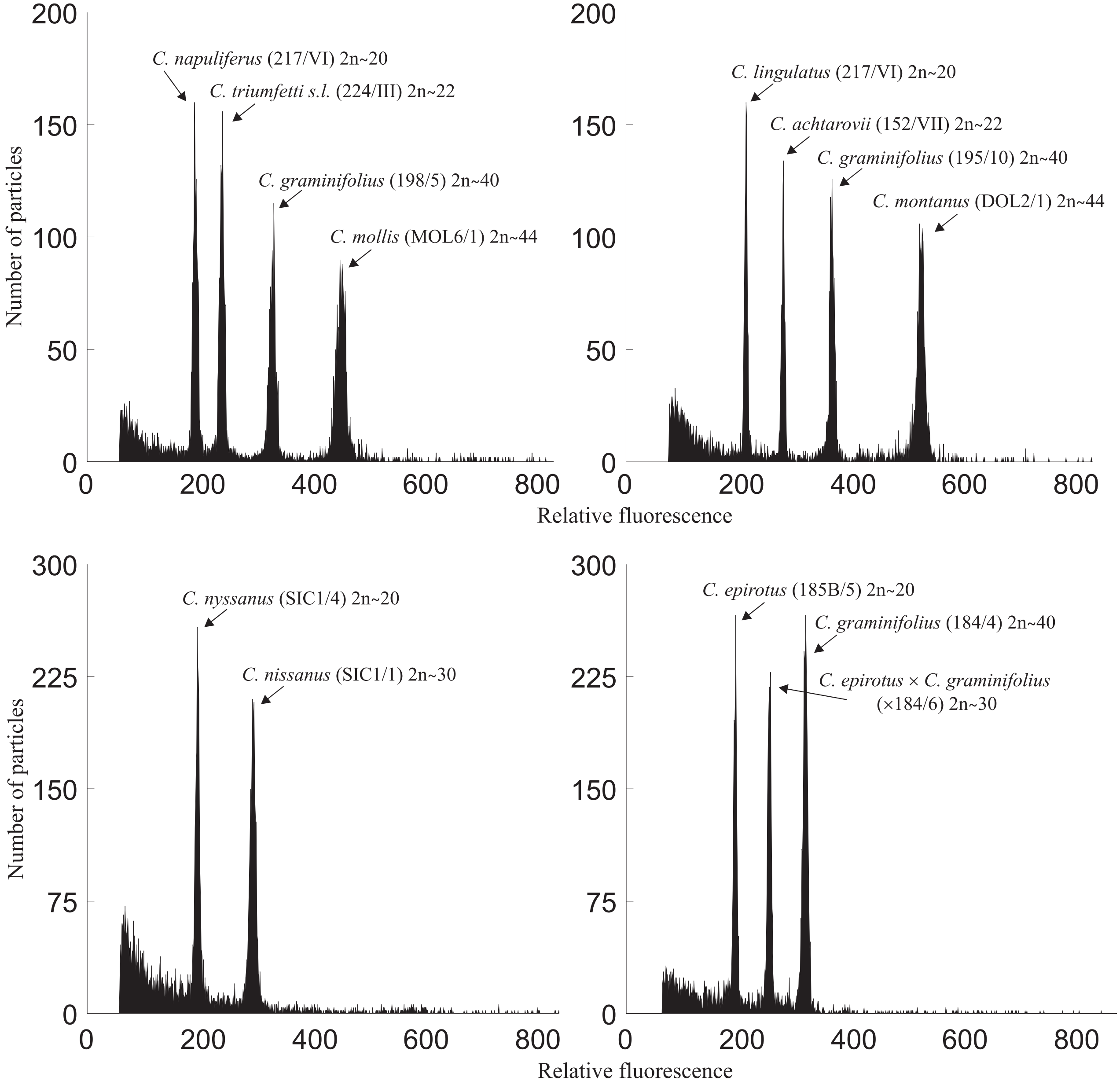

Figure 2 Histograms of relative DNA content of DAPI-stained nuclei.

and in the Carpathians (*C. mollis*). Taxa with the derived base chromosome number $x = 10$ are restricted to southern Europe: cytotype $2n = 20$ is recorded in Spain (*C. lingulatus*), the Balkan Peninsula (*C. epirotus*, *C. napulifer*, *C. orbelicus*, *C. tuberosus* and *C. velenovskyi*) and the Black Sea coast (*C. thirkei*); for cytotype $2n = 40$ four disjunct areas have been identified: southern France, Calabria, Greece (*C. graminifolius*) and Crimea (*C. fuscomarginatus*). Diploids ($2n = 20$ and $2n = 22$) have greater geographical ranges in Europe than their corresponding tetraploids ($2n = 40$ and $2n = 44$).

ECOLOGICAL DIFFERENTIATION OF CYTOTYPES

The distribution of populations in the PCA diagram (Fig. 5) along the first axis was strongly influenced by altitude. BioClim variables are correlated mainly with the second axis: variables indicating humid climate are positively correlated and variables reflecting higher temperatures are negatively correlated. No or only a slight correlation between cytotype distribution and type of biotope or type of bedrock was observed.

Analyses of the ecological differentiation of cytotypes from *C.* section *Protocyanus* show that ecologi-

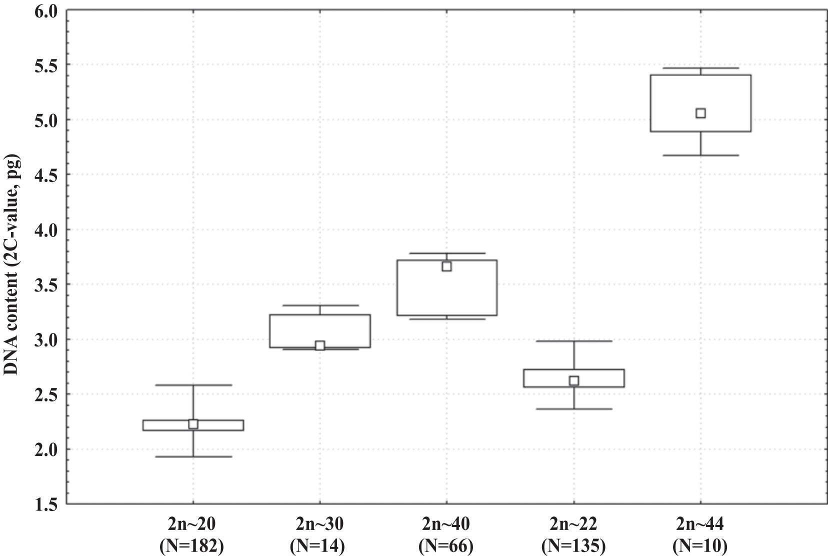

Figure 3 DNA content (represented by 2C-values in pg) of cytotypes of *Cyanus* section *Protocyanus*. Boxes define the 25th and 75th percentiles, squares show medians, whiskers are from minimum to maximum (*N* = number of flow cytometry analyses).

cal boundaries among them are not strictly defined; however, some general preferences are visible (Fig. 5). Diploids ($2n = 20, 22$) are more ecologically diversified than corresponding tetraploids ($2n = 40, 44$). Tetraploid cytotypes occupy similar ecological conditions to diploid cytotypes with the same base chromosome number. Taxa with $x = 10$ prevail in areas combining high altitude with lower precipitation and higher temperatures during warm and dry and cold periods of the year. The two exceptions in the $2n = 20$ cytotype are *C. epirotus*, which occurrs in more humid conditions, and *C. thirkei*, which occupies the extreme position at the left side of the diagram due to low altitude (Fig. 5). Populations of *C. graminifolius* from southern France and Calabria and *C. fuscomarginatus* from Crimea (both $2n = 40$) are concentrated in a group close to the first axis (higher altitudes with intermediate climate conditions), whereas populations of *C. graminifolius* from Greece have much broader ecological amplitude. The $2n = 22$ cytotype represented mainly by taxa from the *C. triumfetti* group is the most ecologically diversified and geographically widespread. Populations from low elevations (up to 600–700 m a. s. l.) with higher temperature and precipitation seasonality and a higher temperature during the driest quarter of the year belong predominantly to the $2n = 22$ cytotype [the *C. triumfetti* group, *C. diospolitanus* and *C. pseudaxillaris* (Stef. & T.Georgiev) Holub]. *Cyanus pichleri* from Bulgaria is the only taxon with $2n = 44$ that is confined to similar conditions. A shift in ecological niches between *C. mollis* and *C. montanus* (both $2n = 44$) is visible, because *C. montanus* is represented mainly by alpine populations occurring in areas with the coldest and the most humid climate.

