Spatio-ecological segregation of diploid and tetraploid cytotypes of *Galium valdepilosum* in central Europe

Ekogeografická diferenciace cytotypů *Galium valdepilosum* ve střední Evropě

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The *Galium pusillum* agg. (*Rubiaceae*), with four species native to the Czech Republic, is a taxonomically challenging complex. Of these, *G. valdepilosum* is particularly interesting because this relict species shows both ploidy (the incidence of diploid and tetraploid cytotypes) and habitat differentiation (occurrence on different soil types, including serpentines). With the aid of DNA flow cytometry, analysis of vegetation samples and a hydroponic cultivation experiment we addressed the cytogeographic pattern, ecological preferences of different cytotypes both across the entire range of distribution and in the contact zone and the plant's response to serpentine edaphic stress. Ploidy distribution in *G. valdepilosum* is parapatric, with a narrow contact zone in southern Moravia. Neither triploids nor mixed 2x-4x populations were found, which together with the restriction of the species to isolated relict habitats, suggest the static character of the contact zone. In general, tetraploids occupied a wider range of habitats and colonized larger geographic areas. Diploids typically occurred in open low-competitive oak-pine forests on acidic soils while their tetraploid counterparts were also able to survive in open basiphilous grasslands with a comparatively higher competitive pressure. Serpentines did not play an important role in ecological sorting of the cytotypes. Cultivation experiments showed that *G. valdepilosum* is likely to be constitutively tolerant to serpentine chemical stress. Relative genome size and ecological data indicate that the serpentine populations from western Bohemia, traditionally referred to as *G. sudeticum*, differ from the type subalpine populations from the Krkonoše Mts and suggest their merger with *G. valdepilosum*.

K e y w o r d s: central Europe, contact zone, cytogeography, ecological sorting, flow cytometry, *Galium sudeticum*, *Galium valdepilosum*, ploidy distribution, polyploidy, serpentine

Introduction

Polyploidy, the possession of three or more complete chromosome sets per nucleus, is a prominent and recurring transition in the evolution of eukaryotic organisms, including land plants (Otto & Whitton 2000). Although polyploidization is often associated with species diversification due to the barriers to gene flow that results from chromosome multiplication, ploidy variation is commonly observed also within taxonomic species (Husband et al. 2013). Many studies of heteroploid species note that different cytotypes have distinct distributions (Suda et al. 2007, Šafářová et al. 2011, Dančák et al. 2012, Krejčíková et al. 2013a). The pattern of ploidy distribution is shaped by the interplay between adaptive and non-adaptive ecological processes (Husband et al. 2013). The adaptive scenario assumes that polyploidy contributes to the acquisition of new genetic, morphological, physiological and/or ecological characteristics (reviewed in Levin 2002) that may modify competitive ability, fitness or ecological tolerance of polyploids compared to their diploid progenitors and ultimately lead to new responses to environmental conditions. As a consequence, different cytotypes can sort along abiotic and/or biotic environmental gradients, both contemporary and past (Husband et al. 2013). Although ecological sorting is widely acknowledged as the key mechanism driving geographic segregation of different cytotypes, several non-adaptive (i.e. environmentally independent) processes can also play a role in shaping ploidy distribution. Among others, spatial segregation of cytotypes can be governed through frequency-dependent mating success, in polyploid systems traditionally referred to as the "minority cytotype disadvantage" (Levin 1975). Present-day ploidy distribution can also reflect the dynamics of genome duplication (e.g. the frequency of unreduced gamete formation) or different dispersal abilities of the cytotypes; for example, widespread cytotypes may have been superior colonizers of habitats that appeared after the retreat of ice shields or due to human activities such as deforestation and agricultural practices (Stebbins 1985, Sonnleitner et al. 2010). However, adaptive and non-adaptive scenarios could not be distinguished on the basis of distributional patterns but the cytotypes should be subjected to a detailed evaluation of their ecological preferences and important biological traits (e.g. vegetation analyses, crossing and transplant experiments, cultivation under manipulated environmental characteristics).

Spatial relationships between cytotypes within species can be categorized as sympatric, parapatric or allopatric, depending on whether they are geographically intermixed, adjacent or disjunct, respectively. When polyploids first arise, they by necessity occur in sympatry with their diploid/lower-polyploid progenitors. Subsequent cytotype expansion or retreat will result in parapatric or allopatric distributions. Contact zones can be quite narrow, eventually comprising only a few populations, as reported in *Chamerion angustifolium* (Husband & Schemske 1998) or *Ranunculus adoneus* (Baack 2004). Cytotype mixtures extending over large areas seem to be less frequent and occur for example in *Galax urceolata* (Burton & Husband 1999), *Solidago altissima* (Halverson et al. 2008) and *Allium oleraceum* (Duchoslav et al. 2010). However, the immediate contact of different cytotypes (i.e. the incidence of mixed-ploidy populations) is often limited even in species with geographically extensive and diffuse contact zones, illustrative examples being *Knautia arvensis* (Kolář et al. 2009), *Vicia cracca* (Trávníček et al. 2010), *Aster amellus* (Castro et al. 2012) or *Odontites vernus* (Koutecký et al. 2012). While most contact zones are formed by two ploidy levels, the last years have seen much more complex population structures, with up to five different co-existing cytotypes (Sonnleitner et al. 2010, Trávníček et al. 2011b, 2012). Investigations into the adaptive significance of ploidy shift first require assessment of potential relationship between intraspecific ploidy variation and environmental factors of occupied sites. Detected associations of ploidy levels with both abiotic (Duchoslav et al. 2010, Sonnleitner et al. 2010, Manzaneda et al. 2012) and biotic (Krejčíková et al. 2013b) parameters provide important clues for explaining the observed cytogeographic patterns.

Published studies addressing cytogeographic patterns and underlying mechanisms in heteroploid species in central Europe usually deal with species of semi-ruderal habitats (*Allium oleraceum*: Duchoslav et al. 2010, Šafářová & Duchoslav 2010, Šafářová et al.

2011; *Knautia arvensis*: Kolář et al. 2009; *Pilosella officinarum*: Mráz et al. 2008; *Spergularia echinosperma*: Kúr et al. 2012; *Vicia cracca*: Trávníček et al. 2010) or non-relict natural sites (*Aster amellus*: Mandáková & Münzbergová 2006, Castro et al. 2012; *Molinia caerulea* agg.: Dančák et al. 2012), whereas species restricted to isolated relict sites, i.e. low-competition habitats with species assemblages usually persisting from the early Holocene, have been largely neglected (but see Suda & Lysák 2001, Suda et al. 2004). Due to their supposed closer association with local environmental conditions, insular-like distribution and long periods of isolation of individual populations, relict species with multiple cytotypes provide novel insights into the structure and dynamics of contact zones between different cytotypes.

A suitable candidate for such an investigation is *Galium valdepilosum* H. Braun (*Rubiaceae*), a diploid-tetraploid member of the *G. pusillum* aggregate (Ehrendorfer 1960, Ehrendorfer et al. 1976). This group, which in central European literature is sometimes treated in a narrower sense as *G. pumilum* aggregate, encompasses four native species in the Czech Republic (Krahulcová & Štěpánková 1998, Štěpánková 2000, Danihelka et al. 2012): (i) widespread octoploid (2n = 8x = 88) *G. pumilum* Murray, (ii) tetraploid (2n = 4x = 44) *G. austriacum* Jacq. restricted to limestone outcrops in Pavlovské vrchy in southern Moravia, (iii) endemic tetraploid *G. sudeticum* Tausch, which has a very unusual distribution pattern, being reported from basiphilous subalpine areas (glacial cirques) in the Krkonoše Mts (historically also from the Hrubý Jeseník Mts) and from comparatively low-lying serpentine outcrops in the Slavkovský les Mts (western Bohemia), and (iv) ploidy-variable *G. valdepilosum*, which includes diploid (2n = 2x = 22) and tetraploid (2n = 4x = 44) populations inhabiting different relict sites (dry grasslands, open forests) on both serpentine and non-serpentine soils. A previous study of the aggregate using conventional chromosome counts (Krahulcová & Štěpánková 1998) provided a rough picture of ploidy distribution in the Czech Republic and its close surroundings and concluded that ploidy variation is not associated with serpentine vs non-serpentine sites. The origin of the tetraploid cytotype (auto- vs allopolyploid) is unclear. Although overall morphological similarities (but with certain quantitative differentiating traits; Štěpánková 2000) and close monoploid genome sizes of both cytotypes (Kolář et al. 2013) would favour autopolyploidy, reticulate patterns of morphological characters, high plasticity and great taxonomic complexity of the whole *G. pusillum* group indicate the need for a multi-species molecular investigation.

The present study builds on our previous research on the *G. pusillum* agg. in deglaciated areas of northern Europe (Kolář et al. 2013) and the karyological investigations in eastern central Europe of Krahulcová & Štěpánková (1998). Using DNA flow cytometry, analysis of habitat preferences and a hydroponic cultivation experiment we addressed the following questions: (i) What are the ranges of diploid and tetraploid *G. valdepilosum* and where is the contact zone between these cytotypes located? (ii) Do both cytotypes co-occur in ploidy-mixed populations? (iii) Do diploid and tetraploid cytotypes differ in their habitat preferences both across the entire range of distribution and in the zone of ploidy contact? (iv) Are there any ploidy-specific differences in growth response of *G. valdepilosum* to serpentine chemical stress? (v) What is the variation in nuclear DNA content within the tetraploid *G. valdepilosum*? Do taxonomically uncertain serpentine populations in western Bohemia, traditionally referred to as *G. sudeticum*, share genome size values with plants of *G. sudeticum* from subalpine type populations or with *G. valdepilosum*?

