Taxonomy and cytogeography of the *Molinia caerulea* complex in central Europe

Taxonomie a cytogeografie komplexu *Molinia caerulea* ve střední Evropě

Martin D a n č á k[1], Martin D u c h o s l a v[2] & Bohumil T r á v n í č e k[2]

[1]*Department of Ecology & Environmental Sciences, Faculty of Science, Palacký University, Šlechtitelů 11, CZ-783 71 Olomouc, Czech Republic, e-mail: martin.dancak@upol.cz;* [2]*Plant Biosystematics and Ecology RG, Department of Botany, Faculty of Science, Palacký University, Šlechtitelů 11, CZ-783 71 Olomouc, Czech Republic, e-mail: martin.duchoslav @upol.cz, bohumil.travnicek@upol.cz*

Dančák M., Duchoslav M. & Trávníček B. (2012): Taxonomy and cytogeography of the *Molinia caerulea* complex in central Europe. – Preslia 84: 351–374.

Perennial grasses belonging to the genus *Molinia* are widespread in most of Europe and consist of a polyploid complex of closely related taxa with a confusing taxonomy. Based on extensive sampling at 241 localities in Europe, four cytotypes were identified based on chromosome counts and results of flow cytometry: tetraploids (2n = 36), hexaploids (2n = 54), octoploids (2n = 72) and dodecaploids (2n = 108). While tetra- and dodecaploids were commonly recorded, octoploids were less common and only two hexaploid individuals were identified. Previously reported decaploid counts (2n = 90) from central Europe are probably erroneous and refer to 2n = 108. The tetraploid cytotype is distributed throughout Europe and broadly sympatric with other cytotypes. Octo- and dodecaploids were spatially separated with dodecaploids occurring in the western, central and south-central part of Europe and octoploids in the east-central and southeastern part of Europe. All quantitative characters measured (lengths of lemmas, anthers, caryopses and stomata, lengths of the longest hair on the callus and diameter of the culm below the panicle) showed a linear trend across ploidy levels. Tetra-, octo- and dodecaploid cytotypes formed almost non-overlapping groupings in principal component and discriminant analyses of morphological characters. The following taxonomic concept of this complex is proposed: *Molinia caerulea* (L.) Moench is a predominantly tetraploid taxon incorporating very rarely reported hexaploid and perhaps also diploid plants; higher cytotypes (2n = 8x, 12x) are considered to be *M. arundinacea* Schrank, consisting of two subspecies: a dodecaploid subspecies occurring in the southern and western part of central Europe and the octoploid *Molinia arundinacea* subsp. *freyi* Dančák in east-central and southeastern Europe.

K e y w o r d s: chromosome numbers, contact zones, distribution, DNA-ploidy level, flow cytometry, *Molinia*, polyploidy, taxonomy

Introduction

Poaceae, one of the most species-rich angiosperm families (ca 10,000 species) exceeding all other families in ecological dominance and economic importance, is well known for its variation in chromosome numbers (Lewis 1980, Gaut 2002), which is partly a consequence of polyploidy since roughly 44% of the extant species in this family are polyploids (DeWet 1986). Several studies have also revealed the rapid and dynamic nature of genome size-evolution in grasses (see Leitch et al. 2010 for review). Variation in ploidy levels occurs also within many grass species (Lewis 1980, Keeler 1998) with frequent presence of multiple cytotypes within populations (e.g. Lumaret et al. 1987, Norrmann & Keeler 1997, Pečinka et al. 2006, Perný et al. 2008). In many grass taxa the current genome is

a complex product of reticulate evolution with multiple occurrences of both allo- and autopolyploidy (Mahelka & Kopecký 2010). Such complex cytogenetic patterns usually complicate taxonomy because various cytotypes (especially autopolyploids) are usually hardly morphologically recognizable and may or may not differ in geographical distribution and/or environmental preferences (Keeler 1998, Soltis et al. 2007). Thus, it is questionable whether treating them as separate taxa is meaningful.

The genus *Molinia* Schrank includes a few species distributed mainly in the temperate zone of Eurasia. The two species (*Molinia japonica* Hackel and *M. hui* Pilger) occurring in the Far East (Japan, Korea, China and Russia; Watson & Dallwitz 1992) are sometimes separated and classified in the genus *Moliniopsis* Hayata. However, this approach is presently not accepted, since the morphological differentiation from European taxa is weak. The most natural is the treatment by Tsvelev (1976), who divided the genus into two sections: section *Molinia* (comprising European taxa) and section *Hayatia* Tzvelev (comprising eastern Asian taxa). The group of taxa that occur in Europe is traditionally called the *Molinia caerulea* complex and represents a dominant taxon and strong competitor in moorlands, fens, heathlands, temporally wet low-productive grasslands and several types of intermittently waterlogged oak and pine forests (Landolt 1977, Chytrý et al. 2001, Taylor et al. 2001, Marrs et al. 2004, Havlová 2006).

The *Molinia caerulea* complex is known for its high morphological variability (Salim et al. 1995), which has led to various taxonomic concepts and consequently to confusions in phytosociological affiliation (Landolt 1977). Although many studies have focused on the ecology of the species, taxonomic confusion has persisted up to the present time. However, for a correct evaluation and mutual comparison of ecological studies that focus on the bioindication value of the species, its possible threat to species diversity in natural habitats and management measures to control it, a correct taxonomical assessment of particular populations is needed. The number of species belonging to this group varies according to author from one (Clayton et al. 2006) to eight (Milkovits & Borhidi 1986), but most frequently two species are recognized: *Molinia caerulea* (L.) Moench and *Molinia arundinacea* Schrank (Frey 1975a, b, Conert 1992, Adler et al. 1994, Marhold & Hindák 1998, Kubát et al. 2002, Rothmaler et al. 2005). These taxa are, however, poorly differentiated as the diagnostic features are mostly of a quantitative nature and some of them are possibly strongly affected by environmental conditions.

The discovery that in this complex the chromosome number is variable has not improved the situation as it indicates that polyploidy may account for the high morphological variation observed within this group. Six ploidy levels are reported for the *M. caerulea* group: diploid (2n = 2x = 18), tetraploid (2n = 4x = 36), hexaploid (2n = 6x = 54), octoploid (2n = 8x = 72), decaploid (2n = 10x = 90) and dodecaploid (2n = 12x = 108). The first few chromosome counts of representatives of *Molinia* were published in the first half of the 20th century and in which 2n = 36 prevails (Tischler 1934, Rohweder 1937, Jeffries in Tischler 1950), although Mattick (in Tischler 1950) also reports 2n = 18. Several students have carried out systematic studies on this genus since the 1970s in the Netherlands (Sterk & ter Laak 1972), Poland (Frey 1973) and Hungary (Milkovits & Borhidi 1986), with the main emphasis on the karyology of the taxa studied. While in the Netherlands and a large part of Poland only the tetraploid cytotype was found (Sterk & ter Laak 1972, Frey 1973) as many as five cytotypes were reported from Hungary: di-, tetra-, hexa-, octo- and decaploid (Milkovits & Borhidi 1986). While hexa- and octoploid cytotypes are not

reported outside Hungary, the decaploid cytotype is reported also from the southern parts of Poland (Frey 1973), Slovakia (Mičieta 1986, Letz et al. 1999), eastern France and Switzerland (Guinochet & Lemée 1950). Dančák (2002) reports an unpublished record of the dodecaploid ploidy level (exactly 2n = 108) that was recorded by A. Krahulcová for one plant from eastern Bohemia (Czech Republic).

Frey (1973) assigns tetraploids to *Molinia caerulea* and decaploids to *M. arundinacea*, a concept that was later adopted in the majority of central European Floras (e.g. Adler et al. 1994, Kubát et al. 2002, Rothmaler et al. 2005). Nevertheless, the Hungarian botanists Milkovits & Borhidi (1986) propose a much more complex approach. They recognize eight species: *Molinia caerulea* with 2n = 2x, *M. arundinacea* with 2n = 4x and 8x, *M. pocsii* Milkovits with 2n = 6x, *M. litoralis* Host with 2n = 8x, *M. ujhelyi* Milkovits with 2n = 10x (all belong to the series *Caerulea*) and *Molinia simonii* Milkovits with 2n = 2x and 4x, *M. hungarica* Milkovits with 2n = 4x and *M. horanszkyi* Milkovits with 2n = 8x (all belong to the series *Hungaricae* Milkovits). This complicated taxonomic treatment is not adopted in any of the major current European Floras, including the Hungarian Flora (Király 2009). However, there have been no attempts to check their results.

The apparently contrasting cytotype compositions recorded in a number of the European regions studied indicate the difficulty of evaluating this polyploid complex: most reports on the variation in the composition of cytotypes in *Molinia* are based on only sketchily sampled plants or are from only a part of an extensive range, which is also likely to result in some polyploids remaining undetected. Also, some of the older counts seem to be wrong possibly because the high numbers of small chromosomes in *Molinia* make accurate counting more difficult and less accurate (Keeler 1998). Several recent papers have shown that the use of flow cytometry, which makes it possible to process more samples, results in a much more accurate picture of the variation in ploidy levels in taxa (Mráz et al. 2008, Kolář et al. 2009, Duchoslav et al. 2010, Šafářová & Duchoslav 2010, Šafářová et al. 2011, Trávníček et al. 2011).

This paper presents a karyotaxonomic study of the *Molinia caerulea* complex mainly in central Europe (Slovakia and the Czech Republic) plus data on the variation in ploidy level in other European countries. The aims were to analyze cytogeographical variation in this complex and to look for any relationship between ploidy level and morphological characters, which might be taxonomically relevant. In addition the taxonomic value of several taxa described by Milkovits & Borhidi (1986) was evaluated.

Material and methods

Plant material

During 1997–2011 plants were collected from 241 natural localities mostly in Slovakia and the Czech Republic and less frequently in other European countries (Austria, Bulgaria, Croatia, Estonia, France, Germany, Greece, Hungary, Italy, Norway, Poland, Romania, Russia, Slovenia, Spain, Sweden) (see Electronic Appendix 1 for locality and herbarium voucher details) and used for counting the number of chromosomes and estimating ploidy using flow cytometry. One to six plants were collected at each locality and transplanted to the experimental garden of Palacký University, Olomouc, Czech Republic. When more individuals were sampled from a locality, an attempt was made to cover as wide a morphological variation as possible. Voucher specimens were collected in situ

from the same rhizome as living plants and the herbarium specimens prepared were later used in the morphometric analysis. The specimens are deposited at OL.

Counting of chromosomes and estimating DNA ploidy level

Squash preparations were prepared from root tips of cultivated plants pre-treated with cold water (approximately 1 °C) for 24 hours. Subsequently, they were fixed in Farmer's fixative (ethanol: glacial acetic acid 3:1) at 4 °C overnight and stored in 70% ethanol. Feulgen hydrolysis was performed in 5N HCl at room temperature for 30 min. After a short rinse in distilled water, the tips were transferred to Schiff's reagent (Lillie 1951) for 45 min. Permanent squash preparations were prepared according to Conger & Fairchild (1953) after maceration in a mixture of pectinase and cellulase for 10 min. at a temperature of 36 °C. Chromosome numbers were counted on at least five intact metaphase plates for each plant.