DISCUSSION

Here we report new chromosome number and ploidy data for perennial *Cyanus* species in Europe and summarize previous records. This is the first time that a geographically complete assembly of cytotypes has been presented for this taxonomically intricate group. Neither biosystematic studies nor those using DNA sequence analyses (Boršić *et al.*, 2011; C. J. Löser, unpubl. data) have sufficiently elucidated the evolutionary relationships among cytotypes of *C.* section *Protocyanus*. The representation of cytotypes on a map is a first step towards this understanding and could serve as a baseline for further research in this section. Detailed cytogeographical studies focusing on higher taxonomic units such as at the genus or section level are still rare (Watanabe, 1986; Bigazzi & Selvi, 2001; Cook & Semple, 2008; Pellicer *et al.*, 2012) in comparison with numerous studies focusing on particular polyploid complexes (e.g. Schönswetter *et al.*, 2007a; Suda *et al.*, 2007; Mráz *et al.*, 2008, 2012; Perný *et al.*, 2008; Kolář *et al.*, 2009; Duchoslav, Šafářová & Krahulec, 2010; Li *et al.*, 2010; Trávníček, Eliášová & Suda, 2010; Koutecký *et al.*, 2011; Trávníček *et al.*, 2012).

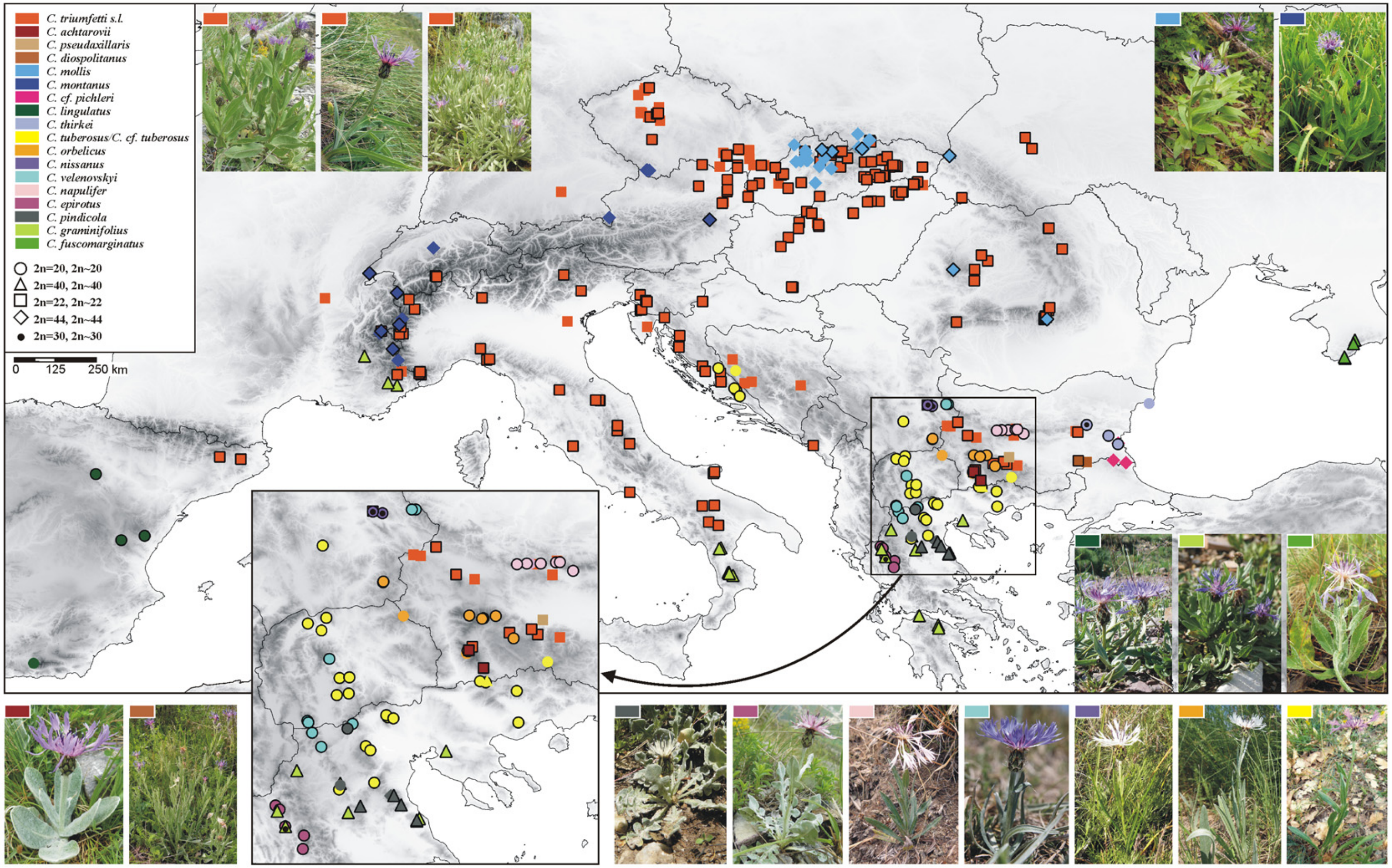

Figure 4 Cytotype distribution of *Cyanus* section *Protocyanus* in Europe. Localities indicated by symbols with black margins are based on our records presented in this paper (Table 2) or published previously; symbols without black margins are based on published records of other authors (see Table S2). Distributions of particular cytotypes are presented separately in Figure S1.