Materials and methods

Field sampling

Plant material was collected from 2009 to 2013 in Austria (12 sites), the Czech Republic (70 sites), Germany (13 sites) and Poland (nine sites). We covered the entire range of *G. valdepilosum* except for populations in central Denmark that are referred to as an endemic subsp. *slesvicense* (Sterner ex Hylander) Ehrendorfer. In addition to the nominate subspecies of *G. valdepilosum* (94 populations), we also included for comparative purposes four serpentine populations from western Bohemia [traditionally determined as *G. sudeticum*, but showing some morphological differences from typical subalpine populations (Štěpánková 2000), which are ecologically close to *G. valdepilosum*], five high-altitude populations of *G. sudeticum* from the Krkonoše Mts and one taxonomically uncertain population from limestone outcrops in the Králický Sněžník Mts (further referred to as *G. pusillum* agg.; see Appendix 1 for details of individual localities). Whenever possible with respect to population size, shoots from at least 10 plants per population were collected and stored in plastic bags in cold conditions until used in the FCM analysis. To avoid collecting the same genet, the distance between the individuals sampled was at least 0.5 m. Herbarium vouchers are deposited in the Herbarium of Charles University in Prague (PRC).

Floristic composition and selected environmental conditions recorded at 52 localities were characterized using vegetation samples (phytosociological relevés), including those of 46 localities of *G. valdepilosum* (covering the entire range of distribution: 7 and 15 diploid-inhabited sites in Lower Austria and Moravia, respectively, and 10, 1, 2, 6, and 5 tetraploid-inhabited sites in Bavaria, Lower Austria, Bohemia, Moravia and Poland, respectively), two serpentine localities of putative *G. sudeticum*, three subalpine localities of *G. sudeticum* and one locality of a taxonomically uncertain member of the *G. pusillum* agg. One vegetation sample per locality was usually recorded, exceptions being three ecologically diverse sites where two samples from distinct vegetation units were recorded; each sample covered an area of 3 × 3 m in areas with an abundance of *Galium* plants (Electronic Appendix 3). In each plot, relative cover of all vascular plant species was quantified using a modified nine-point Braun-Blanquet scale (Braun-Blanquet 1964) and the following environmental parameters were recorded: total vegetation cover, cover of each vegetation layer, slope inclination and orientation, and proportion of bare rock. At 49 localities (Electronic Appendix 5), mixed rhizosphere soil samples were collected at five microsites within the area of the vegetation sample; pH and concentrations of selected elements (C, N, K, Ca, and Mg) were determined in the Analytical Laboratory of the Institute of Botany, Průhonice, CZ (see Kolář et al. 2013 for methodology details).

Flow cytometry

Relative fluorescence intensities of isolated nuclei were estimated using DNA flow cytometry (FCM) following the simplified two-step protocol with DAPI staining and *Bellis perennis* as internal reference standard as detailed in Kolář et al. (2013). In six selected populations (Appendix 1), one individual per population was subjected to more stringent analysis of relative DNA content (following Kolář et al. 2013). For comparative purposes DNA content values of another 17 individuals (from 17 populations) were taken from Kolář et al. (2013). *Galium* accessions with distinct fluorescence intensities were analysed simultaneously in order to confirm between-plant differences observed in runs with an internal standard. Chromosome-counted individuals (Kolář et al. 2013) were used as a reference for the interpretation of FCM histograms.

Hydroponic cultivation

Eight populations were subjected to a hydroponic cultivation experiment aimed at assessing the effects of the major chemical factors associated with serpentine conditions (i.e. low Ca/Mg ratio and high Ni concentrations; Brady et al. 2005, Kazakou et al. 2008) on seedling performance. Due to the acidic pH of *G. valdepilosum*-inhabited serpentine stands (mean pH of 5.5) their responses were compared with those of four acidophilous non-serpentine populations. Two diploid and two tetraploid populations were represented in each group (Fig. 1; see Appendix 1 for details). Mature achenes collected along transects at the original sites were germinated on moist filter paper over a period of three weeks. Vital, undamaged seedlings were then carefully fixed to a floating plastic disc (14 cm in diameter) so that there was an equal distance between each of the experimental plants. There were eight plants (one per population) on each disc, which was placed in a 1-L light-impermeable container filled with a standard nutrient solution as described in Huss-Danell (1978), with a slight modification: $Co(NO_3)_2$ was used instead of $CoSO_4$ as a cobalt source. The seedlings were grown in this nutrient solution for 11 days prior to the start of the experiment. They were then placed into experimental solutions with manipulated concentrations of Mg^{2+} and Ni^{2+} for the next 22 days ($MgSO_4$ and $NiSO_4$ were used as sources of Mg and Ni, respectively; the pH was approx. 7 during the whole experiment). The solutions were replaced every three days with freshly prepared solution and the plants cultivated in a controlled-environment growth cabinet at the Faculty of Science, University of South Bohemia, Czech Republic (for details see Kolář et al. 2014).

To test the individual and combined effects of Ni and Mg on *G. valdepilosum* populations differing in soil type (factor 'substrate at origin') and ploidy level (factor 'ploidy'), we used a mixed-effect full-factorial experimental design. Four experimental treatments were applied: the control (standard nutrient solution), high Ni^{2+}, high Mg^{2+}, and high Ni^{2+} and Mg^{2+}. Based on a preliminary cultivation experiment, the concentrations of Ni^{2+} were set to 0 μM (control) and 30 μM, while the concentrations of Mg^{2+} were set to 0.55 mM (control) and 5.5 mM (i.e. Ca/Mg ratio of 2 and 0.2, respectively). Each experimental unit (= plastic container filled with one of the four experimental solutions) consisted of eight seedlings, one seedling per population. There were eight replicates of each treatment, resulting in 32 experimental units and 256 seedlings. Total root length was used as a proxy of the plant's response to different experimental treatments; the values were obtained from measurements recorded at the beginning and the end of the experiment (following the method described in Kolář et al. 2014).

Statistical analyses

Differences in relative DNA contents were tested in R version 2.15.2 using one-way ANOVA with post-hoc comparisons (Tukey HSD test).

Habitat preferences were based on the species composition of vegetation samples and recorded biotic and abiotic characteristics of the sites. Ellenberg indicator values (EIV),

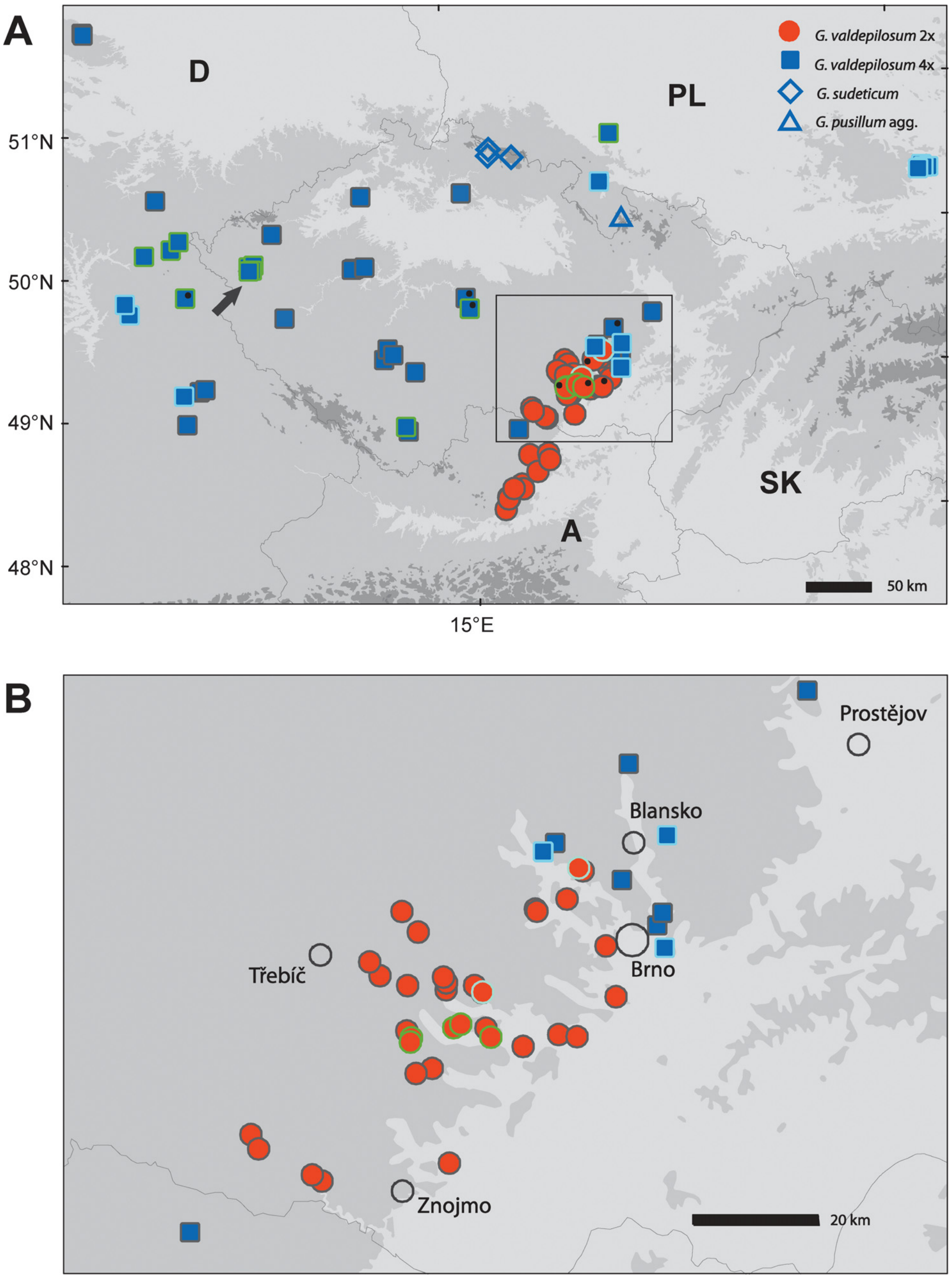


Fig. 1. – Geographic location of populations of *Galium valdepilosum* across the entire study area (A) and in the contact zone in southwestern Moravia (B). Red and blue denote diploids and tetraploids, respectively. Black, light blue and green borders indicate acid, basic and serpentine soils, respectively. The arrow indicates the location of taxonomically reclassified serpentine populations from western Bohemia traditionally referred to as *G. sudeticum*. Populations marked by a black dot were cultivated hydroponically.