DNA ploidy level (Suda et al. 2006) was estimated using flow cytometry (FCM). Nuclear DNA content was measured in young leaf blades and leaf sheaths taken from cultivated plants. Samples of plants with known chromosome numbers (tetra-, hexa- and octoploid) were analyzed simultaneously and the ratio of their G1 peak positions recorded. The DNA ploidy levels of the plants with unknown chromosome numbers were then assessed by their peak position relative to the standard peak of a plant with a known chromosome number (usually a tetraploid plant; see Electronic Appendix 1 for survey of populations that served as internal standards). Approximately half of the plants sampled (incl. those with known chromosome numbers) were reanalysed later and their nuclear DNA content measured with *Zea mays* 'CE-777' (2C = 5.43 pg, Lysák & Doležel 1998; standard used for tetra-, octo- and dodecaploids) or *Pisum sativum* 'Ctirad' (2C = 9.09 pg, Doležel et al. 1998, standard used for hexaploids) as internal standards. *Pisum* was used as an alternative standard for hexaploids, due to overlap of peak positions of *Zea* and hexaploid sample. The ratios between nuclei fluorescence intensity presented in the results are based on propidium iodide staining of *Molinia* cytotypes and the internal standards *Zea mays* 'CE-777' or *Pisum sativum* 'Ctirad', respectively.

Sample preparation followed simplified one-step nuclei isolation procedure using ice-cold LB01 isolation buffer (Doležel et al. 2007). Fresh tissues of a *Molinia* sample (approximately 0.5 cm^2), together with the appropriate amount of the reference standard, were co-chopped with a razor blade in a Petri dish containing 1 ml of isolation buffer. The solution was filtered through nylon mesh (42 μm mesh size) and incubated for at least 5 minutes at room temperature. A flow-through fraction was stained either with 4',6-diamidino-2-phenylindole (DAPI), at a final concentration of 4 $\mu g.ml^{-1}$ (tetraploid *Molinia* as internal standard), or with propidium iodide (PI), at a final concentration of 50 $\mu g.ml^{-1}$ (*Zea mays* or *Pisum sativum* as internal standards, respectively). The relative florescence intensity of DAPI staining was analysed using a Partec PAS ploidy analyser equipped with HBO-100 mercury arc lamp. The relative florescence intensity of PI staining was analysed using a ML CyFlow instrument (Partec GmbH, Münster, Germany) equipped with a diode-pumped solid state green laser (532 nm, 100 mW, Cobolt Samba; Cobolt AB, Stockholm, Sweden). A total of 3000 cells were analysed in each measurement. Peak positions and coefficients of variance (CV) were calculated using the Partec software incorporated in the flow cytometers used. Both DAPI and PI staining yielded histograms with coefficients of variance below 7% for both the standard and the sample in the majority of DNA-ploidy measurements.

Morphometric analyses

Only specimens of plants of known ploidy level were examined morphologically (see Electronic Appendix 1 for the overview). Characters measured included the ones traditionally used for differentiation of the recognized taxa in identification keys and floras (lemma length, anther length, caryopsis length, length of the longest hair on the callus, diameter of the culm below the panicle) plus the length of stomata, which is also potentially useful for the differentiation of cytotypes (Beaulieu et al. 2008). Five to ten measurements of lemma, anther, stomata and caryopsis lengths, depending on availability, were recorded for each plant. Concerning the other two characters (length of the longest hair on the callus, diameter of the culm below the panicle) only one measurement per individual was recorded.

To determine whether the morphology of the cytotypes differed, measurements were made on material of altogether 127 individuals (49 tetraploids, 1 hexaploid, 13 octoploids and 64 dodecaploids) from 76 localities. Firstly, data were analysed using univariate analyses. Pearson correlation coefficients were computed to reveal pairs of highly correlated characters. Either one-way or nested GLM ANOVA was run for comparison of mean values of characters among cytotypes. Set of orthogonal contrasts allowing testing linear and quadratic trend components was used for analysis of response of morphological characters to increasing ploidy level. Bonferroni multiple comparison test at $P = 0.05$ was used to reveal significant differences among means (Zar 1996). The hexaploid cytotype was excluded from the analyses because only one plant was measured. Due to the phenology of the characters analysed, even for an individual plant, it was not possible to measure all the characters. This was apparent mainly in the case of anther length, for which only a limited sample ($n = 24$) was obtained. The analyses were run using Statistica 9 (Statsoft Inc.) software.

Principal component analysis (PCA) (Sneath & Sokal 1973) based on the correlation matrix of the measured characters, except anther length, was used to display the overall pattern in the variation along the first two components extracting most of the original multidimensional character space. The analysis was run with individual plants as objects. The hexaploid cytotype was not included in the analysis because only one plant was measured. In addition seventeen individuals were also not included because of missing data for some characters, resulting in 110 individuals (4x: 39, 8x: 13, and 12x: 58 individuals). To determine the extent of morphological separation between the three cytotypes, canonical discriminant analysis (CDA) was computed on the same data-set (Legendre & Legendre 1998). Parametric classificatory discriminant analysis was performed to estimate the percentage of plants correctly assigned to the predetermined groups (cytotypes), based on the morphological characters measured. The analyses were run using software Canoco for Windows 4.5 (ter Braak & Šmilauer 2002) and NCSS 2001 (Hintze 2001).

Results

Cytotype variation and distribution

The chromosome numbers, estimated by counting the numbers in metaphases in 17 root-tip samples, indicated tetra- ($2n = 4x = 36$; 6 counts), hexa- ($2n = 6x = 54$; 1 count) and octoploid ($2n = 8x = 72$; 4 counts) ploidy levels. Chromosome numbers in each of the remaining six samples exceeded 72 chromosomes (i.e. $2n > 8x$), but we were unable to count them accurately.

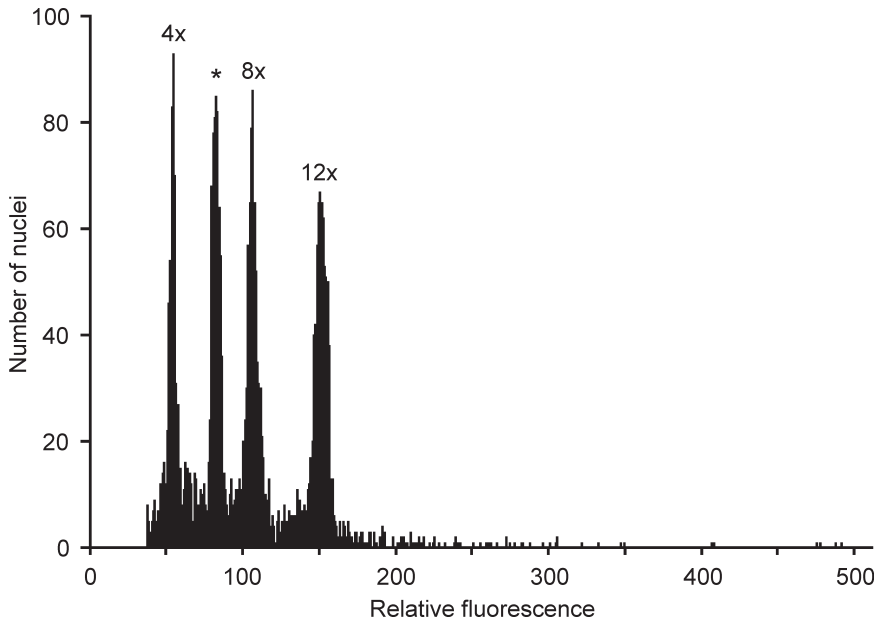

Fig. 1. – Flow cytometric fluorescence histogram showing results of simultaneous analysis of PI-stained nuclei isolated from tetraploid (4x), octoploid (8x) and dodecaploid (12x) plants of the *Molinia caerulea* complex and internal standard *Zea mays* 'CE-777' (marked by asterisk).

DNA ploidy level was determined using flow cytometry for 354 individuals from 241 localities (see Electronic Appendix 1 for a full list of localities). This revealed there were four levels of DNA-ploidy in this material: tetra-, hexa-, octo- and dodecaploid ($2n \approx 12x \approx 108$). Neither diploid ($2n = 2x = 18$) nor decaploid ($2n = 10x = 90$) plants were found. Mean relative fluorescence intensities ± SD for individual cytotypes (setting *Zea mays* as unit value and PI-staining) were as follows: DNA-tetraploids 0.648 ± 0.016 (range 0.602–0.678), DNA-octoploids 1.298 ± 0.039 (range 1.254–1.337) and DNA-dodecaploids 1.840 ± 0.042 (range 1.781–1.929) (Fig. 1). Because hexaploids had a nuclear DNA content similar to that of *Zea mays*, they were measured using *Pisum sativum* 'Ctirad' as the internal standard, resulting in a mean flurescence intensity of 0.590 ± 0.025 (range 0.580–0.629). Fluorescence ratios among the four cytotypes (4x vs. 6x, 8x, 12x) averages 1.00 : 1.52 : 2.00 : 2.84. This preliminary result indicates genome size decreases following polyploidization in dodecaploids (Leitch & Bennett 2004).

The most common were tetraploid (at 46.5% of localities) and dodecaploid cytotypes (43.6%), while the octoploid cytotype was considerably less common (12.5%). Just two plants were found to be hexaploid (0.8%). For 47 localities for which at least two individuals were analysed, there were mixtures of cytotypes at nine (19.5%), consisting of either tetra- and dodecaploids (seven localities) or tetra- and hexaploids (two localities).

There were striking differences in the geographic distributions of the cytotypes (Figs 2, 3). Tetraploids appear to be widely distributed throughout Europe. They were found in Norway (1/1; number of localities of the cytotype / total number of localities studied in a country), Sweden (2/2), Estonia (1/1), Russia (2/2), Poland (3/4), Germany (4/5), the

Fig. 2. – Geographical distribution of tetraploids (red dots) of the *Molinia caerulea* complex and mixed-ploidy-level populations including tetraploids (blue dots: 4x + 12x; violet dots: 4x + 6x) based on our data.

Czech Republic (32/87), Slovakia (37/68), Austria (3/5), Spain (1/1), Greece (1/1), Slovenia (3/6), Bulgaria (6/15) and France (5/6). Dodecaploid plants were found only in the western, (west-) central and south-central part of Europe, i.e. in France (1/6), Germany (1/4), Austria (2/5), the Czech Republic (57/87), Hungary (8/10), western Slovakia (27/68), Slovenia (3/6), Croatia (2/2) and Italy (1/1). On the other hand, octoploids were only found in the east-central and southeastern part of Europe, i.e. in eastern Slovakia (7/68), northeastern Hungary (2/10), the southeastern corner of Poland (1/4), Romania (9/9) and Bulgaria (9/15). Single hexaploid plants were found in two otherwise tetraploid populations (southwestern Slovakia and southern Bohemia).