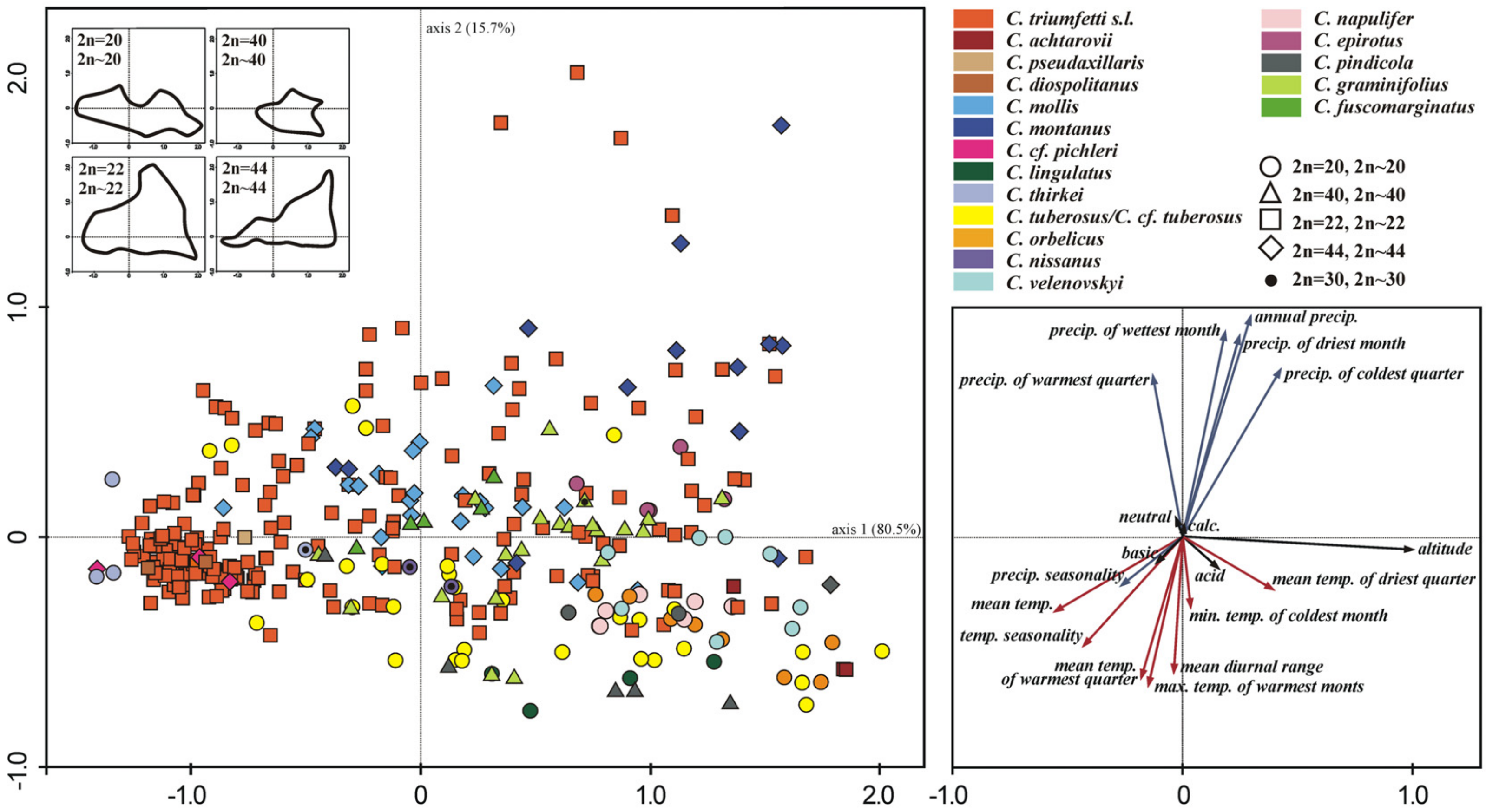

Figure 5 PCA based on 17 environmental and three geographical variables of 358 localities of *Cyanus* section *Protocyanus* in Europe.

PLOIDY AND CHROMOSOME NUMBERS

Subtribe Centaureinae contain three frequent ploidies: diploid, tetraploid and hexaploid (Romaschenko *et al.*, 2004; Šiljak-Yakovlev *et al.*, 2005). Minority ploidies (triploid, pentaploid) have been documented in the tribe only rarely (*Centaurea* L. *s.s. Cheirolophus* Cass.; Garnatje, Garcia Jacas & Vilatersana, 2001; Koutecký, Štěpánek & Baďurová, 2012). Although diploid and tetraploid levels have been frequently reported for perennial *Cyanus* species, the records of triploids reported here are the first for the genus.

Phylogenetic analyses have shown that *Cyanus* belongs to the derived clade of subtribe Centaureinae (Susanna *et al.*, 1995; Garcia-Jacas *et al.*, 2001). The chromosome base numbers of this clade are lower ($x = 12$ or less) than those of the early branching clades of Centaureinae ($x = 13–16$). The descending trend of base chromosome numbers occurs repeatedly in different lineages of Centaureinae and is in agreement with phylogenetic trees inferred from pollen types (Wagenitz & Hellwig, 1996) and DNA sequences (Susanna *et al.*, 1995; Garcia-Jacas *et al.*, 2001). In *Cyanus* higher base chromosome numbers can be regarded as ancestral and are found in both sections: $x = 12$ in annuals of section *Cyanus* and $x = 11$ in perennials of section *Protocyanus* (Wagenitz & Hellwig, 1996; Romaschenko *et al.*, 2004). The base chromosome numbers $x = 8$, 9 and 10 should be considered as derived in the genus *Cyanus*. All these derived numbers are reported for section *Cyanus*, whereas only $x = 10$ and 11 are known for section *Protocyanus*. Regarding results of recent molecular studies (Boršić *et al.*, 2011; C. J. Löser, unpubl. data) showing perennials as phylogenetically sister or ancestral to annuals, we suggest that the perennial ancestor of *Cyanus* had at least $x = 12$. If so then traces of the initial phases of radiation of *Cyanus* have disappeared and have been replaced by derived taxa.

In *C.* section *Protocyanus* some chromosome reports remain doubtful due to miscounting or misidentification, but others can be resolved by re-examination of material from identical locations. For example, a previous count for *C. graminifolius*, $2n = 44$ (Guinochet, 1957), was corrected by recent analyses to $2n = 40$ (Olšavská & Perný, 2009). Another case of ambiguity arises from four tetraploid counts of $2n = 44$ published for the *C. triumfetti* group from southern and eastern Europe. Three counts from the Strandzha Mts and the Black Sea coast (Bulgaria; Sharkova, 1996; Sharkova & Peev, 1997) were later assigned to *C. pichleri* (Bancheva & Denchev, 2000). The tetraploid count for *C. triumfetti s.s.* from Mitoraj near Vinodol (Croatia; Lovrić, 1982) has not yet been reconfirmed. For *C. tuberosus* two different diploid counts, $2n = 20$ (Šiljak-Yakovlev, 1985) and $2n = 22$ (Šiljak-Yakovlev *et al.*, 2005), were reported. We suppose that the number $2n = 22$ from Mt Biokovo refers to a taxon from the *C. triumfetti* group according to our field observations at the locality stated in the publication. There are additional taxa with disparate chromosome records that need to be studied further, e.g. $2n = 20$ (Morales, 1974; present study) vs. $2n = 22$ (Löve & Kjellqvist, 1974; Montserrat-Martí, 1987) for *C. lingulatus*, $2n = 40$ (present study) vs. $2n = 44$ (Strid & Franzén, 1981) for *C. pindicola*. We detected B-chromosomes in both $2n = 20$ and 40 cytotypes (Table 1, Fig. 1A) that could have inflated chromosome numbers in some previous counts.

GENOME SIZE DATA

The clear differences in genome size among plants with different chromosome number (Fig. 3) support the use of flow cytometry as a suitable method to establish ploidy in *C.* section *Protocyanus*. Similar advantages have been reported for other genera (Pellicer *et al.*, 2007, 2012; Kolář *et al.*, 2009). In the dataset presented here, an overlap in genome size was observed between plants with $2n = 20$ and $2n = 22$ (Fig. 3). This was caused mainly by populations of *C. tuberosus* from Croatia with $2n = 20$ and 2C DNA contents of 2.48–2.52 pg, which is close to the populations with $2n = 22$ (2.36–2.89 pg). The DNA contents of the remaining populations with $2n = 20$ were 1.93–2.33 pg (Table 2).