which provide estimates of environmental characteristics inferred from species composition data (Ellenberg 1992), were calculated in JUICE 7.0 (Tichý 2002) based on presence/absence data of herbaceous species of plants. Separate analyses were done for (i) all available vegetation samples of *G. valdepilosum* covering the entire distribution range of the species, and (ii) a subset of vegetation samples from the contact zone between di- and tetraploid cytotypes (i.e. within a radius of 50 km around the town of Brno where immediate contact of both ploidies was recorded). Both unconstrained (using the detrended correspondence analysis, DCA) and constrained (using the canonical correspondence analysis, CCA, with forward selection of environmental variables) ordinations in Canoco for Windows, ver. 4.5 (Lepš & Šmilauer 2003) were used to describe the overall vegetation patterns of the *G. valdepilosum* sites studied. Differences in vegetation composition among vegetation samples recorded at sites of diploid vs tetraploid *G. valdepilosum* were tested in a separate CCA with 'ploidy level' as the only predictor variable. In order to reveal associations of di- vs tetraploid *G. valdepilosum* plants with other plant species, ten co-occurring species with the strongest marginal effects were analysed using the Monte Carlo permutation test (999 permutations, with Bonferroni correction for multiple tests) during the forward-selection linear discriminant analysis in which species abundances (log-transformed) were treated as predictor variables and *Galium* ploidy level as a response (see Lepš & Šmilauer 2003 for details). The biotic characteristics inferred from species composition data (i.e. EIV, species diversity, layer cover) were omitted as predictors in constrained analyses.

Differences in root growth (log-transformed) of *G. valdepilosum* seedlings in response to high concentrations of Mg^{2+} and Ni^{2+} were tested using a hierarchical ANOVA. The effects of substrate at origin, ploidy, Mg and Ni treatments, and all their interactions were tested using a linear model where the experimental container (nested in Mg and Ni treatment interaction) and population of origin (nested in substrate at origin and ploidy interaction) were treated as random and fixed factors, respectively. For comparative purposes, we also performed an analysis aimed at identification of the overall differences in serpentine tolerance among *G. valdepilosum* populations differing in ploidy / soil conditions. A similar ANOVA model was used for this purpose, but with the population of origin (again nested in substrate at origin and ploidy interaction) treated as a factor with random effect. The ANOVA analyses were calculated in Statistica 8 (StatSoft 2007). Note that Statistica uses Satterthwaite's method of denominator synthesis, which finds linear combinations of sources of random variation that serve as appropriate error terms for testing the significance of the respective effect of interest; for this reason the synthesized error mean squares and synthesized error degrees of freedom are also presented.

Results

Cytogeography and variation in relative nuclear DNA content

The FCM analysis of 874 plant samples revealed two different DNA ploidy levels: diploid (338 individuals from 46 localities) and tetraploid (536 individuals from 58 localities). All diploids corresponded to *G. valdepilosum* and were restricted to southern Moravia and Lower Austria. The zone of contact between the plants of the two ploidy levels is located near the town of Brno, where tetraploids in the north-east give way to diploids in the south-west

(Fig. 1). Only the tetraploid cytotype of *G. valdepilosum* was recorded in Bohemia, Germany and Poland. One tetraploid population occurred in northern Austria in an area otherwise dominated by diploids. Subalpine populations of *G. sudeticum* in the Krkonoše Mts were uniformly tetraploid as also were serpentine populations in western Bohemia and a taxonomically-uncertain population on the Polish side of the Králický Sněžník Mts.

While fluorescence intensities of all diploid samples were uniform, there was significant variation in the relative amounts of nuclear DNA ($F_{3,25} = 23.15$, $P < 0.001$) at the tetraploid level. Two groups were identified. The first group encompassed all populations determined as *G. valdepilosum*, four serpentine populations in western Bohemia traditionally referred to as *G. sudeticum* and one calcicolous mountain population in the Králický Sněžník Mts (Fig. 2). The second group with higher fluorescence intensities (mean difference 4.3%) consisted of subalpine populations of *G. sudeticum* in the Krkonoše Mts. Simultaneous FCM analysis (Fig. 3) confirmed the differences in the relative DNA contents of individuals of the putative *G. sudeticum* that originated from the two disjunct geographic areas (western Bohemia and the Krkonoše Mts).

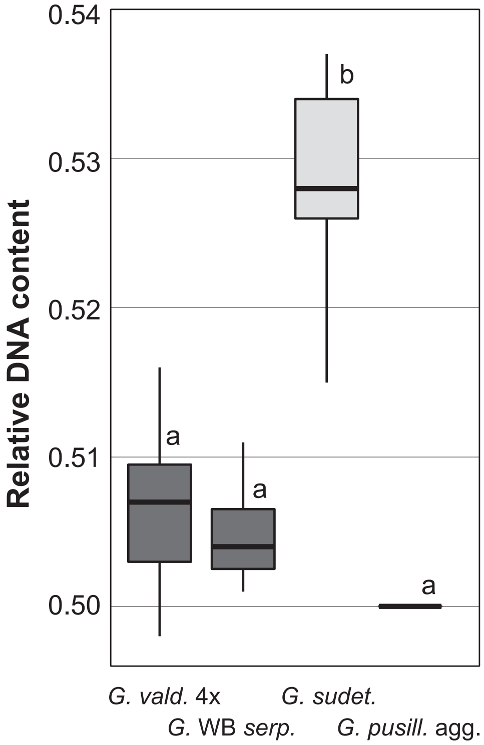


Fig. 2. – Variation in relative nuclear DNA content of *Galium valdepilosum* (23 individuals from 23 populations across the entire range of distribution), *G. sudeticum* from the Krkonoše Mts (four populations), plants inhabiting serpentine sites in western Bohemia traditionally referred to as *G. sudeticum* (four populations) and one taxonomically uncertain *G. pusillum* agg. population from the Králický Sněžník Mts. Fluorescence intensity of *Bellis perennis* was set to a unit value. Each plant was measured three times on different days. Letters indicate significantly different groups at $\alpha = 0.05$. The values represented by lines, boxes and whiskers are median, quartiles and range (min-max), respectively.

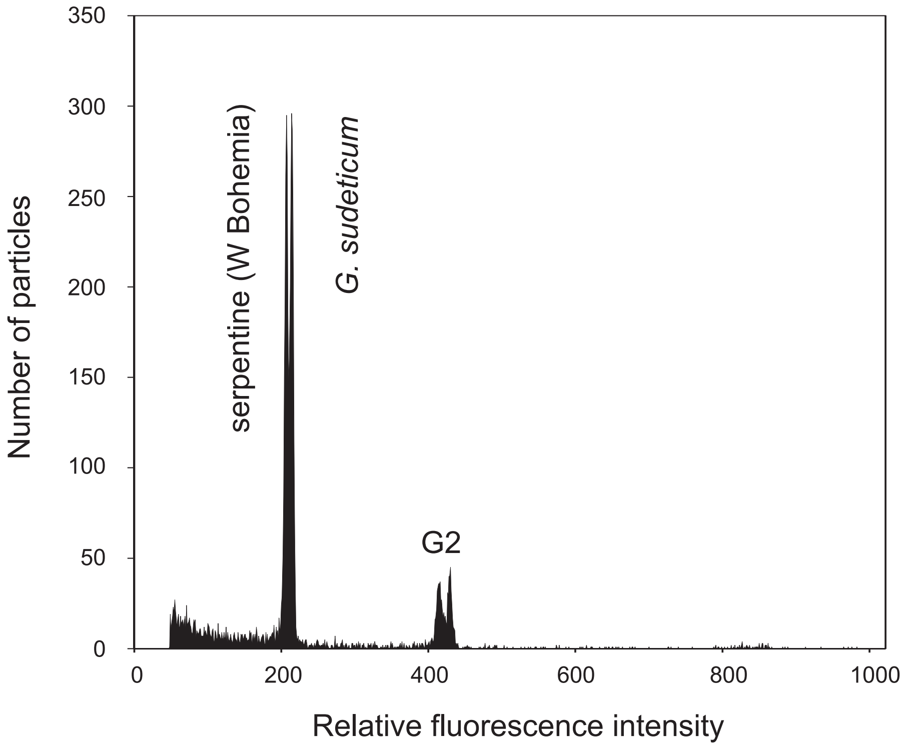


Fig. 3. – Flow cytometric histogram documenting 3.8% divergence in relative nuclear DNA content among simultaneously processed and DAPI-stained accessions of *Galium sudeticum* from the Krkonoše Mts (pop. G172) and plants from serpentine outcrops in western Bohemia traditionally referred to as *G. sudeticum* (pop. G032).

Ecological preferences of different cytotypes

Subalpine populations of *G. sudeticum* in the Krkonoše Mts and the taxonomically uncertain population in the Králický Sněžník Mts are ecologically very distinct from all other populations of *G. valdepilosum* analysed as well as from populations inhabiting serpentine sites in western Bohemia traditionally referred to as *G. sudeticum* (Electronic Appendix 1) and therefore omitted from the following statistical analyses. In contrast, the western Bohemian populations do not ecologically differ from those of *G. valdepilosum* and both groups were therefore merged and included in subsequent analyses.