Fig. 3. – Geographical distribution of octo- (blue dots) and dodecaploids (red dots) of the *Molinia caerulea* complex based on our data.

In central Europe, where the screening was extensive, contrasting patterns of cytotype distributions were observed. Both tetra- and dodecaploid populations were sympatric at the landscape scale and sometimes also at local scales in southern Bohemia (Czech Republic) and the Záhorie region in Slovakia (Fig. 2). On the other hand, partial allopatry to parapatry of cytotypes was recorded in the eastern part of the Czech Republic and Slovakia; for example, only dodecaploids were recorded in eastern Moravia and western Slovakia, tetraploids in southern Slovakia and the high mountains of northern Slovakia and octoploids in eastern Slovakia (Figs 2, 3). In fact, the distributions of octoploids and dodecaploids were allopatric.

Morphological differences among cytotypes

Sizes of the flower parts studied were strongly correlated with each other ($0.75 < r < 0.95$ in all cases) while correlations with diameter of the culm below the panicle were only moderate and ranged from 0.41 (with length of the longest hair on the callus) to 0.65 (with length of the lemma). The hexaploid cytotype was represented by a single specimen therefore the values for hexaploids were estimated but cannot be considered as representative. Ploidy level was significantly correlated with all the characters measured (Table 1). All characters measured showed a linear trend across ploidy levels (4x, 8x, 12x; all contrasts significant at $P \leq 0.05$; Fig. 4). The characters which differentiate the cytotypes best (R^2 of the models > 0.94) are lemma and stoma lengths. No apparent morphological discontinuities were found within the various cytotypes.

The ordination diagram of the PCA, based on individuals (Fig. 5A), indicates that the cytotypes form almost non-overlapping groups, with the tetraploids on the right, octoploids in the centre and dodecaploids on the left of the diagram. Thus, only the first component contributed significantly to their differentiation. However, morphological variation was almost continuous. Characters with the highest correlations (based on eigenvector values) with the first axis were the length of lemma, length of caryopsis and length of stoma (Table 2). Within each cytotype the spread along the second axis suggest important morphological variation mostly correlated with the length of the longest hair on the callus and diameter of the culm below the panicle. The dodecaploids were more spread along the second axis than the other cytotypes, indicating their higher variation in the length of the longest hair on the callus and diameter of the culm below the panicle (Table 1).

Canonical discriminant analysis (CDA) based on individual plants and with the 4x, 8x and 12x cytotypes defined as three groups showed clear separation among cytotypes ($F_{10, 206} = 47.8$, $P < 0.001$, Fig. 5B). It is obvious from Figure 5B that for discriminating among the cytotypes only the first canonical function is necessary since the groups are separately positioned along the first axis. Characters exhibiting the highest correlations with the canonical axis were the length of stoma, length of lemma and length of caryopsis (Table 2). The parametric method of analysis based on probability models resulted in a high number of plants being correctly classified in terms of cytotype (92.3% of tetraploids, 100% of octoploids and 96.6% of dodecaploids, respectively).

Table 1. – Descriptive statistics (arithmetic mean, standard deviation, minimum and maximum) of the morphological characters studied in particular cytotypes of the *Molinia caerulea* complex. Differences among cytotypes were tested by either one-way ANOVA (*) or nested ANOVA. Different superscripts indicate significant differences among means at P = 0.05 (Bonferroni multiple comparison test). Hexaploids were excluded from all analyses because only one plant was measured.

Character	4x			6x			8x			12x			F	P
	min	mean (sd)	max	min	mean (sd)	max	min	mean (sd)	max	min	mean (sd)	max		
Length of stoma (μm)	22	30 (3)^a	39	30	33 (2)	36	25	35 (3)^b	44	28	41 (3)^c	58	180.4	< 0.001
Length of lemma (mm)	2.3	3.3 (0.4)^a	4.5	3.4	3.8 (0.2)	4.2	3.3	4.0 (0.4)^b	5.4	3.4	4.8 (0.6)^c	6.5	70.7	< 0.001
Length of anthers (mm)	1.6	2.2 (0.2)^a	2.5	2.4	2.6 (0.1)	2.7	2.1	2.5 (0.2)^a	3.0	2.4	3.1 (0.3)^b	3.8	26.0	< 0.001
Length of caryopsis (mm)	1.4	1.9 (0.2)^a	2.4	1.8	2.0 (0.1)	2.0	2.0	2.3 (0.2)^b	2.5	2.0	2.7 (0.2)^c	3.9	100.1	< 0.001
Length of the longest hair on the callus (mm)*	0.0	0.3 (0.2)^a	0.7	–	0.2	–	0.2	0.4 (0.2)^b	0.8	0.2	0.6 (0.2)^c	1.1	47.5	< 0.001
Diameter of the culm below the panicle (mm)*	0.6	0.9 (0.2)^a	1.3	–	1.0	–	0.6	1.0 (0.2)^b	1.5	0.7	1.3 (0.2)^c	1.9	44.1	< 0.001

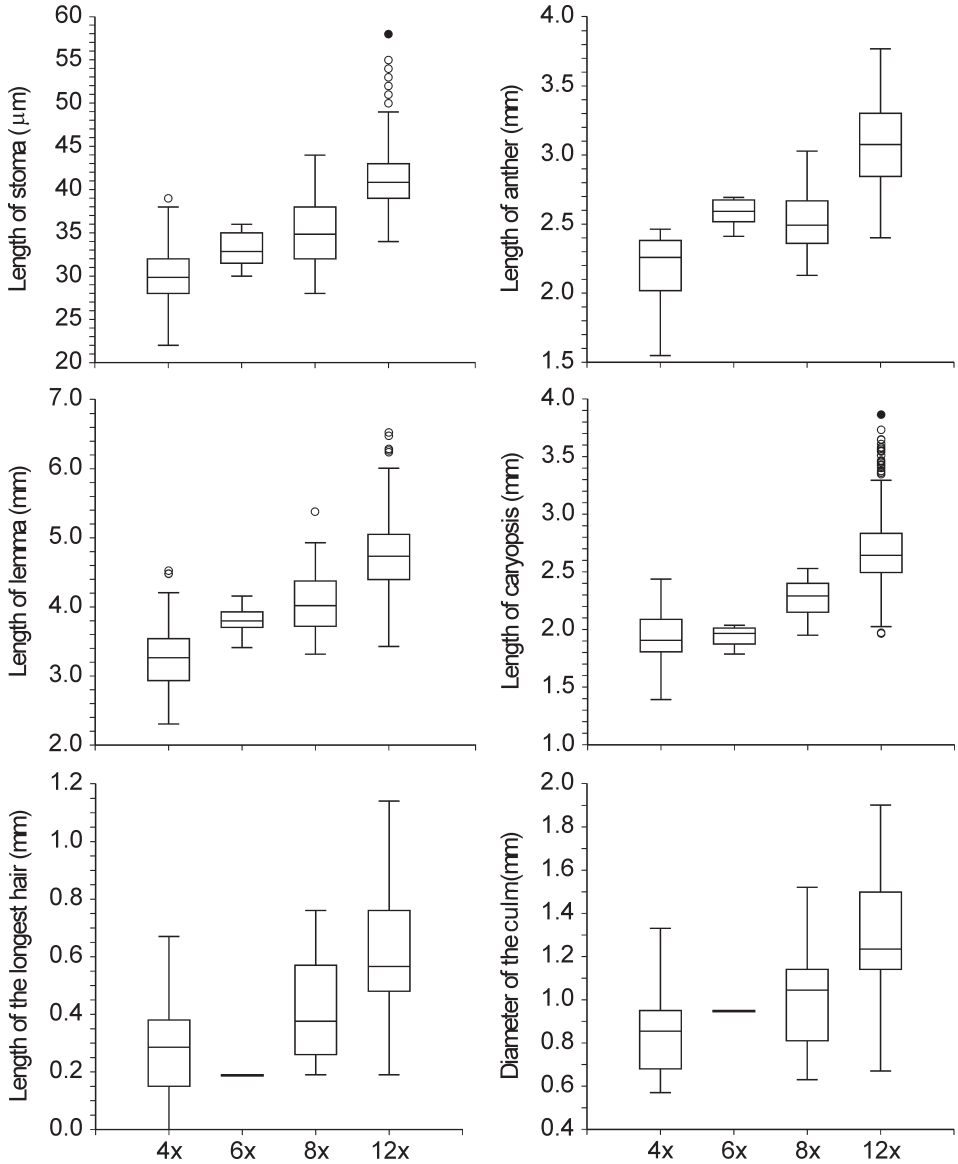

Fig. 4. – Box plots showing variation in the lengths of the lemma, caryopsis, stoma and anther, length of the longest hair on the callus and diameter of the culm below the panicle for four cytotypes (2n = 4x, 6x, 8x, 12x) of *Molinia caerulea* complex. Note only one hexaploid plant was measured.