A decrease of genome size per basic chromosome set (Cx-value), often observed in polyploid series (Leitch & Bennett, 2004; Pellicer *et al.*, 2007), was revealed within *C.* section *Protocyanus* between diploids with $2n = 20$ (mean $2C = 2.22 \pm 0.13$ pg) and tetraploids with $2n = 40$ (mean $2C = 1.77 \pm 0.12$ pg). However, the Cx-values were relatively constant between diploids (2.64 ± 0.11 pg) and tetraploids (2.55 ± 0.15 pg) with $x = 11$ (this study; Olšavská *et al.*, 2012). A previous detailed study revealed that 2C-values at the diploid level (the *C. triumfetti* group) varied by 21% and at the tetraploid level (the *C. montanus* group) by 13% (Olšavská *et al.*, 2012). Significant between-species differences in genome size indicate that this attribute may be used as a supportive taxonomic marker in the *C. triumfetti* and *C. montanus* groups. The variation in genome size within $2n = 20$, $2n = 30$ and $2n = 40$ cytotypes is currently under study (K. Olšavská *et al.*, unpubl. data).

EVOLUTIONARY SIGNIFICANCE OF CYTOTYPES

Knowledge of the allopatric/sympatric distribution and coexistence of cytotypes and frequencies of intercytotype crossing is helpful for understanding evolutionary processes (Schönswetter *et al*., 2007a; Mráz *et al*., 2012; Trávníček *et al*., 2012). Notably, in section *Protocyanus* a sympatric distribution is mainly observed for: (1) taxa differing in ploidy, e.g. the *C. triumfetti* group (2*n* = 22) co-occur with *C. montanus* or *C. mollis* (2*n* = 44); and (2) taxa with the same ploidy but different base chromosome numbers, e.g. the *C. triumfetti* group (2*n* = 22) co-occur with *C. napulifer* or *C. nissanus* (2*n* = 20) (Fig. 4, Fig. S1). In contrast, taxa with the same chromosome number from any of the three groups of section *Protocyanus* do not co-occur and form parapatric contact zones (Fig. 4, Fig. S1). Experimental hybridizations showed that taxa of the *C. triumfetti* group (2*n* = 22) hybridized easily and produced viable progeny (Olšavská & Löser, 2013). This argues for the importance of allopatry in speciation of taxa with the same chromosome number. Geographical isolation has been shown to lead to speciation in several plant groups with the same chromosome number (e.g. Watanabe, 1986; Martín-Bravo *et al*., 2010). The reproductive barrier caused by karyological differences seems to be sufficient enough to prevent hybridization in section *Protocyanus*. Reproductive incompatibilities between the *C. triumfetti* group (2*n* = 22) and the *C. montanus* group (2*n* = 44) were confirmed by hybridization experiments (Olšavská & Löser, 2013). Strong reproductive isolation between diploids and tetraploids has also frequently been observed in the closely related genus *Centaurea* (Koutecký *et al*., 2011; Mráz *et al*., 2012). In section *Protocyanus*, this barrier is broken only rarely, as in the case of *C. epirotus* and *C. graminifolius*. Triploids found in the Mitsikeli Mts (western Greece) are apparently hybrids between *C. epirotus* and *C. graminifolius* combining morphological characters of both parents (e.g. light pinkish-purple florets and white-tomentose leaves on both sides are typical for *C. epirotus*, and stems 20–30 cm high with several lanceolate leaves are typical for *C. graminifolius*). Extensive gene flow among the cytotypes of *C.* section *Protocyanus* is suggested by molecular analyses (C. J. Löser, unpubl. data), but this is the first direct evidence of ongoing hybridization in *Cyanus*. In contrast, triploid plants of *C. nissanus* do not differ morphologically from diploids of this species and are probably autopolyploids that have originated through unreduced gamete formation (Ramsey & Schemske, 1998). Plants of the *C. triumfetti* group growing nearby in the Svrljiska Planina Mts (eastern Serbia) do not contribute to the origin of these triploids. Therefore, gene flow from diploids to tetraploids with identical base chromosome numbers via unreduced gametes is expected to occur but probably only rarely.

KARYOLOGICAL DATA AND PHYLOGENETICS OF *C.* SECTION *PROTOCYANUS*

Cyanus section *Protocyanus* is an example of a plant group in which ploidy is stable within taxa/groups of closely related taxa, in contrast to the numerous examples of plant groups that show chromosome variability at the population or individual level [e.g. *Allium oleraceum* L. (Duchoslav *et al*., 2010); *Gymnadenia conopsea* (L.) R.Br. complex (Trávníček *et al*., 2012); *Knautia arvensis* Coult. agg. (Kolář *et al*., 2009); *Phleum pratense* L. group (Perný *et al*., 2008); *Pilosella alpicola* F.W.Schultz & Sch. Bip. complex (Šingliarová, Hodálová & Mráz, 2011); and *Senecio carniolicus* Willd. (Schönswetter *et al*., 2007a)]. Changes in base chromosome number/ploidy in section *Protocyanus* were thus probably rare events and may have occurred early in the diversification of the section.

All four cytotypes are represented in Europe either by polymorphic groups of similar taxa with a broad distribution (the *C. triumfetti* group 2*n* = 22; *C. montanus* and *C. mollis* 2*n* = 44; the *C. napulifer* group 2*n* = 20; *C. graminifolius* and *C. fuscomarginatus* 2*n* = 40) or by morphologically distinct and narrow endemic taxa restricted to the Balkans (*C. achtarovii* and *C. diospolitanus* 2*n* = 22; *C. epirotus* 2*n* = 20; *C. pindicolus* 2*n* = 20, 40). These endemics may represent remnants/relicts of past diversification episodes on the European continent.

As we have shown, chromosomal data are useful characters for helping to decipher lineage diversification in section *Protocyanus*. These types of data should not be overlooked in biosystematic studies of such complex groups, especially when molecular data provide incongruent phylogenetic signals (Boršić *et al*., 2011). For example, knowledge about tetraploids of the *C. montanus* group with 2*n* = 44 in Europe is controversial, and no definitive conclusion about their origin has yet been reached. Analyses of the ITS region showed relationships of *C. montanus s.s.* (2*n* = 44) to *C. epirotus* (2*n* = 20) on the one hand and *C. maramarosiensis* (Jáv.) Dostál (2*n* = 44) and *C. mollis* (2*n* = 44) to *C. achtarovii* (2*n* = 22) and Balkan members of the *C. napulifer* group (2*n* = 20) on the other (Boršić *et al*., 2011). The current distribution of these taxa is distinct and it is also probable that their glacial refugia and distributions during interglacials did not overlap. Therefore, relationships of these taxa are likely to have been established in the early stages of diversification of section *Protocyanus*. *Cyanus mollis* and *C. montanus s.s.* have been considered as autopolyploids which evolved from taxa of the *C. triumfetti* group. This hypothesis is supported by similarities in their morphological characters, flavonoid glycoside variation patterns, complementary chromosome numbers, shape of chromosomes and genome size (Baksay, 1957; Gonnet, 1993; Olšavská *et al*., 2012). However, the above-mentioned similarities between the *C. montanus* and *C. triumfetti* groups might also reflect an extinct common diploid ancestor.

BIOGEOGRAPHICAL REMARKS

Cytotype distribution and ecological data can shed light on the role of historical processes such as migration and expansion from different refugia using various migration corridors (Mráz *et al*., 2008; Perný *et al*., 2008). In the formation of the European flora two main climatic events played a key role: the Messinian salinity crisis (5.5–4.5 Mya) and the Pleistocene glaciations (2.588–0.0117 Mya; Petit, Hampe & Cheddadi, 2005). These climatic events might be invoked to explain the distribution pattern of the *Cyanus* cytotypes in Europe.