Floristic composition of sites inhabited by *G. valdepilosum* is primarily shaped by soil pH, concentration of Ca, organic C content and serpentine-specific Ca/Mg ratio (Monte Carlo test, $P = 0.001$). At these sites five other environmental parameters (concentration of Mg, cover of rocks, tree/shrub and moss layers, and altitude) were marginally significant (i.e. $P < 0.05$ yet not passing the significance level defined by Bonferroni correction).

Sites of di- and tetraploid cytotypes significantly differed in floristic composition both across the entire range of their distribution and in the contact zone (Monte Carlo test, both $P = 0.001$). Despite this differentiation, linear discriminant analysis revealed only a few species that were significantly associated with a particular cytotype of *G. valdepilosum. Arrhenatherum elatius*, *Genista pilosa* and *Pimpinella saxifraga* were associated with diploids while juvenile *Rubus idaeus* and *Galium album* with tetraploids (in vegetation samples from the entire range and contact zone, respectively).

Diploids of *G. valdepilosum* mostly occurred in open forests on nutrient-poor acidic or serpentine soils and, in general, had a narrower ecological niche than their tetraploid counterparts (Fig. 4). Tetraploids were ecologically more divergent and occupied two major types of habitats across their entire distribution: (i) acidic or serpentine sites and (ii) base-rich non-forested sites such as relatively species-rich rocky/continental grassland (see also Table 1). Although both di- and tetraploids grow on serpentine soils the environmental conditions where tetraploids grow differ. Ecological segregation of both cytotypes was more pronounced in the zone where they come into contact (Fig. 4). While diploids usually occurred in acidophilous open forests (including serpentine sites), tetraploids preferred lime-rich stands with a dense herbaceous cover.

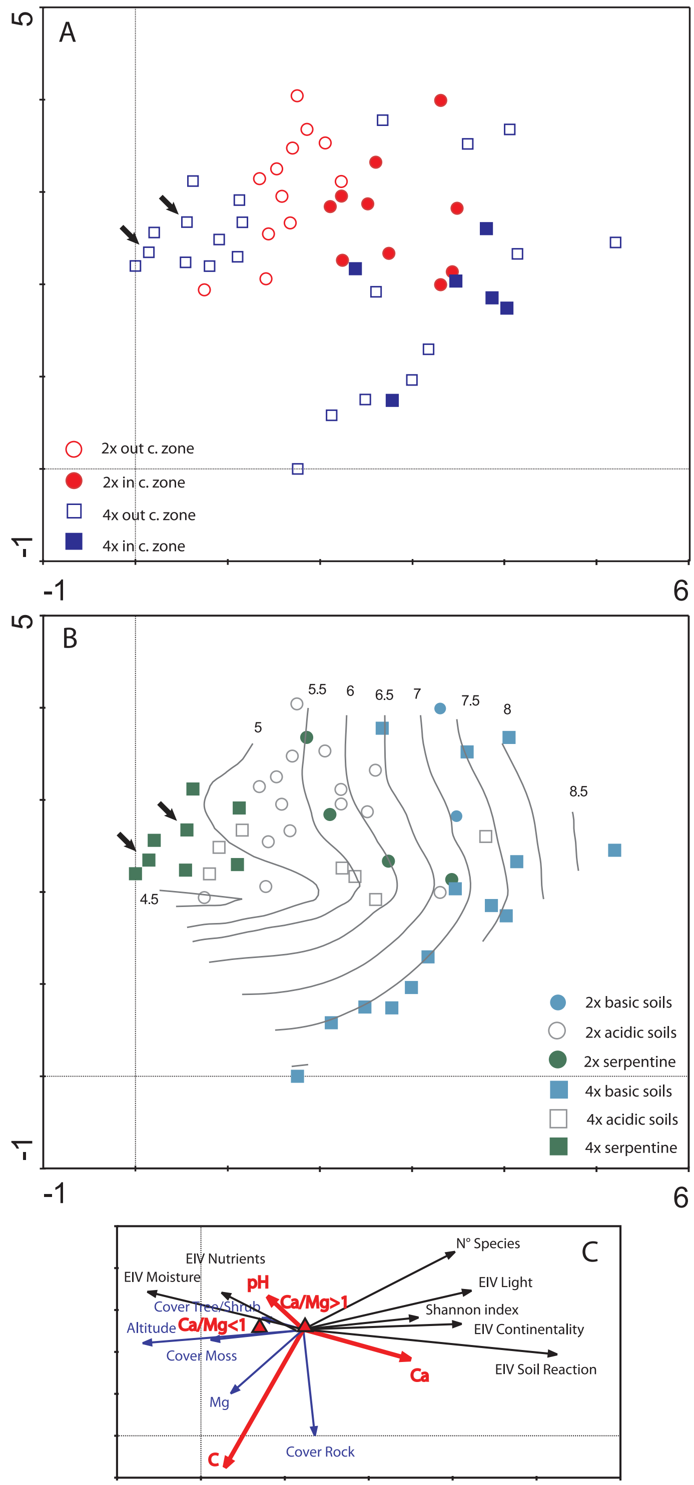
Response to serpentine chemical stress

At high concentrations of Mg the roots of seedlings of *G. valdepilosum* grew significantly less, whereas the effect of high Ni was obvious only in its interaction with Mg (slightly better growth at a high Mg + Ni concentration than at a high concentration of Mg; Table 2). In general, *Galium* plants of serpentine vs non-serpentine origin and of different ploidy levels responded to Mg and Ni stress in a similar way (Table 2). The root growth of the two serpentine tetraploid populations was better than that of both their diploid and non-serpentine counterparts, irrespective of the actual concentrations of Mg and/or Ni in the solution (Fig. 5; see also Electronic Appendix 2 for response of individual populations). However, the effects of ploidy level ($F_{1,207} = 2.34$, $P = 0.20$) and substrate at origin ($F_{1,207} = 6.83$, $P = 0.06$) were not significant in the ANOVA model with population treated as a random-effect factor, which makes generalizing about this difference tenuous.

Discussion

This study increased our understanding of the karyological and ecological differentiation of the *G. pusillum* agg. in central Europe, particularly that of *G. valdepilosum*, which is a declining species restricted to various relict habitats, whose centre of distribution is in the Czech Republic. In addition to providing a detailed picture of the distributions of individuals with different ploidy levels at various spatial scales, the data also provides the first evidence that the taxonomic relationships of some populations may need to be reassessed.

Fig. 4. – Habitat preferences of di- and tetraploid cytotypes of *Galium valdepilosum*. The patterns in floristic composition of 50 vegetation samples are visualized using detrended correspondence analysis (the first and second ordination axes explain 5.4% and 3.8% of the total variation, respectively). (A) Diploid (red) and tetraploid (blue) localities within the contact zone (filled symbols) and beyond (empty symbols). (B) Vegetation samples labelled according to the major soil type (base-rich: blue, acidic: white, and serpentine: green) as determined by geological bedrock, soil pH and Ca/Mg ratio (diploid: circle, tetraploid: square). The contour lines depict pH values modelled by loess smoother from the measured values of individual vegetation samples. (C) Environmental variables significantly (red lines) and marginally significantly (blue lines) influencing floristic composition of *Galium* sites, and variables inferred from species composition data (black lines) passively projected on the plot. Serpentine populations from western Bohemia traditionally referred to as *G. sudeticum* are marked by an arrow. ▶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4x serpentine
C
N° Species
EIV Nutrients
EIV Moisture
pH
Ca/Mg>1
EIV Light
Cover Tree/Shrub
Shannon index
Altitude
Ca/Mg<1
EIV Continentality
Cover Moss
Ca
EIV Soil Reaction
Mg
Cover Rock
C

Table 1. – Ploidy levels, relative amounts of nuclear DNA and habitat preferences of the taxa/populations of *Galium pusillum* agg. investigated. Relative DNA content is given as mean ± SD; fluorescence intensity of *Bellis perennis* (internal reference standard) was set to a unit value. Nomenclature of vegetation units follows Chytrý et al. (2007, 2013).

Taxon/population	No. of populations	Ploidy level	Relative DNA content*	Geological substrate	Associated vegetation
G. valdepilosum H. Braun	46	2x	0.259±0.006	various silicate rocks, serpentine, rarely basic conglomerate and limestone	*Quercion petraeae*, *Quercion roboris*, *Dicrano-Pinion sylvestris*, rarely *Erico carneae-Pinion* (on serpentines)
	48	4x	0.506±0.005	various silicate rocks, serpentine, limestone, rarely chalk (Poland) and vulcanite	*Quercion roboris*, *Quercion petraeae*, *Dicrano-Pinion sylvestris*, rarely *Diantho lumnitzeri-Seslerion*, *Cirsio-Brachypodion pinnati* (in Poland), *Erico carneae-Pinion* (in Bavaria)
G. pusillum agg. from serpentines in western Bohemia traditionally referred to as *G. sudeticum*	4	4x	0.505±0.004	serpentine	*Dicrano-Pinion*
G. sudeticum Tausch	5	4x	0.528±0.008	base-rich substrates in glacial cirques (erlan, carbonate)	*Agrostion alpinae*
G. pusillum agg. from the Králický Sněžník Mts	1	4x	0.500	limestone	cf. *Tilio platyphyli-Acerion*

Table 2. – The effects of different concentrations of Mg and Ni, ploidy level and soil from which the plants originated (serpentine vs non-serpentine) on the total root length of *Galium valdepilosum* plants in hydroponic cultivation. Statistically significant results are in bold: *P < 0.05, ***P < 0.001. Dependent variables were log transformed prior to the analysis.