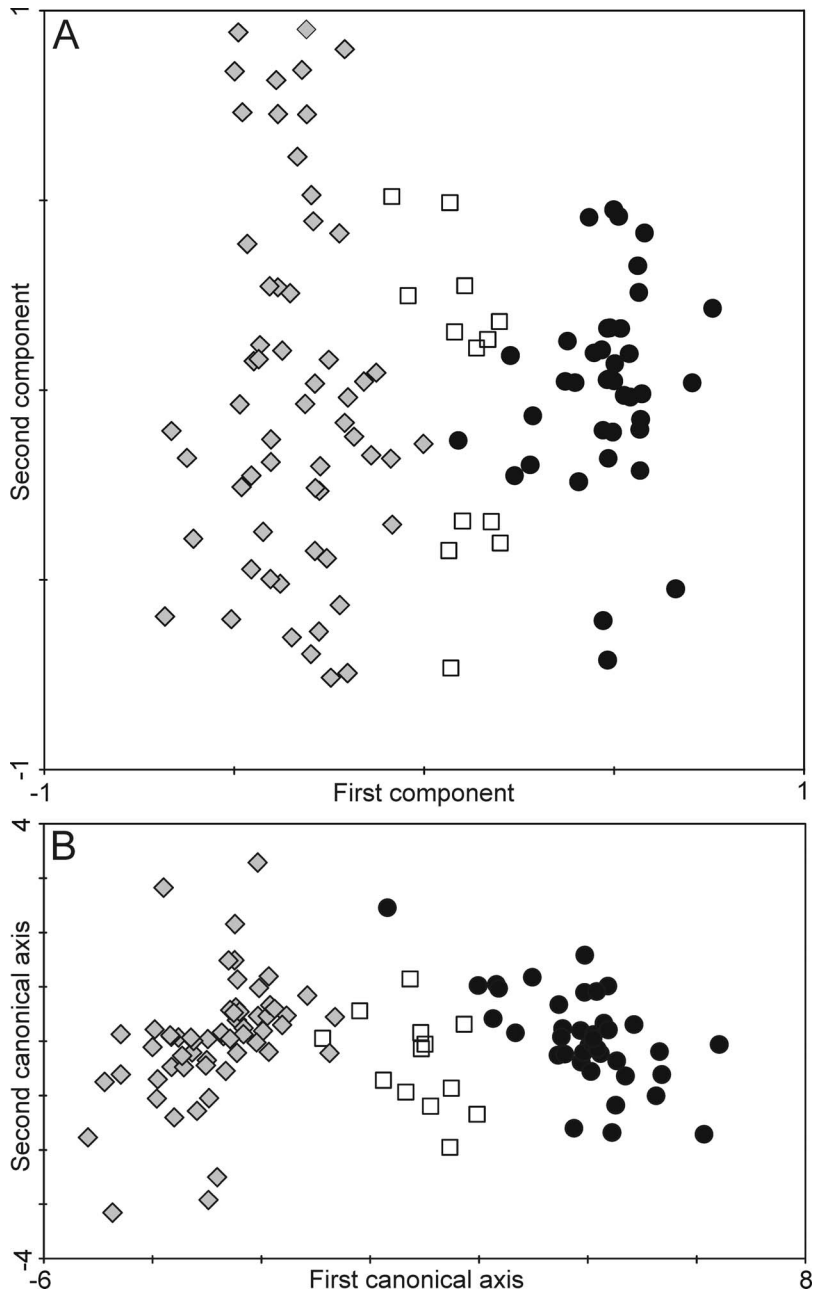

Fig. 5. – Biplot of principal component analysis (PCA; A) and a plot of the canonical variate scores of the canonical discriminant analysis (B) of 117 individuals of *Molinia cauerulea* complex based on five morphological characters. First two components of PCA explain 71.9% and 11.7% of the variation, respectively. Circles – tetraploid plants, squares – octoploid plants, diamonds – dodecaploid plants.

Table 2. – Results of morphometric analyses of *Molinia caerulea* complex. PCA – eigenvectors expressing correlations of the characters measured with the principal component axes of PCA based on 117 individuals (see Fig. 5A), CDA – total canonical structure expressing correlations of the characters measured with the canonical axes (see Fig. 5B).

Character	PCA		CDA	
	First component	Second component	First axis	Second axis
Length of stoma	–0.888	–0.045	–0.611	–0.006
Length of lemma	–0.936	0.106	–0.552	0.684
Length of caryopsis	–0.901	0.054	–0.544	–0.183
Length of the longest hair on the callus	–0.763	0.442	–0.311	–0.136
Diameter of the culm below the panicle	–0.727	–0.613	–0.291	0.078

Discussion

Cytotype diversity and distribution

Although altogether six cytotypes (2n = 18, 36, 54, 72, 90 and 108) are reported for central Europe by various authors, our screening included just four cytotypes (2n = 36, 54, 72 and 108). We did not find any diploid (2n = 18) or decaploid (2n = 90) plants.

There are two reports of diploid plants, the first by Mattick-Ehrensberger in 1949 (in Tischler 1950) for which the locality is unknown, however Frey (1973) and Tutin (1975) suppose it is situated in Tyrol (Austria). This record has frequently been regarded as incorrect (cf. Dobeš & Vitek 2000) nevertheless there are another two localities recorded for Hungary, one of which at least consists of a mixture of di- and tetraploid plants (Milkovits & Borhidi 1986). There are several possible explanations for such incidental occurrences of diploids. They may be simply a consequence of inaccurate counting of chromosomes, i.e. they do not exist. This seems the most likely scenario, because the karyological counts of Milkovits & Borhidi (1986) frequently conflicted with their other results (for details, see also below) and of that in other studies. Alternatively, diploids may have been formerly a more widely distributed cytotype, which retreated as a result of climate changes during the quaternary period or due to competition with polyploids. They may also have arisen recently within tetraploid populations via a reduction in chromosome number. It is known in some other plant species that individuals with a lower ploidy level can occur within polyploid populations (Dunwell 2010). However, this is quite a rare phenomenon in natural populations and probably has no evolutionary importance (Dunwell 2010).

Although extensive material was analysed from regions where decaploids (2n = 90) were previously reported, no decaploid plants were found. Instead, DNA-dodecaploids (2n ≈ 108) were frequent in these regions. Unfortunately, attempts to estimate the exact number of chromosomes from squash preparations were not successful and therefore we had to base our estimations on flow cytometry. However, we believe that some of the counts of 2n = 90 recorded for central Europe are wrong and should be 2n = 108. Several lines of evidence support this conclusion. First, the flow cytometry showed that all the plants studied, which were of a higher ploidy level than octoploid, were of the same ploidy level and their relative DNA content was approximately 1.5 times higher than that of octoploids, suggesting DNA-dodecaploids. Second, when we measured the DNA-ploidy level of the dodecaploid plant,

for which Krahulcová (cited in Dančák 2002) counted exactly 2n = 108, it was identical with the supposed DNA-dodecaploids. These facts led us to the assumption that previous counts of 2n = 90 for central Europe may refer to 2n = 108. However, whether any decaploids occur in western Europe (France and Switzerland), from where the first counts of 2n = 90 were published (Guinochet & Lemée 1950), is not yet clear.

Hexaploid plants were reported by Milkovits & Borhidi (1986) based on an uncertain counts of either 2n = 50 or 54 for two localities in northwestern Hungary, while we recorded them as a minority cytotype within two tetraploid populations in Slovakia and the Czech Republic. Concerning the hexaploids recorded by us, their rarity indicate that they are probably generated within tetraploid populations via fusion of unreduced and reduced gametes but are subsequently eliminated from the population due to a minority cytotype exclusion process (Levin 1975). A similar situation is recorded in e.g. *Cardamine yezoensis*, where the higher-ploidy levels do not appear to form their own populations, but occur in mixed ploidy-level populations (Marhold et al. 2010). However, this hypothesis does not apply to the supposed Hungarian "hexaploids" (= *Molinia pocsii* Milkovits 1986), because we only found dodecaploids, while Milkovits & Borhidi report tetra-, hexa- and octoploids in northwestern Hungary. Also Milkovits & Borhidi's description in the Latin diagnosis rather suggests dodecaploid plants. Therefore, we consider their "hexaploid" count as incorrect.

Concerning the high ploidy levels in low ploidy populations, we can neither exclude the generation of octoploids in tetraploid populations or dodecaploids in octoploid populations, although no 4x+8x, 4x+6x+8x or 8x+12x populations were found. This may suggest that either neopolyploidy is an extremely rare process in *Molinia* or that some cytotypes are reproductively isolated. However, due to less intensive within-population sampling we cannot exclude the possibility that we simply did not detect such mixed populations. Significant influence of sampling strategy on detection of a population's cytotype composition has recently been demonstrated (Šafářová & Duchoslav 2010, Šafářová et al. 2011).

The analysis of the distributions of the major cytotypes revealed clear patterns. Because of their wider distribution we assume that tetraploids are older than higher ploidy cytotypes and at least originated during late Pleistocene. Tetraploids occur (with variable frequency) almost all over Europe and are sympatric with other common cytotypes (2n = 8x, 12x) at a large scale but at a local scale their pattern of distribution is mosaic-like with a few small areas of single-cytotypes in regions sampled in detail (Figs 2, 3). Given that cytotypes differ in their ecological tolerances, Hewitt (1988) shows that the depth of contact zones and their structure depends on the spatial pattern of the habitats supporting each cytotype. Although not directly measured by us, we suggest that ecological differentiation of *Molinia* cytotypes (4x vs. 8x + 12x) is at least partially responsible for the observed pattern. The analysis of the database and our observations indicate that tetraploids most frequently occurred in relic habitats including bogs and both acidic and calcium-rich fens, mires, subalpine and alpine herbaceous and cliff vegetation, whereas octo- and dodecaploids typically occurred in several types of intermittently waterlogged oak and pine forests, calcium-rich mires, heathlands, meadow springs, temporally wet, low-productive grasslands and dry grasslands, and anthropic sites such as forest tracks, roadside ditches, secondary forests, etc. However, the ecological niches of cytotypes partially overlap, which results in rare mixed 4x + 12x populations, e.g. in fen and intermittently wet meadows, springs and pine forests. This accords with the observations of Guinochet & Lemée (1950), Frey (1975a) and Landolt (1977) on the ecology of tetra- and "decaploid"

(= dodecaploid?) cytotypes. In order to assess the phytosociological importance of *Molinia* taxa a detailed study of the ecological requirements of the different cytotypes is needed (see also Landolt 1977).

On the other hand there is no contact zone between octo- and dodecaploid cytotypes, which are vicariate. Absence of direct contact (ranges separated by ca. 100 km) between octo- and dodecaploids suggests that cytotype allopatry is not a result of recent postzygotic barriers between cytotypes (i.e. results of a balance between dispersion rates and frequency-dependent selection against hybrids) or of differences in their ecological niches because both octo- and dodecaploids inhabit similar habitats (M. Dančák, pers. obs.). We propose that the present-day allopatry of octo- and dodecaploids originated in refugia in southern Europe (probably in the Balkans) during the late Pleistocene. The octoploids evolved from tetraploids (via fusion of unreduced gametes) and consequently were partially spatially separated as a result of their broader environmental tolerance. The dodecaploids evolved later from octoploids in a similar way (fusion of reduced and unreduced gametes). Due to strong competition with octoploids they were forced to separate spatially. When the climate started to warm up both cytotypes followed expanding forests. The octoploids expanded north-eastwards and the dodecaploids north-westwards. The Pannonian lowlands (especially the eastern part), where there are no suitable habitats for octo- and dodecaploids were important in determining the spatial separation (and recent allopatry) of both cytotypes. This is highly speculative, but such mechanisms were previously proposed to account for the formation and present-day distribution of autopolyploid lineages of many plants (Parisod et al. 2010). In order, to confirm this hypothesis, data on the genetic patterns of *Molinia* cytotypes throughout their distributions are needed.

Morphological differentiation of the cytotypes

Both Guinochet & Lemée (1950) and Frey (1975a) have shown there is a correlation between chromosome number and morphology in species of *Molinia*. Frey (1975a) reports that each of the two cytotypes in Poland (*M. caerulea* with 2n = 36 and *M. arundinacea* with 2n = 90) corresponds to a particular phenotype. He records that the size of the generative characters studied (length of lemma, length of panicle, diameter of pollen grains) and the length of stomata correlated well with the chromosome number, i.e. the values for 2n = 36 plants were lower than those for 2n = 90 plants. However, the variation within and overlap between cytotypes in size of a number of vegetative characters (length of the longest leaf and height of the plant) means these characters are unsuitable for differentiating the taxa (Frey 1975a). This is in line with Salim et al. (1995), who report big differences in vegetative characters between populations of the same cytotype from acid moorlands and an alkaline waste tip, i.e. tetraploid *Molinia caerulea*. Since we also recorded considerable variation in the size of vegetative characters (e.g. height of plants, length of panicle, looseness of panicle) of the material sampled, we focused mainly on quantitative morphological characters measured on generative parts.