The westward colonization of Europe by *Cyanus* perennials is considered more likely given that the assumed evolutionary centre and highest species diversity of *Cyanus* is in the eastern Mediterranean (Hellwig, 2004). Given the current distribution patterns of *Cyanus* cytotypes in Europe this favours the hypothesis of several different routes of colonization in the past (Fig. 4, Fig. S1). The European mountain ranges (mainly the Alps and Carpathians) might have served as migration routes for plants with a base chromosome number of $x = 11$ (*C. mollis*, *C. montanus*, the *C. triumfetti* group) as they do not currently occur in southern Europe. In the Mediterranean, intervening land-mass connections resulting from sea-level oscillations during the Messinian salinity crisis and during the last glaciation might have played important roles in spreading cytotypes with $x = 10$, which today are concentrated across this region, including the Balkan Peninsula. However, such land bridges and connections were probably not suitable for dispersal of mountain species. The explanation might be that the eastern Mediterranean ancestor had wider ecological amplitude than the present species. This hypothesis agrees with the current situation; for example, Greek plants of *C. graminifolius* occur from 700 to 2100 m a.s.l. on various types of bedrock, whereas the western Mediterranean populations are more specialized (*C. graminifolius* in southern France occurs at 1500–1900 m a.s.l. on limestone and in southern Italy it occurs at 1500–1700 m a.s.l. on limestone and granite). The current distribution of species with $x = 10$ covers several refugia detected around the Mediterranean Basin (High and Middle Atlas, Hautes Alpes, southern Calabria and several refugias in Iberia; Médail & Diadema, 2009) where populations might have persisted and diverged.

Speciation in the *C. triumfetti* group ($2n = 22$), with incomplete reproductive isolation (Olšavská & Löser, 2013), may have been triggered predominantly by extensive geographical radiation. Because the current distribution of the *C. triumfetti* group in Europe lies in the area influenced by the Pleistocene glaciations (Fig. 4, Fig. S1C), scattered glacial local refugia in the Pyrenees, Alps, Carpathians, Dinarides or Apennines (Tribsch & Schönswetter, 2003; González-Sampériz *et al*., 2005; Mitka *et al*., 2007) have played a key role in the persistance and alteration of genetic variability of these plants. Such diversification may explain a genetic gap between Alpine, Czech + Austrian and western Carpathians + Pannonian populations of the *C. triumfetti* group documented by amplified fragment length polymorphism (AFLP) and genome size variation (Olšavská *et al*., 2011, 2012), also expressed in different levels of reproductive isolation (Olšavská & Löser, 2013). Also, the morphological differentiation of species of the *C. napulifer* group, which share the same chromosome number $2n = 20$ and close genetic relationships of which are documented by nuclear ribosomal DNA sequences (Boršić *et al*., 2011; C. J. Löser unpubl. data), may have resulted from allopatric speciation processes in isolated Balkan mountains as has been suggested for other alpine plants in southern Europe (Paun *et al*., 2005; Kučera, Marhold & Lihová, 2010).

In contrast to the general trend that polyploids are more widespread than their corresponding diploids (Watanabe, 1986; te Beest *et al*., 2012), both diploid cytotypes from section *Protocyanus* ($2n = 20$ and $2n = 22$) have wider geographical ranges in Europe than corresponding tetraploid cytotypes ($2n = 40$ and $2n = 44$) (Fig. S1). The spreading potential of the *C. triumfetti* group, represented by the $2n = 22$ cytotype, is illustrated by the general distribution patterns shown in Figure 4. The distribution patterns also indicate that competition between taxa with different base chromosome numbers is likely to have occured in the past. For example, range expansion of *C. graminifolius* in Italy and France might have been limited by the spreading of populations of the *C. triumfetti* group. By contrast, *C. graminifolius* and *C. lingulatus* have wider distributions in Greece, the Iberian Peninsula and Atlas Mts where the *C. triumfetti* group does not occur (Fig. 4, Fig. S1).

CIRCUMSCRIPTION OF THE *C. TRIUMFETTI* GROUP AND *C. GRAMINIFOLIUS*

We have shown that populations with $2n = 22$ that are morphologically similar to typical *C. triumfetti s.s.*, i.e. those belonging to the *C. triumfetti* group, form a geographical continuum ranging from northern Iberia, the Alps, Central Europe and the Carpathians to the Apennine and Balkan Peninsulas (excluding Greece) (Fig. 4, Fig. S1C). Taxa included in this group were classified by previous authors as subspecies or varieties of *C. triumfetti* (Dostál, 1976; Greuter, 2006–2009) or were classified as sections, subsections or series (von Hayek, 1901; Stefanov & Georgiev, 1931; Cherepanov, 1963; Table S1). The group is morphologically diverse but clearly distinguished by a combination of morphological characters: a mesomorphic habit; an absence of long rhizomes and temporary fusiform or tuberous storage roots; longer and paler fimbriae in comparison with the margin of involucral bracts (Olšavská *et al*., 2011). The species fitting this circumscription of the *C. triumfetti* group with $2n = 22$ form a separate clade in phylogenetic analyses of nuclear DNA (Boršić *et al*., 2011; C. J. Löser, unpubl. data). The group is found in a wide range of climatic/ecological conditions and altitudes and has the largest niche envelope (Fig. 5). Nevertheless, there is a common preference of warm sites with southern exposures.

The presented circumscription of the *C. triumfetti* group should help to resolve persisting taxonomic problems with this highly polymorphic complex. Based on the data gathered in this study we recommend refraining from using the name *C. triumfetti* outside the above-mentioned area. For example, the name *C. triumfetti* (or *Centaurea axillaris* Willd., *nom. illeg.*) has been frequently used for plants with creeping rhizomes similar to *C. graminifolius* in Turkey (Wagenitz, 1975), Caucasus (Fedorov, 1969) and Israel (Danin, 2006), which makes the *C. triumfetti* group a widely distributed taxon (Dostál, 1976; Greuter, 2006–2009). This taxonomic inconsistency of the *C. triumfetti* group has arisen partially from a nomenclatural problem, which makes names *Centaurea axillaris* and *Cyanus graminifolius* (synonyms *Centaurea variegata* Lam. and *Centaurea seusana* Chaix) synonymous (see Olšavská *et al*., 2011).

We discovered that Greek and Calabrian plants previously assigned to *Centaurea variegata*, *Centaurea seusana* or *Cyanus pichleri* (Halácsy, 1902; Zángheri, 1976; Pignatti, 1982; Gamal-Eldin & Wagenitz, 1991) have a base chromosome number $x = 10$ and are tetraploid ($2n = 4x = 40$). This supports the recognition of the above-mentioned Greek and Calabrian plants as *C. graminifolius*. Use of the name *C. pichleri* for these Greek populations is unsuitable as they do not posses ascending shoots from a terminal rosette, a typical character given for *C. pichleri* (Boissier, 1875). The question of whether *C. pichleri* is the correct name for Bulgarian plants with a similar morphology needs to be studied further. The name *C. graminifolius* is also currently used by some authors for morphologically similar plants from the Iberian Peninsula and the Atlas Mts (Greuter, 2006–2009; Muñoz & Devesa, 2010). In contrast to tetraploid *C. graminifolius*, these plants are diploid and we therefore prefer their traditional recognition as a separate species, *C. lingulatus*.

TAXONOMIC TREATMENT OF *CYANUS* SECTION *PROTOCYANUS* AND *CYANUS TERNOPOLIENSIS*

Cyanus *section* Protocyanus *(Dobrocz.) Olšavská,* ***comb. nov.***

Basionym: Centaurea subgenus *Cyanus* section *Protocyanus* Dobrocz. in Bot. Zhurn. (Kiev) 6: 64, 1949.