Factor/Interaction	Effect	Effect df	Synthesized error df	MS	Synthesized error MS	F
Experimental container	random	28	207	0.191	0.108	**1.77***
Population	fixed	4	207	0.635	0.108	**5.89*****
Mg	fixed	1	28	1.303	0.191	**6.82***
Ni	fixed	1	28	0.065	0.191	0.34
Ploidy	fixed	1	207	1.489	0.108	**13.80*****
Substrate at origin	fixed	1	207	4.339	0.108	**40.22*****
Mg × Ni	fixed	1	28	1.199	0.191	**6.27***
Ploidy × Mg	fixed	1	207	0.003	0.108	0.03
Ploidy × Ni	fixed	1	207	0.005	0.108	0.05
Substrate at origin × Mg	fixed	1	207	0.044	0.108	0.40
Substrate at origin × Ni	fixed	1	207	0.039	0.108	0.36
Ploidy × Substrate at origin	fixed	1	207	2.054	0.108	**19.04*****
Ploidy × Mg × Ni	fixed	1	207	0.111	0.108	1.03
Substrate at origin × Mg × Ni	fixed	1	207	0.338	0.108	3.13
Ploidy × Substrate at origin × Mg	fixed	1	207	0.025	0.108	0.23
Ploidy × Substrate at origin × Ni	fixed	1	207	0.022	0.108	0.20
Ploidy × Substrate at origin × Mg × Ni	fixed	1	207	0.014	0.108	0.13
Error		207		0.108		

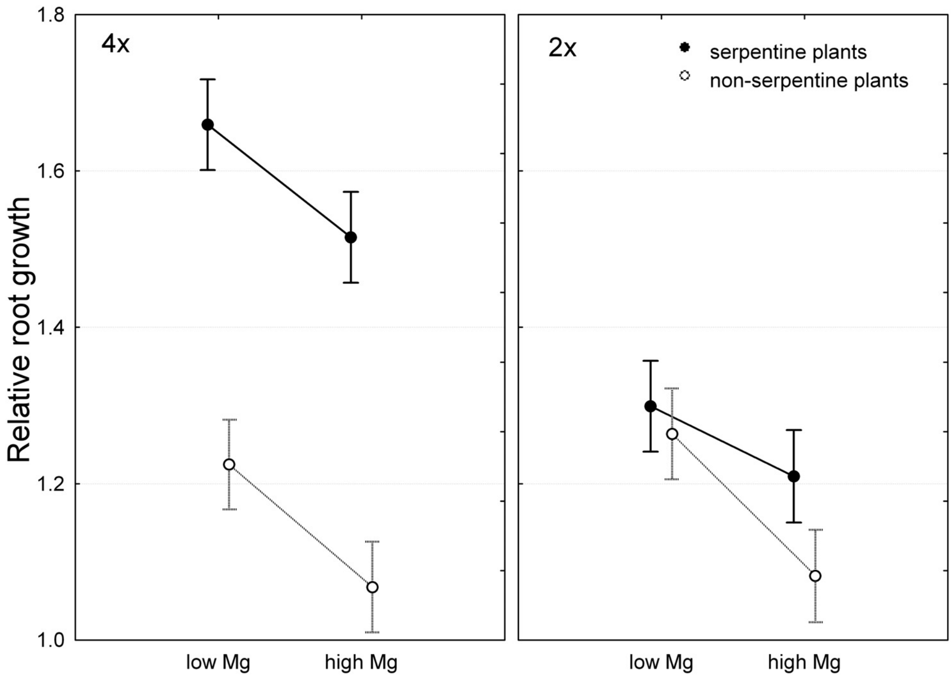

Fig. 5. – Differences recorded in the growth of the root system of diploid and tetraploid seedlings of *Galium valdepilosum* originating from serpentine vs non-serpentine soil when grown in low and high concentrations of Mg. Symbols and vertical bars denote unweighted means and standard errors of the mean, respectively.

Cytogeography of Galium valdepilosum and the underlying mechanisms

The overall cytogeographic pattern inferred from the FCM analysis of nearly 100 populations spread across the entire distribution of *G. valdepilosum* corresponds well with the incidence of different ploidy levels based on the conventional karyological counts of Krendl (1993) and Krahulcová & Štěpánková (1998). This is slightly different from that in the review of Ehrendorfer (1962), partly because he includes chromosomal data of M. Piotrowicz (published in Skalińska et al. 1961), which includes diploid populations from Małopolska upland in southern Poland. In contrast, our thorough investigation of the same geographic area (including searches for all the populations reported by M. Piotrowicz) revealed either only tetraploid individuals or failed to confirm the occurrence of the species. We can only speculate about the reasons for this discrepancy, which include species misidentification, incorrect chromosome counting (other chromosome counts from that area detected only tetraploids; Kucowa & Mądalski 1964) or even extinction of diploid cytotypes in situ (the species seems to be strongly declining particularly at localities with xerothermous grassland; see also Zarzycki & Kaźmierczakowa 2001 and Grulich 2012). The map in Ehrendorfer (1962) also shows a few diploid populations in central Bohemia. However, these records cannot be verified and should be treated with caution because neither exact localities nor references are provided in the original work.

Ploidy distribution in *G. valdepilosum* can best be described as parapatric, i.e. with closely adjacent but not overlapping ranges. Despite intensive sampling in the contact zone (the majority of *Galium* tufts was checked for ploidy in large populations while all individuals were examined in small populations), we did not find any mixed 2x-4x populations or a minority cytotype such as a triploid. This suggests very low rates of neopolyploid formation and/or establishment, leaving very little room for inter-ploidy interactions. Consequently, the contact zone seems to be a non-dynamic system, which contrasts with many other recently investigated intraspecific heteroploid systems in central Europe that frequently comprised cytotype-mixed populations and odd ploidies (e.g. *Allium oleraceum*: Duchoslav et al. 2010; *Gymnadenia conopsea*: Trávníček et al. 2011b; and *Hieracium echioides*: Trávníček et al. 2011a). The static character of the contact zone is further underlined by the overall species' preferences for open relict stands, in which populations of such heliophilous and competitively weak plants are spatially isolated, possibly for many generations (in extreme cases since the spread of closed forests in the middle Holocene; Ložek 1973, Lang 1994). Geographic segregation of different cytotypes is widely considered to be the most important prezygotic reproductive barrier, with many examples described in the literature (see Husband & Sabara 2004, Kron et al. 2007, Šafářová & Duchoslav 2010, Husband et al. 2013).

The analysis of environmental conditions recorded at the localities showed that, despite being restricted to relict habitats, *G. valdepilosum* can grow in a wide range of different soils (including acidic, basic and serpentine soils; Electronic Appendix 5) and different types of vegetation (floristic composition of which is also largely determined by soil parameters). Although we found no evidence for strong inter-ploidy niche divergence (either across the entire range of the species' distribution or in the contact zone), some ecological trends can be discerned. In particular, while diploids typically occurred in open low-competitive oak-pine forests on acidic soils, their tetraploid counterparts were also able to survive in open basiphilous grasslands with comparatively high competitive pressure. In general, tetraploids occupied a wider range of habitats and also colonized larger geographic areas.

Serpentines do not play an important role in inter-ploidy niche segregation and serpentine/non-serpentine differentiation merely reflects colonization history (i.e. diploids occur on serpentines in 2x-dominated areas and vice versa). Serpentine and non-serpentine *G. valdepilosum* populations also do not differ morphologically (Štěpánková 1997). In addition, the results of our cultivation experiment (populations responded in a similar way irrespective of the type of soil they normally grow in) indicate that response to serpentine chemical stress seems to be a constitutive trait common for both serpentine and non-serpentine diploid and tetraploid populations of *G. valdepilosum*. Such constitutive tolerance to serpentine stress implies that the species appears to be somehow "preadapted" to the principal chemical challenges of serpentine substrates such as low Ca/Mg ratio and high Ni content. Our hypothesis of serpentine "preadaption" of *G. valdepilosum* is supported by the high number of spatially isolated serpentine localities (almost all large areas of serpentine on the Hercynian massif) inhabited by the species, which most likely were independently colonized from nearby non-serpentine areas. The absence of local adaptation to high heavy metal toxicity is documented for several plant complexes, including *Silene dioica* (Westerbergh 1994), *Thlaspi goesingense* (Reeves & Baker 1984) and *Th. montanum* (Boyd & Martens 1998). Moreover, even plants that do not grow on serpentines can tolerate extremely low Ca/Mg ratios, such as *Phacelia dubia* var. *georgiana*, which is restricted to dry and nutrient poor granite outcrops (Taylor & Levy 2002), i.e. similar areas to those inhabited by *G. valdepilosum*. In summary, serpentine sites seem to have served as an easily colonized refugium for *G. valdepilosum*, but had no influence on the ecological sorting of its cytotypes. This is in marked contrast with another thoroughly investigated central European di-tetraploid complex, *Knautia arvensis*, which includes a distinct serpentine-tolerant genetic lineage comprising diploid and local autotetraploid populations (Kolář et al. 2012, 2014).

Taxonomic implications

The taxonomy of the *G. pusillum* species complex in Europe is challenging due to the high number of phenotypically similar taxa and small differences in the diagnostic characters, mainly in their fruit (Ehrendorfer et al. 1976). Misidentifications are common and literature records not accompanied by herbarium vouchers are likely to be unreliable (Štěpánková 2000).

Galium sudeticum described from the Krkonoše Mts (Tausch 1835) is traditionally reported from two other geographic areas in the Czech Republic (Ehrendorfer et al. 1976, Štěpánková 2000): (i) the glacial cirque Velká Kotlina in the Hrubý Jeseník Mts (not recently rediscovered despite repeated intensive searches, including our own), and (ii) serpentine outcrops in the Slavkovský les in western Bohemia (first referred to as *G. sudeticum* by Ehrendorfer 1956). Its peculiar distribution (high-altitude habitats in the Sudeten Mts vs comparatively lower-lying, more than 200 km distant serpentine sites) has been long noted and considered comparable to some other arcto-alpine species that occur in isolated serpentine areas (Krahulcová & Štěpánková 1998). Nevertheless, certain morphological differences between subalpine and serpentine populations of the putative *G. sudeticum* (Štěpánková 2000) require further detailed study.