Multivariate analyses at the level of individual plants resulted in a fairly good separation of the cytotypes, indicating that morphological differentiation between the cytotypes does exist. However, tight proximity of groups without clear discontinuities in the PCA diagram (Fig. 5A) together with the results of univariate analyses of the characters studied showed partial overlap in these characters between cytotypes. This is especially the case of

the length of the longest hair on the callus and the diameter of the culm below the panicle. These two characters were very variable within the cytotypes and are generally of less importance for the differentiation among cytotypes (Fig. 4, Table 2). CDA that weights characters to stress the between-group variation component yielded a satisfactory degree of differentiation among the cytotypes and also showed that characters with the highest loadings in the PCA (length of stoma, length of lemma, length of caryopsis) showed also high correlations with the first canonical axis in the CDA. Parametric classificatory discriminant analysis based on a probability model resulted in a high accuracy of cytotype identification (> 90%) but also suggest that identification of tetra- and dodecaploids is less reliable than that of octoploids. However, due to the small sample of octoploids studied their morphological variation could be underestimated.

Our results confirm that tetraploid plants correspond morphologically with *M. caerulea* and dodecaploid plants with *M. arundinacea*, as reported by Frey (1975a). Also the mean values of stoma length reported for tetraploids by Guinochet & Lemée (1950) is similar to those reported here. The values of the lengths of the lemma and stoma for dodecaploids were in the same range as those reported by Frey for "decaploids". Octoploid plants were intermediate between tetra- and dodecaploids in all the characters measured, although no significant differences in mean values between tetraploids and octoploids were observed in the length of the anthers. This was mainly due to small sample size and consequently lower power of the multiple-comparison test.

There were no morphological discontinuities suggesting additional subdivision in any of the cytotypes, which agrees with previous studies (Sterk & ter Laak 1972, Frey 1975a). Frey (1975a) distinguishs two varieties within *M. caerulea*, typical var. *caerulea* and var. *subspicata* Boenn., which differ in panicle shape (being much contracted in var. *subspicata*). However var. *subspicata* is merely an extreme form of *M. caerulea* and is not clearly separated from its typical morphotypes. Our field observations also suggest that panicle shape can be considerably modified by environmental conditions.

Taxonomic treatment of different cytotypes: how many taxa occur in central Europe?

In contrast to most taxonomic treatments of the *M. caerulea* complex, which recognise two species in central Europe, *Molinia caerulea* (L.) Moench and *Molinia arundinacea* Schrank (Frey 1975a, b, Conert 1992, Adler et al. 1994, Marhold & Hindák 1998, Kubát et al. 2002, Rothmaler et al. 2005), Milkovits & Borhidi (1986) set up an entirely new taxonomic concept of this complex. They recognise eight species forming two parallel polyploid series. Five of them (*Molinia caerulea* with 2n = 2x, *M. arundinacea* with 2n = 4x and 8x, *M. pocsii* Milkovits with 2n = 6x, *M. litoralis* Host with 2n = 8x and *M. ujhelyi* Milkovits with 2n = 10x) belong to the series *Caerulea* with an Atlantic-Alpine distribution. Three other species (*Molinia simonii* Milkovits with 2n = 2x and 4x, *M. hungarica* Milkovits with 2n = 4x and *M. horanszkyi* Milkovits with 2n = 8x) belong to the series *Hungaricae* Milkovits with a Pannonian-Balkan distribution. Although at first glance this concept seems to be reliable, there are a number of reasons why we question Milkovits & Borhidi's conclusions. (i) As described earlier, within the material currently studied and in accordance with previous studies (Sterk & ter Laak 1972, Frey 1975a), no significant morphological discontinuities among individuals of the same ploidy level were detected. (ii) Chromosome numbers reported by Milkovits & Borhidi mostly disagree with morphology

and habitat preferences of the taxa mentioned. The values of the morphological traits of 6x, 8x, 10x and partially also even of 4x (in the case of *M. arundinacea* sensu Milkovits & Borhidi, non Schrank) correspond to those of our 12x. Moreover, the authors did not give any details of the methods they used to obtain the data or of the respective sample sizes. (iii) Type specimens of the newly described taxa are missing from the BP herbarium and were probably never deposited there (Somlyay, in litt.). (iv) The geographical distribution of the cytotypes reported by Milkovits & Borhidi is speculative and partially clearly wrong. Nearly all of their conclusions concerning the distribution of particular cytotypes (species) obviously disagree with all other studies on chromosome counts (cf. Sterk & ter Laak 1972, Frey 1973, our data). Milkovits & Borhidi report the diploid, hexaploid and octoploid species of series *Caerulea* to be common in some parts of western and northern Europe (e.g. Austria, Germany, Switzerland), although there are no records (except for one dubious record of 2n = 18, Mattick-Ehrensberger in Tischler 1950 from Austria) from these regions to support this conclusion. Additionally they consider the decaploid plants named *Molinia ujhelyi* to be a Pannonian endemic, although Guinochet & Lemée (1950) and Frey (1973) formerly reported this chromosome number for plants from France and Poland. Considering all the facts the claims by Milkovits and Borhidi are not supported by any evidence.

Soltis et al. (2007) recently argued for accepting autopolyploidy as a significant mechanism of speciation based on geographical, ecological and evolutionary differences and reproductive isolation between cytotypes and for practical reasons (e.g. conservation of rare cytotypes), even if morphological variation prevents the reliable identification of cytotypes. Rowley (2007) suggests a more flexible approach to the taxonomic classification of autopolyploids, which also includes infraspecific categories. Our results showed that tetra-, octo- and dodecaploid cytotypes are partially morphologically differentiated, differ markedly in geographic distribution and show at least partial habitat differentiation. These results suggest that it is possible to distinguish three taxa in central Europe. One of them is cytologically variable (predominantly the tetraploid, sporadically the hexaploid; perhaps also the diploid?) and two others are cytologicaly uniform (octoploid and dodecaploid). Tetraploid plants were previously assigned to *M. caerulea* (Sterk & ter Laak 1972, Frey 1973) and "decaploids" to *M. arundinacea* (Frey 1973). Our results support this concept with the correction that the right chromosome number for *Molinia arundinacea* s. str. (at least in central Europe) is not 2n = 90, but 2n = 108. We prefer to include hexaploids (and diploids if they exist) in *M. caerulea* as they are very rare and found by us always within tetraploid populations. They probably do not form their own populations, do not have their own distributional range and do not differ ecologically from tetraploids. On the contrary, the octoploid cytotype forms populations on its own and has a distinct distribution. Octoploids are clearly spatially separated from dodecaploids, with which they share the same (or very similar) habitat preferences (M. Dančák, pers. obs.) and general habit. Thus, even though the characters of the octoploid plants examined were intermediate between those of tetraploid and dodecaploid plants, it is obvious that they are related to dodecaploid *M. arundinacea*. Therefore we suggest treating octoploids and dodecaploids as subspecies of *M. arundinacea*. In this approach the dodecaploid plants are assigned to the subsp. *arundinacea* and octoploid plants to the newly described subsp. *freyi* (see below). A similar approach is adopted in the taxonomic treatment of the *Scilla bifolia* agg. (Trávníček et al. 2009). In this group, three species (*Scilla kladnii* Schur,

S. vindobonensis Speta and *S. bifolia* L.) that are ecogeograhically different are recognised. One of them (*S. bifolia*) is composed of three geographically vicariant types, which differ karyologically (ploidy level) but not entirely ecologically: subsp. *bifolia* has an alpine distribution and can be either diploid or tetraploid, subsp. *buekkensis* (Speta) Soó is tetraploid with a carpathian distribution and subsp. *spetana* (Keresty) Trávn. is hexaploid with a pannonian distribution. On the other hand, Somlyay et al. (2006) use a different taxonomic category (i.e. species level) for the classification of diploid and tetraploid cytotypes of the *Muscari botryoides* agg., which differ in their geographic distribution and are reproductively isolated, but are morphologically and ecologically very similar. Nevertheless, we agree with the reasoning of Rowley (2007) that when the cytotypes are not sufficiently differentiated, taxonomic classification using infraspecific categories is more suitable.

Milkovits & Borhidi (1986) divide the octoploid cytotype into two taxa. They consider one to be *Molinia litoralis* Host and the other *M. horanszkyi*. As the name *Molinia litoralis* refers very probably to dodecaploid plants (the taxon is described from the Adriatic coast), it cannot be used for octoploid plants. Also the name *Molinia horanszkyi* is very probably a synonymy of *M. arundinacea* subsp. *arundinacea*, because in their Latin diagnosis the authors report a lemma length of 5.8 mm, which corresponds with our dodecaploids, despite the declared octoploid chromosome number. Milkovits & Borhidi either miscounted the chromosome number or incorrectly measured the length of the lemmas of the plants. It is most likely they miscounted the number of chromosomes. Since no typus was found in BP herbarium, we also visited the locus classicus of *Molinia horanszkyi* in the Mátra Mts (small lake called Pisztrángos-tó near Mátraháza). Unfortunately, we did not find any *Molinia* plants there. However, we found a dodecaploploid population close to the locus classicus (3.5 km NNW), which supports our opinion.

With respect to morphology and ecogeography, the name *Molinia hungarica* Milkovits should be synonymised with *M. caerulea* and the name *M. ujhelyi* Milkovits with *M. arundinacea* subsp. *arundinacea*. The name established by Milkovits & Borhidi (1986) for the allegedly diploid and tetraploid species *Molinia simonii* is here (with respect to morphology, ecology and cytology) considered a synonym of *M. caerulea*. The name *M. pocsii* is very probably a synonym of *M. arundinacea* subsp. *arundinacea* (plants about 120–180 cm high, lemma 5.5 mm long), despite the declared hexaploid chromosome number.

Pobedimova (1949) describes *Molinia euxina* Pobed. from southern Ukraine that should differ from both *M. caerulea* and *M. arundinacea* (see also Frey 1975). The first author studied type material deposited in herbarium LE and found that it belongs to the taxon *Molinia caerulea* s. str. Therefore, the name *Molinia euxina* Pobed. is a synonym of *Molinia caerulea* (L.) Moench.

Identification key and taxonomic treatment of taxa from the *Molinia caerulea* group in central Europe

In central Europe the *Molinia caerulea* complex is represented by three taxa differing in morphology and chromosome number: *Molinia caerulea* (L.) Moench, *M. arundinacea* Schrank subsp. *arundinacea* and *M. arundinacea* subsp. *freyi* Dančák. Below is an identification key, an overview of accepted taxa together with morphological descriptions and brief commentary on their karyological variability and geographical distribution.