Type(Dobrochayeva & Kotov in Ukrayins'k. Bot. Zhurn. 14: 43, 1962): *Cyanus montanus* (L.) Hill

Synonymy:

≡ *Centaurea* section *Cyanus* [rankless] *Perennes* Boiss. in Fl. Orient. 3: 636. 1875.

Cyanus ternopoliensis *(Dobrocz.) Olšavská,* ***comb. nov.***

Basionym: Centaurea ternopoliensis Dobrocz. in Bot. Zhurn. (Kiev) 6(2): 71. 1949.

Type (Dobrochayeva & Kotov in Ukrayins'k. Bot. Zhurn. 14: 42, 1962): RSS Ucr., prov. Ternopoliensis, distr. Berezhany. Quercetum in montibus N versus ab oppido. 11. VI 1915. Legit. Michelson, in Herbario Instituti Botanici Ac. Sc. URSS conservatur.

Note: The new combination is necessary because based on our field observations *Cyanus ternopoliensis* should not be identified with *Cyanus strictus* (Waldst. & Kit.) Soják as already proposed by Greuter (2006–2009). This fact is also highlighted by Dobrochayeva (1949).

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REFERENCES

Baksay L. 1957. The cytotaxonomy of the species *Chrysanthemum maximum* Ram., *Centaurea montana* L., *Serratula lycopifolia* (Vill.) Kern., and *Bupleurum falcatum* L., ranging in Europe. *Annales Historico-Naturales Musei Nationalis Hungarici* **8:** 155–168.

Baltisberger M. 1991. Cytological investigations of some Greek plants. *Flora Mediterranea* **1:** 157–173.

Baltisberger M. 2012. *Centaurea pindicola*. In: Marhold K, ed. IAPT/IOPB chromosome data 13. *Taxon* **61:** 889, E2–E3.

Bancheva S. 1998. Reports (970–976). In: Kamari G, Felber F, Garbari F, eds. Mediterranean chromosome number reports – 8. *Flora Mediterranea* **8:** 273–280.

Bancheva S. 1999. Reports (1050–1054). In: Kamari G, Felber F, Garbari F, eds. Mediterranean chromosome number reports – 9. *Flora Mediterranea* **9:** 323–387.

Bancheva S, Denchev C. 2000. Occurrence and taxonomic investigation of *Centaurea pichleri* Boiss. (Asteraceae) in Bulgaria. *Phytologia Balcanica* **6:** 167–175.

Bancheva S, Greilhuber J. 2006. Genome size in Bulgarian *Centaurea s.l.* (Asteraceae). *Plant Systematics and Evolution* **257:** 95–117.

Bancheva S, Raimondo FM. 2003. Biosystematic studies of seven Balkan species from genus *Cyanus* (Compositae). *Bocconea* **16:** 507–527.

Bancheva S, Stoyanov S. 2009. A new species of *Cyanus* (Asteraceae, Centaureinae) from southeastern Bulgaria. *Novon* **19:** 421–425.

te Beest M, Le Roux JJ, Richardson DM, Brysting AK, Suda J, Kubešová M, Pyšek P. 2012. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany* **109:** 19–45.

Bigazzi M, Selvi F. 2001. Karyotype morphology and cytogeography in *Brunnera* and *Cynoglottis* (Boraginaceae). *Botanical Journal of the Linnean Society* **136:** 365–378.

Boissier E. 1875. *Flora Orientalis III*. Geneva, Basle.

Borhidi I. 1957. *Centaurea achtarovii* Urumoff dans les Carpathes orientaux. Annotations sur le groupe *Centaurea triumfettii* All. *Annales Historico-Naturales Musei Nationalis Hungarici* **7:** 219–225.

Boršić I, Susanna A, Bancheva S, Garcia-Jacas N. 2011. *Centaurea* sect. *Cyanus*: nuclear phylogeny, biogeography and life-form evolution. *International Journal of Plant Sciences* **172:** 238–249.

ter Braak CJF, Šmilauer P. 2002. *CANOCO reference manual and CanoDraw for Windows user's guide. Software for Canonical Community Ordination (version 4.5)*. Wageningen & České Budějovice: Biometris.

Cherepanov SK. 1963. Podrod *Cyanus*. In: Bobrov EG, Cherepanov SK, eds. *Flora SSSR 28*. Leningrad: Botanicheskii institut V. L. Komarova Akademii Nauk SSSR, 387–418.

Compagnoni B, Galluzo F, eds. 2004. *Geological map of Italy 1:1250000*. Florence: S.E.L.C.A.

Cook RE, Semple JC. 2008. Cytogeography of *Solidago* subsect. Glomeruliflorae (Asteraceae: Astereae). *Botany* **86:** 1488–1496.

Dadlez R, Marek S, Pokorski J, eds. 2000. *Mapa geologiczna Polski bez utwórow kenozoiku 1:1000000*. Warszawa: wydawnictvo kartografyczne Polskej agencji ekologicznej S.A.

Danin A, ed. 2006. (continuously updated). *Flora of Israel online*. Available at: http://flora.huji.ac.il/browse.asp. Accessed 22 February 2012.

Dobrochayeva DM. 1949. Voloshky URSR, ikh poshyrennya ta istoriya rozvytku. *Botanichnyi Zhurnal* **6:** 63–77.

Doležel J, Greilhuber J, Suda J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols* **2:** 2233–2244.

Doležel J, Sgorbati S, Lucretti S. 1992. Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. *Journal of Plant Physiology* **85:** 625–631.

Dostál J. 1969. Taxonomická studie o čeledích Lycopodiaceae a Asteraceae a o rodech Lycopodium a Centaurea. Acta Universitatis Palackianae Olomucensis. *Facultas Rerum Naturalium. Series 2. Biologica* **31:** 5–35.

Dostál J. 1976. *Centaurea* L. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, eds. *Flora Europaea 4*. Cambridge: Cambridge University Press, 254–301.

Duchoslav M, Šafářová L, Krahulec F. 2010. Complex distribution patterns, ecology and coexistence of ploidy levels of *Allium oleraceum* (Alliaceae) in the Czech Republic. *Annals of Botany* **105:** 719–735.

Fedorov AA, ed. 1969. *Chromosome numbers of flowering plants*. Leningrad: Nauka.

Gabaldón V, ed. 1994. *Mapa geológico de la Península Iberíca, Baleares y Canarias 1:1000000*. Madrid: Villena S. A.

Gamal-Eldin E, Wagenitz G. 1991. *Centaurea* L. In: Strid A, Tan K, eds. *Mountain flora of Greece 2*. Edinburgh: Edinburgh University Press, 488–525.

Garcia-Jacas N, Susanna A, Garnatje T, Vilatersana R. 2001. Generic delimitation and phylogeny of the subtribe Centaureinae (Asteraceae): a combined nuclear and chloroplast DNA analysis. *Annals of Botany* **87:** 503–515.

Garnatje T, Garcia Jacas N, Vilatersana R. 2001. Natural triploidy in *Centaurea* and *Cheirolophus* (Asteraceae). *Botanica Helvetica* **111:** 25–29.

Ghaffari SM, Kelich K. 2006. New or rare chromosome counts of some angiosperm species from Iran. *Iranian Journal of Botany* **12:** 81–86.

Gömürgen AN, Adigüzel N. 2001. Chromosome numbers and karyotype analysis of *Centaurea tchihatcheffii* Fisch. et Mey. (Compositae, Cardueae). *Ot Sistematik Botanik Dergisi* **8:** 83–86.