This paper contributed to clarifying the taxonomic status of isolated western Bohemian populations traditionally referred to as *G. sudeticum*. Currently the available evidence supports the merger of these serpentine populations with *G. valdepilosum*. First, serpentine plants in western Bohemia share the same nuclear DNA C-values with all the other samples determined as *G. valdepilosum* analysed but differ significantly from those of individuals of *G. sudeticum* in the Krkonoše Mts. Genome size is usually stable at low taxonomic levels and intraspecific variation often indicates taxonomic heterogeneity (Kron et al. 2007, Loureiro et al. 2010). Consequently, genome size has repeatedly proved to be a useful marker for circumscribing species/subspecies and resolving complex low-level taxonomies (Ekrt et al. 2010, Suda et al. 2010). Another clue comes from the study of their ecological preferences. Environmental conditions at serpentine localities in western Bohemia are virtually identical to those at neighbouring Bavarian serpentines, which host plants invariably identified as *G. valdepilosum* (Noack 1983). In addition, recent morphological investigations (F. Ehrendorfer, pers. comm.) also support the placing of western Bohemian serpentine populations in *G. valdepilosum*. Available data thus suggest that the name *G. sudeticum* should be applied only to subalpine populations currently restricted to the Krkonoše Mts and formerly also occurring in the Hrubý Jeseník Mts. Phenotypic and genome size (Kolář et al. 2013) analyses further indicate that the subalpine populations of *G. sudeticum* are closely related to the highly polymorphic *G. anisophyllon* Villars, which inhabits various neutral to basiphilous subalpine areas in the Alps and Carpathians (Ehrendorfer 1958, Ehrendorfer et al. 1976). The precise taxonomic assignment of serpentine *Galium* populations traditionally referred to as *G. sudeticum* should therefore wait for a detailed assessment of their morphological variation and genetic relationships to other high-altitude taxa.

Finally, we found one distinct but taxonomically uncertain population on a limestone outcrop in the Králický Sněžník Mts in Poland. Although these tetraploid plants are geographically close to the historical *G. sudeticum* occurrence in the Hrubý Jeseník Mts they are ecologically closest to the Alpine-Carpathian species *G. anisophyllon* (note that the Carpathian species *Sesleria tatrae* also occurs on the same outcrop; Fabiszewski 1989). Nevertheless, these plants clearly differ from both *G. anisophyllon* and *G. sudeticum* in their relative genome sizes, and their taxonomic status remains to be clarified.

See http://www.preslia.cz for Electronic Appendices 1–5.

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Souhrn

Okruh svízele maličkého (*Galium pusillum* agg.) patří k taxonomicky obtížným skupinám středoevropské květeny. Česká flóra zahrnuje čtyři původní druhy, mezi nimi svízel moravský (*G. valdepilosum*), který je zajímavý díky své ploidní variabilitě (existence diploidních a tetraploidních populací) a růstu na reliktních stanovištích na různých podkladech (bazické, silikátové, hadcové). Pomocí průtokové cytometrie, fytocenologického snímkování a hydroponického kultivačního pokusu jsme sledovali geografické rozšíření obou cytotypů, jejich ekologické preference (jak v rámci celého areálu druhu, tak v kontaktní zóně) a odezvu na simulovaný hadcový stres. Rozšíření cytotypů je parapatrické, s úzkou kontaktní zónou na jižní Moravě v okolí Brna. Nepodařilo se nalézt žádné triploidní jedince ani ploidněsmíšené populace, což ukazuje, že kontaktní zóna tohoto druhu vázaného na izolovaná reliktní stanoviště je z evolučního hlediska „strnulá“. Tetraploidi mají větší areál a současně se vyskytují na širším spektru stanovišť. Diploidi obecně preferují otevřené dubo-borové lesy na kyselých půdách, zatímco tetraploidi jsou schopni růst i v zapojenější travinné vegetaci na bazických půdách. Hadcové substráty nehrají v ekologické diferenciaci cytotypů žádnou významnou roli a hostí diploidní i tetraploidní populace. Výsledky kultivačního pokusu svědčí o tom, že druh *G. valdepilosum* je obecně tolerantní k hadcovým podmínkám (obdobná odpověď hadcových i nehadcových populací na chemický stres). Relativní velikost genomu i ekologické charakteristiky ukazují, že hadcové populace ze Slavkovského lesa, které byly v minulosti určovány jako *G. sudeticum*, jsou velmi pravděpodobně odlišné od typového výskytu *G. sudeticum* v Krkonoších a spíše patří ke *G. valdepilosum*.

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Appendix 1. – Details of the localities of *Galium valdepilosum* and *G. sudeticum* sampled that were included in this study. Population codes correspond to Kolář et al. (2013); * populations used in the hydroponic cultivation experiment; number of individuals analysed by flow cytometry in round brackets; relative fluorescence intensity (setting internal reference standard, *Bellis perennis*, to a unit value) in square brackets, [n] values newly published in this article, [k] values published in Kolář et al. (2013); No/No: number of vegetation and soil samples, respectively.

Galium valdepilosum **H. Braun**

G020 – 2x (6) CZ, Jihomoravský kraj: Moravský Krumlov – Rokytná, oak forest on slope above right bank of Rokytná river, 0.8 km ESE of the church in Rokytná, open oak-hornbeam forest, basic conglomerate, 270 m a.s.l., coll. F. Kolář, M. Dortová, 3. 8. 2009, 49°03'43.2"N, 16°19'50.0"E

G021* – 2x (6) [0.251][k] 1/1 CZ, Vysočina: Mohelno, pine forest along the road Dukovany – Mohelno, 1.1 km S of the church in Mohelno, open pine forest, serpentine, 330 m a.s.l., coll. F. Kolář, M. Dortová, 3. 8. 2009, 49°06'13.4"N, 16°11'29.1"E

G022 – 2x (5) CZ, Vysočina: Tasov, ruins of Dub castle, 1.4 km SW of Tasov, rocky stands at castle ruins, silicate, 400 m a.s.l., coll. F. Kolář, M. Dortová, 1. 8. 2009, 49°16'53.2"N, 16°04'42.9"E

G025 – 2x (4) CZ, Vysočina: Březník, stone wall below forest road from Vlčí kopec to Březník, 500 m SW of point 426 m, 2.8 km SE of the church in the village, open acidophilous oak forest, silicate, 350 m a.s.l., coll. F. Kolář, 6. 9. 2009, 49°09'27.1"N, 16°09'53.2"E

G026 – 2x (4) CZ, Vysočina: Sedlec, south facing slopes in the meander on the right bank of Oslava river, 2.4 km E of the church in the village, open acidophilous oak forest, silicate, 400 m a.s.l., coll. F. Kolář, 6. 9. 2009, 49°10'4.3"N, 16°09'59.4"E

G027 – 2x (10*)* CZ, Vysočina: Dukovany, pine forest with *Sesleria* on north facing slopes of Jihlava river (Mohelno damm), 2.3 km NE of the church in the village, open pine forest with *Sesleria*, serpentine, 276 m a.s.l., coll. F. Kolář, 7. 9. 2009, 49°05'58.1"N, 16°10'29.4"E

G028* – 2x (10) [0.257][k] 1/1 CZ, Vysočina: Lhánice, open pine forest above forest road from Dolní mlýn to Lhánice, 1.4 km SSW of the village, open pine forest, serpentine, 350 m a.s.l., coll. F. Kolář, 8. 9. 2009, 49°04'48.5"N, 16°15'29.6"E

G029 – 2x (10) CZ, Jihomoravský kraj: Jamolice, oak forest along forest road from Senorady to Templštejn castle, 2.5 km NNW of the village, open acidophilous oak forest, silicate, 301 m a.s.l., coll. F. Kolář, 8. 9. 2009, 49°05'39.4"N, 16°14'56.3"E

G061* – 2x (10) [0.268][k] 1/1 CZ, Jihomoravský kraj: Želešice, rocks in open forest on the steep slope above right bank of Bobrava river at the W edge of the quarry, 1.7 km NW of the church in the village, rocks in open forest, amphibolite, 252 m a.s.l., coll. F. Kolář, M. Dortová, 4. 8. 2010, 49°07'26.9"N, 16°33'15.8"E

G062 – 2x (10) [0.257][k] 1/1 CZ, Jihomoravský kraj: Dolní Kounice, open pine forest on rocky slope of the Šibeničný vrch (296 m) above right bank of Jihlava river, 800 m W of the church in the town, open pine forest on rocky slope, diorite, 225 m a.s.l., coll. F. Kolář, M. Dortová, 4. 8. 2010, 49°04'10.8"N, 16°27'22.7"E

G063 – 2x (1) CZ, Jihomoravský kraj: Moravské Bránice, open oak forest on the slopes above right bank of Jihlava river, 1.5 km SW of the railway station, open oak forest, granite, 233 m a.s.l., coll. F. Kolář, M. Dortová, 4. 8. 2010, 49°04'30.2"N, 16°24'52.1"E

G065* – 2x (10) [0.257][k] 1/1 CZ, Jihomoravský kraj: Chudčice, slope above forest road at the "U Tří křížů" crossing, 1 km SSE of the church in the village, slope above road in the open oak forest, basic conglomerate, 314 m a.s.l., coll. F. Kolář, M. Dortová, 5. 8. 2010, 49°16'41.9"N, 16°27'43.5"E

G090 – 2x (10) 1/1 CZ, Jihomoravský kraj: Vranov nad Dyjí, at the Ledové sluje caves, 2.5 km SE of the town, open forest, silicate, 370 m a.s.l., coll. M. Kubešová, J. Suda, 21. 7. 2010, 48°53'04.4"N, 15°50'35.5"E