1a Plants (15–) 30–90 (–150) cm tall; culm thin below the panicle, usually not exceeding 1.3 mm in diameter; leaf blades narrow, the widest leaves usually 5–10 mm wide; spikelets 4–6 (–8) mm long; lemmae of lower flowers (2.3–) 2.7–3.7 (–4.5) mm long, obtuse to acute; rachilla and callus glabrous or covered with very short bristle-like hairs; caryopses (1.4–) 1.7–2.2 (–2.4) mm long; undehisced anthers (1.5–) 1.8–2.4 (–2.5) mm long; stomata on the adaxial side of the leaf blade (22–) 27–34 (–39) μm long. – Panicle usually contracted, with short erect branches (rarely with longer loose branches). 2n = 18 ?, 36, 54 ..... ***M. caerulea*** (L.) Moench

1b Plants (50–) 90–200 (–250) cm tall; culm thick below the panicle, usually exceeding 1.3 mm in diameter; leaf blades broad, the widest leaves usually 8–15 mm wide; spikelets (5–) 6–9 mm long; lemmae of lower flowers (3.3–) 3.5–5.5 (–6.5) mm long, acute to obtuse; rachilla and callus usually covered with long conspicuous bristle-like hairs; caryopses (1.9–) 2.0–3.1 (–3.8) mm long; undehisced anthers (2.1–) 2.2–3.6 (–3.8) mm long; stomata on the adaxial side of the leaf blade (28–) 30–45 (–58) μm long. – Panicle usually open, with long loose branches (rarely with short and erect branches). 2n = 72, 90 ?, 108 ..... ***M. arundinacea*** Schrank

01a Lemmae of lower and middle flowers (3.3–) 3.5–4.5 (–5.4) mm long; stomata (28–) 30–40 (–44) μm long; caryopses (1.9–) 2.0–2.5 (–2.6) mm long; undehisced anthers (2.1–) 2.2–2.8 (–3.0) mm long. 2n = 72 ..... ***M. a.* subsp. *freyi*** Dančák

01b Lemmae of lower and middle flowers (3.4–) 4.0–5.5 (–6.5) mm long; stomata (34–) 37–45 (–58) μm long; caryopses (2.0–) 2.4–3.1 (–3.8) mm long; undehisced anthers (2.4–) 2.6–3.6 (–3.8) mm long. 2n = 108 ..... ***M. a.* subsp. *arundinacea***

Molinia caerulea (L.) Moench, Methodus 183, 1794.

Typus: specimen no. 85.1 in LINN (lectotypus Trist & Sell 1988: 154).
Synonymy:
≡ *Aira caerulea* L., Sp. Pl. 63, 1753.
≡ *Melica caerulea* (L.) L., Mant. Pl. Altera 325, 1771.
≡ *Enodium caeruleum* (L.) Gaudin, Agrost. Helv. 1: 145, 1811.
≡ *Amblytes caerulea* (L.) Dulac, Fl. Hautes-Pyrénées 80, 1867.
= *Aira atrovirens* Thuill., Fl. Env. Paris, ed. 2, 1: 37, 1799.
= *Molinia depauperata* Lindl., Syn. Brit. Fl., ed. 2, 307, 1835.
= *M. minor* (Holandre) Holandre, Fl. Moselle, ed. 2, 813, 1842.
= *M. obtusa* Peterm., Flora (Regensb.) 27: 235, 1844.
= *M. euxina* Pobed., Bot. Mater. Gerb. Bot. Inst. Akad. Nauk SSSR 11: 36, 1949.
= *M. hungarica* Milk., Symb. Bot. Upsal. 27 (2): 143, 1987 (“1986”).
= *M. simonii* Milk., Symb. Bot. Upsal. 27 (2): 142, 1987 (“1986”).
= *M. simonii* var. *major* Milk., Symb. Bot. Upsal. 27 (2): 142, 1987 (“1986”).
– *M. varia* Schrank, Baier. Fl. 1: 334, 1789, nom. illeg.
– *M. variabilis* Wibel, Prim. Fl. Werth. 115, 1799, nom. illeg.

D e s c r i p t i o n: Perennial, caespitose herbaceous plant, culm (15–) 30–100 (–150) cm tall. Diameter of culm below the panicle 0.5–1.3 (–2.0) mm. Leaf blade 2.5–8.0 (–10.0) mm wide (the widest leaves 5–10 mm wide); stomata on the adaxial side of leaf blade (22–) 27–34 (–39) μm long. Panicle usually straight, contracted or sometimes loose (5–) 10–30 (–50) cm long, branches erect, straight and rigid, rarely lax, the lower 0.5–10.0 (–20.0) cm long, pedicels usually 2–4 mm long; spikelets with 2–5 flowers, 4–6 (–8) mm long, violet, dark blue, blue-green or green, rachilla usually glabrous or covered with very short bristle-like hairs; glumes ovate to lanceolate, 2.5–3.0 (–3.5) mm long, obtuse to acute, non-carinate, hyaline, glabrous, lower glume 1-nerved or without nerve, upper glume 1–3-nerved slightly longer than the lower. Lemma ovate to lanceolate, (2.3–) 2.7–3.7 (–4.5) mm long, obtuse to acute, 3-nerved, glabrous; palea lanceolate, equal to lemma, obtuse to truncate, hyaline, 2-nerved; undehisced anthers (1.5–) 1.8–2.4 (–2.5) mm long; pollen grains 20–30 μm in diameter. Caryopsis is elliptical in outline, (1.4–) 1.7–2.2 (–2.4) mm long, brown. Flowering period VI–IX. 2n = 18?, 36, 64.

D i s t r i b u t i o n: According to Hultén & Fries (1986) and Conert (1992) it is distributed from the British Isles to southern Urals and from northern Norway to the Pannonian Lowlands, the Alps and the northwest part of the Iberian Peninsula. It is rare in or completely missing from the Mediterranean. In Asia it occurs in a small area in western Siberia (between the Irtysh River and the Ural Mountains), in the Caucasian area and in the eastern Mediterranean (Turkey, Lebanon, Syria, and Israel). In Africa it is reported to occur in Morocco, Algeria, Tunisia, Ethiopia and Kenya. It has been introduced into the USA and Canada (Hitchcock & Chase 1951, Conert 1992).

Molinia arundinacea Schrank, Baier. Fl. 1: 336, 1789.

Typus: Niederbayern, Auwälder bei Isargemünd bei Deggendorf, H. Paul 1935 in M (neotypus Conert 1981: 9).

D e s c r i p t i o n: Perennial, caespitose herbaceous plant, culm (50–) 90–200 (–250) cm tall. Diameter of the culm below the panicle (0.8–) 1.3–2.0 (–2.5) mm. Leaf blade (5–) 6–12 (–17) mm wide (the widest leaves usually 8–15 mm wide); stomata on the adaxial side of the leaf blade (30–) 37–45 (–58) μm long. Panicle usually straight, sometimes reflexed at the apex, in the lower part very loose and wide, with lax branches (25–) 30–60 (–75) cm long, lower branches (4–) 8–20 (–30) cm long, spreading to drooping, sometimes flexuous, rarely short and erect, pedicels usually 2–6 mm long; spikelets with 2–5 flowers, (5–) 6–9 mm long, usually tinged violet, sometimes green, callus and adjacent part of rachilla usually conspicuously hairy with 0.3–0.7 mm long bristle-like hairs, rarely almost glabrous; glumes lanceolate 3.0–5.5 mm long, acute, hyaline, non-carinate, hyaline, glabrous, lower glume 1-nerved, upper glume 1–3-nerved, slightly longer than the lower. Lemma lanceolate, (3.4–) 4.0–5.5 (–6.5) mm long, acuminate to acute, rarely obtuse, glabrous, 3 (–5) –nerved, midvein scabridulous; palea lanceolate, almost equal to lemma, obtuse, hyaline, 2-nerved; undehisced anthers (2.4–) 2.6–3.6 (–3.8) mm long; pollen grains 30–40 μm in diameter. Caryopsis is elliptical in outline, (2.0–) 2.4–3.1 (–3.8) mm long, dark brown. Flowering period VII–IX. 2n = 72, 90?, 108.

D i s t r i b u t i o n: According to Frey (1976), Conert (1992) and our data it occurs in central and southeastern Europe, westwards to eastern France, eastwards to central Romania, northwards to southern Poland and southwards to Bulgaria. *Molinia arundinacea* (as *M. litoralis*) is also sometimes reported to occur in the Caucasian area (Grossheim 1949, Tsvelev 1976, Galushko 1978), which is accepted by Conert (1981, 1992). Maire (1955) distinguishes two forms of *Molinia arundinacea* from North Africa: var. *africana* Maire and var. *rivulorum* (Pomel) Trabut, both having long, loose panicles and very long lemmas resembling *M. caerulea* var. *litoralis* (= *M. arundinacea*). However, he reports n = 18 for both these taxa.

Molinia arundinacea subsp. *arundinacea*

Synonymy:
≡ *M. caerulea* var. *arundinacea* (Schrank) Peterm., Bot. Exc. Leipzig 553, 1846.
≡ *M. caerulea* subsp. *arundinacea* (Schrank) K. Richt., Pl. Eur. 1: 72, 1890.
= *Melica caerulea* var. *major* Roth, Tent. Fl. Germ. 2: 103, 1789.
= *Enodium sylvaticum* Link, Enum. Hort. Berol. Alt. 1: 80, 1821.
≡ *Molinia sylvatica* (Link) Link, Hort. Berol. 1: 197, 1827.
= *Molinia altissima* Link, Hort. Berol. 1: 197, 1827.
≡ *M. caerulea* subsp. *altissima* (Link) Domin, Preslia 13–15: 39, 1935.

= *M. litoralis* Host, Fl. Austriac. 1: 118, 1827.
≡ *M. caerulea* subsp. *litoralis* (Host) Braun-Blanq., Sched. Fl. Rhaet. Exs. **7**: 184, 1924.
= *M. arundinacea* var. *robusta* Milk., Symb. Bot. Upsal., 27 (2): 141, 1987 (“1986”).
= *M. horanszkyi* Milk., Symb. Bot. Upsal. 27 (2): 143, 1987 (“1986”).
= *M. pocsii* Milk., Symb. Bot. Upsal. 27 (2): 141, 1987 (“1986”).
= *M. ujhelyi* Milk., Symb. Bot. Upsal. 27 (2): 142, 1987 (“1986”).

2n = 90?, 108

D i s t r i b u t i o n: This subspecies occurs in the western part of the species range. According to cytological data it occurs in France, Switzerland, Germany, the Czech Republic, southern Poland, western Slovakia, Austria, Hungary, Slovenia and Croatia. We expect it to occur also in Bosnia and Herzegovina.

Molinia arundinacea subsp. *freyi* Dančák, subsp. nova

Typus: Hungary, Abaúj-Zemplén County, Zemplén Mts., village of Telikbánya, roadside ca 4.3 km SE of the village centre, ca 350 m a. s. l., 48° 27' 29" N, 21° 23' 44" E, M. Dančák 17. IX. 2011 in OL (holotype).
Synonymy:
– *M. horanszkyi* auct., non Milk., Symb. Bot. Upsal. 27 (2): 143, 1987 (“1986”).