Gonnet JF. 1993. Flavonoid glycoside variation in wild specimens of *Centaurea triumfetti* (Compositae) and comments on its relationships with *Centaurea montana* based on flavonoid fingerprints. *Biochemical Systematics and Ecology* **21:** 389–396.

González-Sampériz P, Valero-Garcés BL, Carrión JS, Peña-Monné JL, García-Ruiz JM, Martí-Bono C. 2005. Glacial and lateglacial vegetation in northeastern Spain: new data and a review. *Quaternary International* **140–141:** 4–20.

Greuter W. 2006–2009. Compositae (pro parte majore). In: Greuter W, von Raab-Straube E, eds. *Compositae. Euro+Med Plantbase – the information resource for Euro-Mediterranean plant diversity*. Available at: http://ww2.bgbm.org/EuroPlusMed, accessed 22 January 2013.

Grisebach AHR. 1846. *Spicilegium Florae Rumelicae et Bithynicae 2*. Brunsvigae.

Guinochet M. 1957. Contribution à l?étude caryologique du genre Centaurea L. sens. lat. Bulletin de la Société d'Histoire Naturelle de l'Afrique du Nord. *Algiers.* **48:** 282–300.

Halácsy E. 1902. *Conspectus florae Graecae II*. Lipsiae.

von Hayek A. 1901. Die Centaurea-Arten Osterreich-Ungarns. Denkschriften der Kaiserlichen Akademie der Wissenschaften, Wien. *Mathematisch-Naturwissenschaftliche Klasse* **72:** 595–773.

Hellwig FH. 2004. Centaureinae (Asteraceae) in the Mediterranean: history of ecogeographical radiation. *Plant Systematics and Evolution* **246:** 137–162.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25:** 1965–1978.

Kaya Z, Bancheva S. 2009. A new species of *Cyanus* (*Centaurea* p.p.) sect. Napuliferi (Asteraceae) from Turkey. *Novon* **19:** 175–177.

Kolář F, Štech M, Trávníček P, Rauchová J, Urfus T, Vít P, Kubešová M, Suda J. 2009. Towards resolving the *Knautia arvensis* agg. (Dipsacaceae) puzzle: primary and secondary contact zones and ploidy segregation at landscape and microgeographic scales. *Annals of Botany* **103:** 963–974.

Koutecký P, Badurová T, Štech M, Košnar J, Karásek J. 2011. Hybridization between diploid *Centaurea pseudophrygia* and tetraploid *C. jacea* (Asteraceae): the role of mixed pollination, unreduced gametes, and mentor effects. *Biological Journal of the Linnean Society* **104:** 93–106.

Koutecký P, Štěpánek J, Bad'urová T. 2012. Differentiation between diploid and tetraploid *Centaurea phrygia*: mating barriers, morphology and geographic distribution. *Preslia* **84:** 1–32.

Kučera J, Marhold K, Lihová J. 2010. *Cardamine maritima* group (Brassicaceae) in the amphi-Adriatic area: a hotspot of species diversity revealed by DNA sequensces and morphological variation. *Taxon* **59:** 148–164.

Kuzmanov B, Georgieva S. 1987. Reports. In: Löve Á, ed. IOPB Chromosome number reports XCIV. *Taxon* **36:** 284.

Leitch IJ, Bennett MD. 2004. Genome downsizing in polyploid plants. *Biological Journal of the Linnean Society* **82:** 651–663.

Levin DA. 2002. *The role of chromosomal change in plant evolution*. Oxford: Oxford University Press.

Li D, Liu Y, Zhong C, Huang H. 2010. Morphological and cytotype variation of wild kiwifruit (*Actinidia chinensis* complex) along an altitudinal and longitudinal gradient in central-west China. *Botanical Journal of the Linnean Society* **164:** 72–83.

Löve Á, Kjellqvist E. 1974. Cytotaxonomy of Spanish plants. IV. – Dicotyledons: Caesalpiniaceae – Asteraceae. *Lagascalia* **4:** 153–211.

Lovrić A-Z. 1982. Reports. In: Löve Á, ed. IOPB chromosome number reports LXXVII. *Taxon* **31:** 762–763.

Martín-Bravo S, Valcárcel V, Vargas P, Luceńo M. 2010. Geographical speciation related to Pleistocene range shifts in the western Mediterranean mountains (*Reseda* sect. Glaucoreseda, Resedaceae). *Taxon* **59:** 466–482.

Médail F, Diadema K. 2009. Glacial refugia influence plant diversity patterns in the Mediteraneaen Basin. *Journal of Biogeography* **36:** 1333–1345.

Mitka J, Sutkowska A, Ilnicki T, Joachimiak AJ. 2007. Reticulate evolution of high-alpine *Aconitum* (Ranunculaceae) in the eastern Sudetes and western Carpathians (Central Europe). *Acta Biologica Cracoviensia Series Botanica* **49:** 15–26.

Montserrat-Martí JM. 1987. Números cromosomáticos de plantas occidentales, 420–421. *Anales del Jardin Botánico de Madrid* **43:** 425–426.

Morales FMJ. 1974. Reports. In: Löve Á, ed. IOPB chromosome number reports XLVI. *Taxon* **23:** 805.

Mráz P, Šingliarová B, Urfus T, Krahulec F. 2008. Cytogeography of *Pilosella officinarum* (Compositae): altitudinal and longitudinal differences in ploidy level distribution in the Czech Republic and Slovakia and the general pattern in Europe. *Annals of Botany* **101:** 59–71.

Mráz P, Španiel S, Keller A, Bowmann G. Farkas A, Šingliarová B, Rohr PR, Broennimann O, Müller-Schärer H. 2012. Antropogenic disturbance as a driver of microspatial and microhabitat segregation of cytotypes of *Centaurea stoebe* and cytotype interactions in secondary contact zones. *Annals of Botany* **110:** 615–627.

Muñoz AF, Devesa JA. 2010. Revisión taxonómica del complejo de *Centaurea Cyanus* L. (*Centaurea* sect. *Cyanus*, Asteraceae) en la Península Ibérica. *Acta Botanica Malacitana* **35:** 23–55.

Olšavská K, Löser CJ. 2013. Mating system and hybridization of the *Cyanus triumfetti* and *C. montanus* groups (Asteraceae). *Folia Geobotanica*. doi: 10.1007/s12224-013-9155-3.

Olšavská K, Perný M. 2009. *Cyanus graminifolius*. In: Marhold K, ed. IAPT/IOPB chromosome data 7. *Taxon* **58:** 181–183.

Olšavská K, Perný M, Kučera J, Hodálová I. 2011. Biosystematic study of the *Cyanus triumfetti* group in Central Europe. *Preslia* **83:** 59–98.

Olšavská K, Perný M, Mártonfi P, Hodálová I. 2009. *Cyanus triumfettii* subsp. *triumfettii* (Compositae) does not occur in the western Carpathians and adjacent parts of Pannonia: karyological and morphological evidence. *Nordic Journal of Botany* **27:** 21–36.

Olšavská K, Perný M, Španiel S, Šingliarová B. 2012. Nuclear DNA content variation among perennial taxa of the genus *Cyanus* (Asteraceae) in Central Europe and adjacent areas. *Plant Systematics and Evolution* **298:** 1463–1482.

Paun O, Lehnebach C, Johansson JT, Lockhart P, Hörandl E. 2005. Phylogenetic relationships and biogeography of *Ranunculus* and allied genera (Ranunculaceae) in the Mediterranean region and in the European Alpine System. *Taxon* **54:** 911–930.