G091 – 2x (7) 1/1 CZ, Jihomoravský kraj: Vranov nad Dyjí, zizgzag bends of the road to Lesná, approx 500 m E of the town, open oak forest, silicate, 393 m a.s.l., coll. M. Kubešová, J. Suda, 21. 7. 2010, 48°53'42.4"N, 15°49'18.8"E

G092 – 2x (10) 1/1 CZ, Jihomoravský kraj: Déšov, bank of the road in pine forest on the left bank of Želetavka river, opposite to Koberův mlýn, 3 km SSW of the village, bank of the road in pine forest, silicate, 367 m a.s.l., coll. M. Kubešová, J. Suda, 22. 7. 2010, 48°57'47.8"N, 15°41'20.4"E

G093 – 2x (10) [0.255][k] 1/1 CZ, Jihomoravský kraj: Bítov, *Pinus nigra*-*Quercus* forest on the rocky slope above Vranovská přehrada dam, close to the castle, *Pinus nigra*-oak forest on the rocky slope, silicate, 368 m a.s.l., coll. M. Kubešová, J. Suda, 22. 7. 2010, 48°56'26.3"N, 15°42'13.5"E

G094 – 2x (10) 1/1 CZ, Jihomoravský kraj: Těšetice, oak forest on the right bank of the Těšetice dam, oak forest, silicate, 352 m a.s.l., coll. M. Kubešová, J. Suda, 22. 7. 2010, 48°53'43.6"N, 16°08'16.8"E

G095 – 2x (10) 1/1 AT, Niederösterreich: Melk, pine forest next to the road 3 km N of the town, pine forest, silicate, 385 m a.s.l., coll. M. Kubešová, J. Suda, 23. 7. 2010, 48°16'03.9"N, 15°20'11.6"E

G096 – 2x (10) [0.257][k] 1/1 AT, Niederösterreich: Benking, 800 m ENE of the village, road bank below beech forest, silicate, 652 m a.s.l., coll. M. Kubešová, J. Suda, 23. 7. 2010, 48°20'28.8"N, 15°22'27.7"E
G097 – 2x (10) AT, Niederösterreich: Benking, 1.3 km ENE of the village, road bank in a beech and pine forest, silicate, 667 m a.s.l., coll. M. Kubešová, J. Suda, 23. 7. 2010, 48°20'34.3"N, 15°22'51.9"E
G098 – 2x (1) AT, Niederösterreich: Viessling, along the road L7133 SE of the village, road bank in a beech and pine forest, silicate, 658 m a.s.l., coll. M. Kubešová, J. Suda 23. 7. 2010, 48°21'00.3"N, 15°22'22.9"E
G099 – 2x (10) [0.261][k] 1/1 AT, Niederösterreich: Grossheinrichschlag, sunny river bank 2 km E of the village, sunny river bank, silicate, 642 m a.s.l., coll. M. Kubešová, J. Suda, 23. 7. 2010, 48°24'38.6"N, 15°26'09.4"E
G100 – 2x (1) AT, Niederösterreich: Waldschlössl, along Reichaueramt forest road 2.5 km SW of the village, forest, silicate, 552 m a.s.l., coll. M. Kubešová, J. Suda, 23. 7. 2010, 48°26'11.7"N, 15°31'32.1"E
G102 – 2x (10) 1/1 AT, Niederösterreich: Rosenburg am Kamp, pine forest on the NW slope above the village, pine forest, silicate, 327 m a.s.l., coll. M. Kubešová, J. Suda, 24. 7. 2010, 48°38'15.3"N, 15°37'40.3"E
G103 – 2x (10) 1/1 AT, Niederösterreich: Schönberg am Kamp, pine forest, pine forest, silicate, 240 m a.s.l., coll. M. Kubešová, J. Suda, 24. 7. 2010, 48°31'00"N, 15°42'00"E
G104 – 2x (10) [0.249][k] 1/1 AT, Niederösterreich: Limberg, clearing in oak forest SW of the village, clearing in oak forest, silicate, 407 m a.s.l., coll. M. Kubešová, J. Suda, 24. 7. 2010, 48°35'32.4"N, 15°50'07.0"E
G105 – 2x (10) 1/1 AT, Niederösterreich: Eggenburg, clearing in oak forest S of the village, clearing in oak forest, silicate, 385 m a.s.l., coll. M. Kubešová, J. Suda, 24. 7. 2010, 48°38'16.0"N, 15°49'34.4"E
G117 – 2x (23) [0.266][k] 1/1 CZ, Jihomoravský kraj: Malhostovice, Drásovský kopeček rock, 1 km SSW of the village, steppe, limestone, 300 m a.s.l., coll. P. Koutecký, 30. 5. 2011, 49°19'26"N, 16°29'43"E
G118 – 2x (17) [0.268][k] CZ, Jihomoravský kraj: Malhostovice, Zlobice reserve, 2 km S of the village, open forest, silicate, 350 m a.s.l., coll. M. Štech, 30. 5. 2011, 49°19'07"N, 16°30'17"E
G155 – 2x (2) CZ, Jihomoravský kraj: Lažánky, rocks near Bílý brook (Bítýška), 1.7 km S of the village, scree in forest, silicate, 417 m a.s.l., coll. M. Kubešová, J. Suda, 30. 6. 2011, 49°16'02.5"N, 16°23'10.8"E
G156 – 2x (13) [0.267][k] CZ, Jihomoravský kraj: Lažánky, rocks near Bílý brook (Bítýška), 1.7 km S of the village, rock in forest, silicate, 336 m a.s.l., coll. M. Kubešová, J. Suda, 30. 6. 2011, 49°15'49.9"N, 16°23'22.4"E
G158 – 2x (4) 1/1 CZ, Jihomoravský kraj: Ketkovice, along the way from the village to Ketkovický hrad castle, 1.5 km SW of the village, edge of open forest, silicate, 380 m a.s.l., coll. F. Kolář, 5. 7. 2011, 49°08'56.7"N, 16°14'49.2"E
G159 – 2x (5) 1/1 CZ, Jihomoravský kraj: Ketkovice, old limestone quarry along the way from the village to Ketkovický hrad castle, 1.5 km SW of the village, rocky grassland in old quarry, limestone, 370 m a.s.l., coll. F. Kolář, 5. 7. 2011, 49°08'57.1"N, 16°14'53.7"E
G160 – 2x (1) CZ, Jihomoravský kraj: Ketkovice, rocky otcrops above Chvojnice river, 2.3 km W of the village, open forest with rocky outcrops, silicate, 370 m a.s.l., coll. F. Kolář, 5. 7. 2011, 49°09'35.7"N, 16°13'51.3"E
G161 – 2x (3) CZ, Vysočina: Hrotovice, W facing slopes of the Milačka brook, S border of the village, rocky outcrop in open oak forest, silicate, 400 m a.s.l., coll. F. Kolář, 6. 7. 2011, 49°06'02.4"N, 16°03'59.5"E
G162 – 2x (3) CZ, Vysočina: Hrotovice, serpentine outcrops in coniferous forest 2.2 km SSE of the village, open coniferous forest, serpentine, 390 m a.s.l., coll. F. Kolář, 6. 7. 2011, 49°05'22.3"N, 16°04'31.4"E
G163 – 2x (8) 1/1 CZ, Vysočina: Rouchovany, N facing slope below ruin of castle Mstěnice, 3 km NW of the village, open pine forest, serpentine, 360 m a.s.l., coll. F. Kolář, 6. 7. 2011, 49°04'59.2"N, 16°04'19.8"E
G164 – 2x (3) CZ, Vysočina: Tavíkovice, slopes above left bank of Rokytná river, 0.7 km NE of the village, open oak wood, silicate, 350 m a.s.l., coll. Z. Kaplan, 6. 7. 2011, 49°02'26.1"N, 16°07'02.9"E
G165 – 2x (13) 1/1 CZ, Vysočina: Zahrádka, along a small road to Naloučanský Mlýn, open forest margin, silicate, 390 m a.s.l., coll. F. Kolář, 7. 7. 2011, 49°14'53.6"N, 16°06'43.4"E
G166 – 2x (10) -/1 CZ, Vysočina: Vladislav, rocks on the left bank of Jihlava River, ca 0.4 km SEE of the centre of the town, shady grassy terraces at the foot of a rock, granitoid (granodiorite), 390 m a.s.l., coll. P. Koutecký, 7. 7. 2011, 49°12'33.7"N, 15°59'37.9"E
G167 – 2x (5) CZ, Vysočina: Koněšín, steep slope of Jihlava River valley, left bank of Dalešice river dam, secondary spruce forest, gneiss, 410 m a.s.l., coll. P. Koutecký, 7. 7. 2011, 49°11'13.2"N, 16°00'54.1"E
G168 – 2x (1) CZ, Vysočina: Přešovice, SW facing slope 2 km SE of the village, open forest, silicate, 350 m a.s.l., coll. J. Janáková, 7. 7. 2011, 49°02'07"N, 16°04'45"E
G169 – 2x (1) CZ, Jihomoravský kraj: Náměšť nad Oslavou, slope above left bank of Chvojnice river, 300m ENE of Čertův most, 3.5 km ESE of the railway station, small rock in river canyon, silicate, 350 m a.s.l., coll. J. Prančl, 7. 7. 2011, 49°10'36.5"N, 16°09'38.8"E

G170 – 2x (5) CZ, Vysočina: Hartvíkovice, E facing slope above water reservoir, 800 m SSW of the village, open forest with rocks, silicate, 400 m a.s.l., coll. F. Kolář, 8. 7. 2011, 49°10'07"N, 16°04'36"E

G234 – 2x (5) AT, Niederösterreich: Krems-Land, Wachau: ca. 1,5 km NE Dürnstein, Mähntalgraben, wayside and forest edge of an acidophilic, thermophilic forest, gneiss, 370 m a.s.l., coll. C. Pachschwöll, 11. 6. 2011, 48°24'09"N, 15°32'09"E