D i a g n o s i s: Ab *M. arundinacea* subsp. *arundinacea* lemmae spicules inferiores et medialis (3,3–) 3,5–4,5 (–5,4) mm longae, stomata (28–) 30–40 (–44) μm longa, caryopsis (1,9–) 2,0–2,5 (–2,6) mm longa, antherae indehiscentis (2,1–) 2,2–2,8 (–3,0) mm longae differt. Numero chromosomatico 2n = 72.

This subspecies is named after the excellent Polish botanist Ludwik Frey, author of the modern taxonomic concept of the *Molinia caerulea* group.

2n = 72

D i s t r i b u t i o n: This subspecies occurs in the eastern (south-eastern) part of the species range. Based on cytological data it grows in eastern Slovakia, south-eastern Poland, north-eastern Hungary, Romania and Bulgaria (Hájek et al. 2005 sub *M. horanszkyi*). We expect it to occur also in the Carpathian part of Ukraine and eastern part of Serbia.

See http://www.preslia.cz for Electronic Appendix 1

Acknowledgements

We thank several collectors for collecting samples from some of the populations. We thank J. Doležel, I. Doležalová, L. Šafářová, M. Bartoš and especially M. Hroneš and M. Jandová for their help with flow-cytometry and J. Čihalíková for help with the karyological analyses. Our thanks also go to three reviewers for their constructive comments and advice on previous version of this manuscript. The first author thanks Michal Hájek, who inspired him to study the *Molinia caerulea* group, helped a lot with field work and was always keen to discuss the topic. J. W. Jongepier and T. Dixon kindly revised the English. This work was supported by the Grant Agency of the Czech Republic (grant numbers 206/01/1115, 206/07/0706) and by internal grants from Palacký University (IGA PřF 2011/3, 2012/1).

Souhrn

V Evropě se vyskytující zástupci rodu bezkolenec (*Molinia* Schrank) se všichni řadí do okruhu *Molinia caerulea*. Taxonomické hodnocení tohoto komplexu je ztíženo výskytem polyploidie a velké morfologické variability a s tím souvisejícím velkým množstvím publikovaných jmen a nomenklatorických kombinací. Většina současných středoevropských flór akceptuje závěry studií polského botanika Freye a rozlišuje v rámci tohoto komplexu dva typy, nejčastěji hodnocené jako druhy *Molinia caerulea* a *M. arundinacea*. Radikálně nové taxonomické členění okruhu *M. caerulea* publikovali maďarští autoři Milkovits & Borhidi (1986), kteří tuto skupinu rozštěpili na dvě paralelní polyploidní řady (série) a popsali několik nových taxonů (druhů a variet). Ve své práci přinášíme výsledky karyologické a morfometrické analýzy vzorků řady populací okruhu *M. caerulea* a z toho vycházející taxonomické hodnocení této skupiny. Na základě studia 241 lokalit na území Evropy byly identifikovány tetraploidní (2n = 36), hexaploidní (2n = 54), oktoploidní (2n = 72) a dodekaploidní (2n ≈ 108) cytotypy. Nebyl potvrzen výskyt diploidů (ojediněle uváděných v literatuře a vztahovaných k *M. caerulea*) a také dříve často uváděný výskyt dekaploidů (2n = 90), jimž bylo přisuzováno jméno *M. arundinacea*. Údaje o dekaploidech ze střední Evropy jsou s největší pravděpodobností chybné a ve skutečnosti reprezentují dodekaploidy, mylné mohou být i údaje o diploidech. Tetraploidní cytotyp je roztroušeně rozšířený v celé Evropě. Hexaploidní cytotyp byl nalezen velmi vzácně a pouze v populacích tetraploidního cytotypu, ze kterého pravděpodobně může recentně vznikat. Okto- a dodekaplodní cytotypy jsou alopatricky rozšířené, přičemž dodekaploidní cytotyp se vyskytuje v západní a střední Evropě, na jih až po severozápadní Chorvatsko, zatímco oktoploidní cytotyp roste ve východní části střední Evropy a v jihovýchodní Evropě. Rozměry studovaných kvantitativních morfologických znaků (délky pluchy, obilky, prašníku, průduchů, délka nejdelšího chlupu na vřetenu klásku, tloušťka báze stébla pod květenstvím) se signifikantně lišily mezi cytotypy a lineárně se zvětšovaly s rostoucí ploidní úrovní. Některé dříve užívané znaky (počet klásků v květenství, ochlupení listů, výška rostliny) jsou nevhodné pro rozlišení cytotypů. Výsledky našeho studia jsou v podstatě v souladu s taxonomickou klasifikací skupiny navrženou polským botanikem Freyem (i když s určitými korekcemi) a naopak prakticky vůbec nepotvrzují taxonomické pojetí skupiny, jak ho publikovali maďarští autoři Milkovits & Borhidi. Na základě výsledků našich karyologických a morfometrických analýz a při zohlednění základních ekogeografických charakteristik jednotlivých rozlišených cytotypů a nomenklatorických poznatků předkládáme následující klasifikaci okruhu *M. caerulea*: tetraploidní populace převážně reliktních stanovišť řadíme k druhu *M. caerulea*, ekologickými nároky si blízké (vázané většinou na louky a lesní světliny) oktoploidní a dodekaploidní populace k druhu *M. arundinacea*, přičemž dodekaploidi náležejí k subsp. *arundinacea* (západní a střední Evropa) a oktoploidi k nově popsané subsp. *freyi* (východní část střední Evropy a jihovýchodní Evropa). Tyto taxony lze morfologicky rozlišit podle následujícího určovacího klíče:

1a Rostliny (15–) 30–90 (–150) cm vysoké; stéblo pod latou tenké, jeho tloušťka obvykle nepřesahuje 1,3 mm; listové čepele úzké, nejširší 5–10 mm široké; klásky malé, 4–6 (–8) mm dlouhé; pluchy dolních květů v klásku (2,3–) 2,7–3,7 (–4,5) mm dlouhé, tupé (až zašpičatělé) ; vřeteno klásku a ztlustlina na bázi pluchy (callus) lysé nebo pokryté velmi krátkými štětinovitými chlupy; obilky (1,4–) 1,7–2,2 (–2,4) mm dlouhé; neotevřené prašníky (1,5–) 1,8–2,4 (–2,5) mm dlouhé; průduchy na svrchní straně listových čepelí (22–) 27–34 (–39) µm dlouhé. – Lata obvykle stažená, s krátkými vzpřímenými větvemi (vzácněji řídká, s delšími, více rozestálými větvemi). 2n = 18?, 36, 54 ..... ***M. caerulea*** (L.) Moench

1b Rostliny (50–) 90–200 (–250) cm vysoké; stéblo pod latou dosti tlusté, obvykle silnější než 1,3 mm; listové čepele poměrně široké, nejširší často až 8–15 mm; klásky (5–) 6–9 mm dlouhé; pluchy dolních květů v klásku (3,3–) 3,5–5,5 (–6,5) mm dlouhé, zašpičatělé (až tupé) ; vřeteno klásku a ztlustlina na bázi pluchy (callus) obvykle pokryté nápadnými dlouhými štětinovitými chlupy; obilky (1,9–) 2,0–3,1 (–3,8) mm dlouhé; neotevřené prašníky (2,1–) 2,2–3,6 (–3,8) mm dlouhé; průduchy na svrchní straně listových čepelí (28–) 30–45 (–58) µm dlouhé. – Lata obvykle řídká, s dlouhými, často poněkud rozestálými větvemi (vzácněji s kratšími a více vzpřímenými větvemi). 2n = 72, 90 ?, 108 ..... ***M. arundinacea*** Schrank 2

2a Pluchy dolních a středních květů v klásku (3,4–) 4,0–5,5 (–6,5) mm dlouhé; průduchy (34–) 37–45 (–58) µm dlouhé; obilky (2,0–) 2,4–3,1 (–3,8) mm dlouhé; neotevřené prašníky (2,4–) 2,6–3,6 (–3,8) mm dlouhé. 2n = 108 ..... **subsp. *arundinacea***

2b Pluchy dolních a středních květů v klásku (3,3–) 3,5–4,5 (–5,4) mm dlouhé; průduchy (28–) 30–40 (–44) µm dlouhé; obilky (1,9–) 2,0–2,5 (–2,6) mm dlouhé; neotevřené prašníky (2,1–) 2,2–2,8 (–3,0) mm dlouhé. 2n = 72 ..... **subsp. *freyi*** Dančák

References

Adler W., Oswald K. & Fischer R. (1994): Exkursionsflora von Österreich. – Verlag Eugen Ulmer, Stuttgart & Wien.

Beaulieu J. M., Leitch I. J., Patel S., Pendharkar A. & Knight C. A. (2008): Genome size is a strong predictor of cell size and stomatal density in angiosperms. – New Phytol. 179: 975–986.

Chytrý M., Kučera T. & Kočí M. (eds) (2001): Katalog biotopů České republiky [Habitat catalogue of the Czech Republic]. – AOPK, Praha.

Clayton W. D., Harman K. T. & Williamson H. (2006 onwards): GrassBase: the online world grass flora. – URL: http://www.kew.org/data/grasses-db.html.

Conert H. J. (1981): Über das Rohrartige Pfeifengras, *Molinia arundinacea* Schrank. – Ber. Bayer. Bot. Ges. 52: 5–14.

Conert H. J. (1992): *Molinia* Schrank. – In: Hegi G. (ed.), Illustrierte Flora von Mitteleuropa, 1/3: 133–140, Paul Parey Verlag, Berlin & Hamburg.

Conger A. D. & Fairchild L. M. (1953): A quick-freeze method for making smear slides permanent. – Stain Tech. 28: 281–283.

Dančák M. (2002): Taxonomický okruh *Molinia caerulea* ve střední Evropě [Taxonomical group of *Molinia caerulea* in Central Europe]. – Zpr. Čes. Bot. Společ. 37: 35–41.

DeWet J. M. J. (1986): Hybridization and polyploidy in the *Poaceae*. – In: Soderstrom T., Hilu K. W., Campbell C. S. & Barkworth M. E. (eds), Grass systematics and evolution, p. 188–194, Smithsonian Institution Press, Washington.

Dobeš C. & Vitek E. (2000): Documented chromosome number checklist of Austrian vascular plants. – Verlag des Naturhistorischen Museums, Wien.

Doležel J., Greilhuber J. & Suda J. (2007): Estimation of nuclear DNA content in plants using flow cytometry. – Nature Protoc. 2: 2233–2244.

Doležel J., Lucretti S., Meister A., Lysák M., Nardi L. & Obermayer R. (1998): Plant genome size estimation by flow cytometry: inter-laboratory comparison. – Ann. Bot. 82 (Suppl. A): 17–26.

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

Dunwell J. M. (2010): Haploids in flowering plants: origins and exploitation. – Plant Biotech. J. 8: 377–424.

Frey L. (1973): Karyological differentiation in the genus *Molinia* Schrank in Poland. – Fragm. Flor. Geobot. 19: 389–396.