Pellicer J, Clermont S, Houston L, Rich TCG, Fay MF. 2012. Cytotype diversity in the *Sorbus* complex (Rosaceae) in Britain: sorting out the puzzle. *Annals of Botany* **110:** 1185–1193.

Pellicer J, Garcia S, Garnatje T, Dariimaa S, Korobkov AA, Vallès J. 2007. Chromosome numbers in some *Artemisia* (Asteraceae, Anthemideae) species and genome size variation in its subgenus *Dracunculus*: karyological, systematic and phylogenetic implications. *Chromosome Botany* **2:** 45–53.

Perný M, Kolarčik V, Majeský L, Mártonfi P. 2008. Cytogeography of the *Phleum pratense* group (Poaceae) in the Carpathians and Pannonia. *Botanical Journal of the Linnean Society* **157:** 475–485.

Petit RJ, Hampe A, Cheddadi R. 2005. Climate changes and tree phylogeography in the Mediterranean. *Taxon* **54:** 877–885.

Petrova A, Vladimirov V. 2010. Balkan endemics in the Bulgarian flora. *Phytologia Balcanica* **16:** 293–311.

Petrovič S. 1885. *Additamenta ad floram agri Nyssani*. Beograd.

Pignatti S. 1982. *Flora d'Italia 3*. Bologna: Edagricole.

Ramsey J, Schemske DW. 1998. Pathways, mechanisms and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* **29:** 477–501.

Romaschenko K, Ertuğrul K, Susanna A, Garcia-Jacas N, Uysal T, Arslan E. 2004. New chromosome counts in the *Centaurea jacea* group (Asteraceae, Cardueae) and some related taxa. *Botanical Journal of the Linnean Society* **145:** 345–352.

Sánchez-Jiménez I, Hidalgo O, Canela MÁ, Siljak-Yakovlev S, Šolić ME, Vallès J, Garnatje T. 2012. Genome size and chromosome number in *Echinops* (Asteraceae, Cardueae) in the Aegean and Balkan regions: technical aspects of nuclear DNA amount assessment and genome evolution in a phylogenetic frame. *Plant Systematics and Evolution* **298:** 1085–1099.

Schönswetter P, Lachmayer M, Lettner C, Prehsler D, Rechnitzer S, Reich DS, Sonnleitner M, Wagner I, Hülber K, Schneeweiss GM, Trávníček P, Suda J. 2007a. Sympatric diploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the eastern Alps are separated along an altitudinal gradient. *Journal of Plant Research* **120:** 721–725.

Schönswetter P, Suda J, Popp M, Weiss-Schneeweiss H, Brochmann C. 2007b. Circumpolar phylogeography of *Juncus biglumis* (Juncaceae) inferred from AFLP fingerprints, cpDNA sequences, nuclear DNA content and chromosome numbers. *Molecular Phylogenetics and Evolution* **42:** 92–103.

Sharkova S. 1996. Reports (767–772). In: Kamari G, Felber F, Garbari F, eds. Mediterranean chromosome number reports – 6. *Flora Mediterranea* **6:** 253–337.

Sharkova S, Peev D. 1997. Karyological study of some Bulgarian Centaurea (Asteraceae). *Phytologia Balcanica* **3:** 83–87.

Šiljak-Yakovlev S. 1985. Reports. In: Löve Á, ed. IOPB chromosome reports LXXXVIII. *Taxon* **34:** 548.

Šiljak-Yakovlev S, Solic ME, Catrice O, Brown SC, Papes D. 2005. Nuclear DNA content and chromosome number in some diploid and tetraploid Centaurea (Asteraceae: Cardueae) from the Dalmatia region. *Plant Biology* **7:** 397–404.

Šingliarová B, Hodálová I, Mráz P. 2011. Biosystematic study of the diploid–polyploid *Pilosella alpicola* complex with variation in breeding system: patterns and processes. *Taxon* **60:** 450–470.

Stefanov B, Georgiev T. 1931. Prinos za razgranichenie na vidovet' na roda *Centaurea* L. ot sect. *Cyanus* D. C. *Spisanie na bulgarskata akademiya na naukite* **44:** 133–193.

Strid A, Franzén R. 1981. Reports. In: Löve Á, ed. IOPB chromosome number reports LXXIII. *Taxon* **30:** 829–842.

Stuessy TF. 2009. *Plant taxonomy: the systematic evaluation of comparative data*, 2nd edn. New York: Columbia University Press.

Suda J, Weiss-Schneeweiss H, Tribsch A, Schneeweiss GM, Trávníček P, Schönswetter P. 2007. Complex distribution patterns of di-, tetra-, and hexaploid cytotypes in the European high mountain plant *Senecio carniolicus* (Asteraceae). *American Journal of Botany* **94:** 1391–1401.

Susanna A, Garcia Jacas N, Soltis DE, Soltis SS. 1995. Phylogenetic relationships in the tribe Cardueae (Asteraeae) based on ITS sequences. *American Journal of Botany* **82:** 1056–1068.

Swisstopo. 2005. *Geologische Karte der Schweiz 1:500000*. Available at: http://www.swisstopo.admin.ch/internet/swisstopo/de/home/products/maps/geology/geomaps/gm500.html

Trávníček P, Eliášová A, Suda J. 2010. The distribution of cytotypes of *Vicia cracca* in Central Europe: the changes that have occurred over the last four decades. *Preslia* **82:** 149–163.

Trávníček P, Jersáková J, Kubátová B, Krejčíková J, Bateman RM, Lučanová M, Krajníková E, Těšitelová T, Štípková Z, Amardeilh J-P, Brzosko E, Jermakowicz E, Cabanne O, Durka W, Efimov P, Hedrén M, Hermosilla CE, Kreutz K, Kull T, Tali K, Marchand O, Rey M, Schiestl FP, Čurn V, Suda J. 2012. Minority cytotypes in European populations of the *Gymnadenia conopsea* complex (Orchidaceae) greatly increase intraspecific and intrapopulation diversity. *Annals of Botany* **110:** 977–986.

Tribsch A, Schönswetter P. 2003. Patterns of endemism and comparative phylogeography confirm paleoenvironmental evidence for Pleistocene refugia in the Eastern Alps. *Taxon* **52:** 477–497.

Wagenitz G. 1955. Pollenmorphologie und Systematik in der Gattung *Centaurea* L. *s.l. Flora* **142:** 213–279.

Wagenitz G. 1975. *Centaurea* L. In: Davis PH, ed. *Flora of Turkey 5*. Edinburgh: Edinburgh University Press, 465–585.

Wagenitz G, Hellwig FH. 1996. Evolution of characters and phylogeny of the Centaureinae. In: Hind DJN, Beentje HJ, eds. *Compositae: systematics. Proceedings of the International Compositae Conference, Kew, 1994, vol. 1*. Kew: Royal Botanic Gardens, 491–510.

Watanabe K. 1986. Cytogeography of the genus *Eupatorium* (Compositae). A review. *Plant Species Biology* **1:** 99–116.

Zángheri P. 1976. *Flora Italica I*. Padua: Cedam.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Geographical distribution of cytotypes of *Cyanus* section *Protocyanus* in Europe: A, $2n = 20$, $2n{\sim}20$; B, $2n = 40$, $2n{\sim}40$; C, $2n = 22$, $2n{\sim}22$; D, $2n = 44$, $2n{\sim}44$. Localities indicated by symbols with a black margin are based on our records presented in this paper (Table 2) or published previously; symbols without a black margin are based on published records of other authors (see Table S2).
Table S1. Survey of taxonomic concepts of the genus *Cyanus*.
Table S2. Summary of previously published chromosome numbers of *Cyanus* section *Protocyanus* in Europe.