Frey L. (1975a): Taxonomical studies on the genus *Molinia* Schrank in Poland. – Fragm. Flor. Geobot. 21: 21–50.

Frey L. (1975b): *Molinia caerulea* (L.) Moench ssp. *hispanica*, a new subspecies. – Fragm. Flor. Geobot. 21: 463–465.

Frey L. (1976): Present distribution of *Molinia arundinacea* Schrank in Europe. – Fragm. Flor. Geobot. 22: 275–279.

Galushko A. N. (1978): Flora Severnogo Kavkaza 1 [Flora of the North Caucasus 1]. – Izdatelstvo Rostovskogo Universiteta, Rostov.

Gaut B. S. (2002): Evolutionary dynamics of grass genomes. – New Phytol. 154: 15–28.

Grossheim A. A. (1949): Opredelitel rastenij Kavkaza [Key to the flora of Caucasus]. – Gosudarstveno Izdatelstvo Sovetskaja nauka, Moskva.

Guinochet M. & Lemée G. (1950): Contribution à la connaissance des races biologiques de *Molinia coerulea* (L.) Moench. – Rev. Gén. Bot. 57: 565–593.

Hájek M., Hájková P. & Apostolova I. (2005): Notes on the Bulgarian wetland flora, including new national and regional records. – Phytol. Balcan. 11: 173–184.

Havlová M. (2006): Syntaxonomical revision of the *Molinion* meadows in the Czech Republic. – Preslia 78: 87–101.

Hewitt G. M. (1988): Hybrid zones: natural laboratories for evolutionary studies. – Trends Ecol. Evol. 3: 158–167.

Hintze J. (2001): NCSS 2001: Number Cruncher Statistical System. – NCSS, Kaysville.

Hitchcock A. S. & Chase A. (1951): Manual of the grasses of United States. Ed. 2. – Misc. Publ., Washington.

Hultén E. & Fries M. (1986): Atlas of North European vascular plants: north of the Tropic of Cancer I–III. – Koeltz Scientific Books, Königstein.

Keeler K. J. (1998): Population biology of intraspecific polyploidy in grasses. – In: Cheplick G. (ed.), Population biology of biology of grasses, p. 183–207, Cambridge University Press, Cambridge.

Király G. (ed.) (2009): Új magyar füvészkönyv. Magyarország hajtásos növényei. Határozókulcsok [New Hungarian herbal. The vascular plants of Hungary. Identification key]. – Aggteleki Nemzeti Park Igazgatóság, Jósvafö.

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. – Ann. Bot. 103: 963–974.

Kubát K., Hrouda L., Chrtek J. jun., Kaplan Z., Kirschner J. & Štěpánek J. (eds) (2002): Klíč ke květeně České republiky [Key to the flora of the Czech republic]. – Academia, Praha.

Landolt E. (1977): The importance of closely related taxa for the delimitation of phytosociological units. – Vegetatio 34: 179–189.

Legendre P. & Legendre L. (1998): Numerical ecology. – Elsevier Science, Amsterdam.

Leitch I. J., Beaulieu J. M., Chase M. W., Leitch A. R. & Fay M. F. (2010): Genome size dynamics and evolution in monocots. – Journal of Botany 2010: article ID 862516.

Leitch I. J. & Bennett M. D. (2004): Genome downsizing in polyploid plants. – Biol. J. Linn. Soc. 82: 651–663.

Letz R., Uhríková A. & Májovský J. (1999): Chromosome numbers of several interesting taxa of the flora of Slovakia. – Biológia 54: 43–49.

Levin D. A. (1975): Minority cytotypes exclusion in local plant populations. – Taxon 24: 35–43.

Lewis W. H. (1980): Polyploidy in species populations. – In: Lewis W. H. (ed.), Polyploidy: biological relevance, p. 103–144. Plenum, New York.

Lillie R. D. (1951): Simplification of the manufacture of Schiff reagent for use in histochemical procedures. – Stain Tech. 25: 163–165.

Lumaret R., Guillerm J. L., Delay J., Ait Lhaj Loutfi A., Izco J. & Jay M. (1987): Polyploidy and habitat differentiation in *Dactylis glomerata* L. from Galicia (Spain). – Oecologia 73: 436–446.

Lysák M. & Doležel J. (1998): Estimation of nuclear DNA content in *Sesleria* (*Poaceae*). – Caryologia 52: 123–132.

Mahelka V. & Kopecký D. (2010): Gene capture from across the grass family in the allohexaploid *Elymus repens* (L.) Gould (*Poaceae, Triticeae*) as evidenced by ITS, GBSSI, and molecular cytogenetics. – Mol. Biol. Evol. 27: 1370–1390.

Maire R. (1955): Flore de l'Afrique du Nord, Vol. 3. – Paul Lechevalier, Paris.

Marhold K. & Hindák F. (eds) (1998): Zoznam nižších a vyšších rastlín Slovenska [Checklist of non-vascular and vascular plants of Slovakia]. – Veda, Bratislava.

Marhold K., Kudoh H., Pak J. H., Watanabe K., Španiel S. & Lihová J. (2010): Cytotype diversity and genome size variation in eastern Asian polyploid *Cardamine* (*Brassicaceae*) species. – Ann. Bot. 105: 249–264.

Marrs R. H., Phillips J. D. P., Todd P. A., Ghorbani J. & le Duc M. G. (2004): Control of *Molinia caerulea* on upland moors. – J. App. Ecol. 41: 398–411.

Mičieta K. (1986): Karyological study of the Slovak flora XI. – Acta Fac. Rer. Nat. Univ. Comen. Ser. Bot. 33: 51–53.

Milkovits I. & Borhidi A. (1986): Studies of *Molinia caerulea* complexes in Hungary. – Acta Univ. Ups., Symb. Bot. Ups. 27: 139–145.

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 general pattern in Europe. – Ann. Bot. 101: 59–71.

Norrmann G. A. & Keeler K. H. (1997): Evolutionary implications of meiotic chromosome behavior, reproductive biology and hybridization in 6x and 9x cytotypes of *Andropogon gerardii* (*Poaceae*). – Am. J. Bot. 84: 201–207.

Parisod C., Holderegger R. & Brochmann C. (2010): Evolutionary consequences of autopolyploidy. – New Phytol. 186: 5–17.

Pečinka A., Suchánková P., Lysák M. A., Trávníček B. & Doležel J. (2006): Nuclear DNA content variation among central European *Koeleria* taxa. – Ann. Bot. 98: 117–122.

Perný M., Kolarčík V., Majeský L. & Mártonfi P. (2008): Cytogeography of the *Phleum pratense* group (*Poaceae*) in the Carpathians and Pannonia. – Bot. J. Linn. Soc. 157: 475–485.

Pobedimova E. (1949): Species nova generis *Molinia* Schrank ex Ucraina. – Bot. Mater. Gerb. Bot. Inst. Akad. Nauk SSSR 11: 34–37.

Rohweder H. (1937): Versuch zur Erfassung der mendenmässigen Bedeckung des Darss und Zingst mit Polyploiden Pflanzen. – Planta 27: 501–549.

Rothmaler W., Jäger W. & Werner K. (eds) (2005): Exkursionsflora von Deutschland. Band 4. Gefässpflanzen: Kritischer Band. – Spektrum Akademischer Verlag, München.

Rowley G. D. (2007): Cytotypes: a case for infraspecific names. – Taxon 56: 983.
Šafářová L. & Duchoslav M. (2010): Cytotype distribution in mixed populations of polyploid *Allium oleraceum* measured at a microgeographic scale. – Preslia 82: 107–126.
Šafářová L., Duchoslav M., Jandová M. & Krahulec F. (2011): *Allium oleraceum* in Slovakia: cytotype distribution and ecology. – Preslia 54: 513–527.
Salim K. A., Gordon D. B., Shaw S. & Smith C. A. (1995): Variation in *Molinia caerulea* (L.) Moench, the Purple Moor Grass, in relation to edaphic environments. – Ann. Bot. 75: 481–489.
Sneath P. A. H. & Sokal R. R. (1973): Numerical taxonomy. Principles and practice of numerical classification. – W. H. Freeman & Co., San Francisco.
Soltis D. E., Soltis P. S., Schemske D. W., Hancock J. F., Thompson J. N., Husband B. C. & Judd W. S. (2007): Autopolyploidy in angiosperms: have we grossly underestimated the number of species? – Taxon 56: 13–30.
Somlyay L., Pintér I. & Csontos P. (2006): Taxonomic studies of the *Muscari botryoides* complex in Hungary. – Folia Geobot. 41: 213–228.
Sterk A. A. & ter Laak H. J. (1972): Over de variabiliteit van *Molinia coerulea* (L.) Moench in Nederland [On the variability of *Molinia coerulea* (L.) Moench in Netherland]. – Gorteria 6: 95–103.
Suda J., Krahulcová A., Trávníček P. & Krahulec F. (2006): Ploidy level vs. DNA ploidy level: an appeal for consistent terminology. – Taxon 55: 447–450.
Taylor K., Rowland P. & Jones H. E. (2001): *Molinia caerulea* (L.) Moench. – J. Ecol. 89: 126–144.
ter Braak C. J. F. & Šmilauer P. (2002): CANOCO reference manual and CanoDraw for Windows User's guide: software for canonical community ordination (version 4.5). – Microcomputer Power, Ithaca.
Tischler G. (1934): Die Bedeutung der Polyploidie für die Verbreitung der Angiospermen. – Bot. Jahb. 67: 1–36.
Tischler G. (1950): Die Chromozomenzahlen der Gefässpflanzen Mitteleuropas. – Uitgeverij W. Junk, Haag.
Trávníček B., Duchoslav M., Šarhanová P. & Šafářová L. (2009): Squills (*Scilla* s. lat., *Hyacinthaceae*) in the flora of the Czech Republic, with taxonomical notes on Central-European squill populations. – Acta Mus. Moraviae, Sci. Biol. 94: 157–205.
Trávníček P., Kubátová B., Čurn V., Rauchová J., Krajníková E., Jersáková J. & Suda J. (2011): Remarkable coexistence of multiple cytotypes of the *Gymnadenia conopsea* aggregate (the fragrant orchid): evidence from flow cytometry. – Ann. Bot. 107: 77–87.
Trist P. J. O. & Sell P. D. (1988): Two subspecies of *Molinia caerulea* (L.) Moench in the British Isles. – Watsonia 17: 153–157.
Tsvelev N. N. (1976): Zlaki SSSR [*Poaceae* of the USSR]. – Izdatelstvo Nauka, Leningrad.
Tutin T. G. (1975): *Molinia* in SW Spain. – Lagascalia 5: 73–75.
Watson. L. & Dallwitz M. J. (1992): The Grass Genera of the World. – CAB International, Wallingford.
Zar J. H. (1996): Biostatistical analysis. Ed. 4. – Prentice Hall, Upper Saddle River.

Received 15 November 2011
Revision received 6 February 2012
Accepted 23 February 2012