G270 – 2x (1) CZ, Jihomoravský kraj: Brno-Kohoutovice, forest N of the town, open forest, silicate, 390 m a.s.l., coll. J. Suda, R. Sudová, 3. 7. 2012, 49°12'07.8"N, 16°32'31.1"E

G001 – 4x (5) CZ, Jihočeský kraj: Holubov, NE facing slope at the margin of Holubovské hadce, 1.1 km E of the railway station in Holubov, open pine forest, serpentine, 490 m a.s.l., coll. F. Kolář, 7. 8. 2009, 48°53'28.6"N, 14°20'24.2"E

G002 – 4x (5) CZ, Jihočeský kraj: Zlatá Koruna, W facing slopes above Vltava river, 500 m NNW of the monastery, open oak-pine forest, silicate, 500 m a.s.l., coll. F. Kolář, 1. 9. 2007, 48°51'31"N, 14°21'57"E

G002-2 – 4x (1) CZ, Jihočeský kraj: Borečnice u Čížové, rocks above right bank of Otava river, 0.8 km NE of the village, rocky slope, silicate, 395 m a.s.l., coll. P. Leischner (voucher CB 64499), 19. 5. 2006, 49°22'04"N, 14°08'52"E

G016* – 4x (6) 1/1 CZ, Středočeský kraj: Nesměřice, S slopes above Želivka river, 1.7 km NW of the village, open and rocky oak forest, silicate, 350 m a.s.l., coll. F. Kolář, M. Dortová, 1. 8. 2009, 49°43'59.7"N, 15°03'54.0"E

G017* – 4x (6) [0.512]^k 1/1 CZ, Středočeský kraj: Bernartice, serpentine pine forest on W slope of Sedlický potok, N of higway bridge, 2.5 km NW of Bernartice, open pine forest, serpentine, 400 m a.s.l., coll. F. Kolář, M. Dortová, 1. 8. 2009, 49°41'18.1"N, 15°06'14.3"E

G019 – 4x (2) CZ, Jihomoravský kraj: Brno-Obřany, oak forest on the slope above right bank of Svratka river, 1.2 km E of the church in Obřany, open oak forest, silicate, 250 m a.s.l., coll. F. Kolář, M. Dortová, 2. 8. 2009, 49°13'33.8"N, 16°39'51.8"E

G023 – 4x (6) CZ, Jihočeský kraj: Červená n. Vltavou, pine-oak wood on the top of the rock above the right bank of Hrejkovický potok brook, 0.5 km E of the church in Červená, mixed forest on a rocky slope, silicate, 389 m a.s.l., coll. F. Kolář, 15. 8. 2009, 49°23'59.2"N, 14°14'59.8"E

G023x – 4x (5) CZ, Ústecký kraj: Boreč, screes on the slopes, mossy scrrees and open birch forest, silicate, 367 m a.s.l., coll. M. Dortová, 15. 8. 2009, 50°30'56.3"N, 13°59'18.9"E

G024 – 4x (6) CZ, Jihočeský kraj: Zvíkovské Podhradí, oak forest on the top of the easternmost rock of the south facing rocky slope "Kopaniny", 250 m N of the Zvíkov castle, open oak-pine forest, silicate, 380 m a.s.l., coll. F. Kolář, 16. 8. 2009, 49°26'30.0"N, 14°11'31.9"E

G033 – 4x (1) D, Sachsen-Anhalt: Altenbrak near Thale, rocks on the SW facing slope, 0.5 km ENE of the village, oak forest on devonian schist rocks, schist, 419 m a.s.l., coll. F. Kolář, J. Chrtek, 15. 7. 2010, 51°43'49.2"N, 10°56'52.8"E

G034 – 4x (3) CZ, Středočeský kraj: Roztoky u Křivoklátu, open forest above rocks 600 m SSE of the railway station, open oak forest, porphyrite, 330 m a.s.l., coll. F. Kolář, 17. 7. 2010, 50°01'4.6"N, 13°52'39.4"E

G035 – 4x (1) CZ, Středočeský kraj: Branov, open forest along the road from the village to Roztoky, 600 m E of the village, bank of the road in open forest, porphyrite, 350 m a.s.l., coll. F. Kolář, 17. 7. 2010, 50°00'41.2"N, 13°51'12.1"E

G036 – 4x (17) [0.512]^k 2/1 PL, Woj. Dolnośląnskie: Łączna near Kłodzko, limestone quarry 500 m N of the NW end of the village, exposed scrrees, slopes of an open pine forest above the quarry, limestone, 466 m a.s.l., coll. F. Kolář, 20. 7. 2010, 50°30'07.8"N, 16°37'12.4"E

G037 – 4x (10) 1/1 PL, Woj. Dolnośląnskie: Tapadla near Dzierżoniów, open forest with serpentine rocks on south facing slope approx. 100 m south of the top of Radunia mountain, open oak-pine forest, serpentine, 258 m a.s.l., coll. F. Kolář, 20. 7. 2010, 50°50'10.7"N, 16°46'37.9"E

G040 – 4x (10) [0.509]^k 1/1 PL, Woj. Małopolskie: Klonów near Miechów, slope above the road to Dale, approx. 100 m N of old limestone quarry, in the village, open parts of basiphilous steppe, chalk, 257 m a.s.l., coll. F. Kolář, 21. 7. 2010, 50°20'28.0"N, 20°10'46.2"E

G041 – 4x (10) 1/1 D, Bayern: Hirschling, slope above river Regen, 500 m N of the village, open pine forest, granite, 400 m a.s.l., coll. F. Kolář, P. Vít, 25. 7. 2010, 49°12'00.6"N, 12°09'39.8"E

G042 – 4x (10) [0.509]^k 1/1 D, Bayern: Königshof near Stefling, west facing slopes of a side valley north of Regen river, 100 m N of Königshof, exposed scrrees, slopes of an open pine forest above the quarry, granite, 378 m a.s.l., coll. F. Kolář, P. Vít, 25. 7. 2010, 49°12'51.3"N, 12°13'17.4"E

G043 – 4x (10) [0.510]^k 1/1 D, Bayern: Fischbach near Kallmünz, limestone rocks at the top of Hutberg hill, east of the village, rocks in open pine forest, limestone, 422 m a.s.l., coll. F. Kolář, P. Vít, 25. 7. 2010, 49°10'21.2"N, 11°59'29.4"E

G044 – 4x (6) 1/1 D, Bayern: Matting near Regensburg, south facing slope above Danube river, approx. 1.4 km NE of the village, rocks in open pine forest, limestone, 374 m a.s.l., coll. F. Kolář, P. Vít, 26. 7. 2010, 48°58'16.5"N, 12°01'05.6"E

G045 – 4x (10) [0.502]^k 1/1 D, Bayern: Schuttersmühle near Pottenstein, forest next to limestone rocks on right bank of Weiher brook, approx 100 m N of the mill, rocks in open spruce forest (close to open pine forest on the rocks), dolomite, 460 m a.s.l., coll. F. Kolář, P. Vít, 26. 7. 2010, 49°45'06.0"N, 11°25'40.5"E

G046 – 4x (10) [0.508]^k 2/1 D, Bayern: Rabenstein, slopes and rocks above left bank of Allsbach brook, approx. 300 m NE of the castle Rabenstein, open pine forest, dolomite, 427 m a.s.l., coll. F. Kolář, P. Vít, 26. 7. 2010, 49°49'27.6"N, 11°22'29.2"E

G047 – 4x (10) [0.507]^k 1/1 D, Bayern: Kupferberg, serpentine rocks near to the top of Peterlenstein, 1.5 km NE of the town, rocks and screes in open pine forest, serpentine, 581 m a.s.l., coll. F. Kolář, P. Vít, 27. 7. 2010, 50°09'25.3"N, 11°35'45.7"E

G048 – 4x (9) 1/1 D, Bayern: Gottzmansgrün near Hof, serpentine rocks "Blauer Fels" in the forest ca 800 m N of the village, rocks in open pine forest, serpentine, 541 m a.s.l., coll. F. Kolář, P. Vít, 27. 7. 2010, 50°11'49.0"N, 11°53'27.4"E

G049 – 4x (2) -/1 D, Thüringen: Burgk a. d. Saale, rock next to a pathway approx. 400 m SE of bridge of the road from Schleiz to Remptendorf, 1 km SE of the castle, cracks in schist rock, schist, 378 m a.s.l., coll. F. Kolář, P. Vít, 27. 7. 2010, 50°32'44.3"N, 11°43'51.9"E

G050 – 4x (10) [0.500]^k 1/1 D, Bayern: Woja near Wurlitz, open pine forest next to western margin of a serpentine quarry, 600 m south of the village, rocks in open pine forest, serpentine, 542 m a.s.l., coll. F. Kolář, P. Vít, 27. 7. 2010, 50°15'14.4"N, 11°58'30.4"E

G051* – 4x (10) [0.504]^k 1/1 D, Bayern: Erbendorf, serpentine rocks in pine forest 2 km NNW of the village, rocks in open pine forest, serpentine, 525 m a.s.l., coll. F. Kolář, P. Vít, 27. 7. 2010, 49°51'24.3"N, 12°01'53.7"E

G058* – 4x (5) [0.505]^k 1/1 CZ, Jihomoravský kraj: Boskovice, open oak forest on steep slope above Bělá river, 2 km SW of the Boskovice castle, open oak forest on steep slope, basic conglomerate, 358 m a.s.l., coll. F. Kolář, M. Dortová, 3. 8. 2010, 49°28'27.0"N, 16°37'57.2"E

G059 – 4x (10) [0.516]^k 1/1 CZ, Jihomoravský kraj: Blansko – Skalní Mlýn, open north-facing limestone rocks, 300 m SW of the Skalní Mlýn mill, open north-facing rocks, limestone, 493 m a.s.l., coll. F. Kolář, M. Dortová, 4. 8. 2010, 49°21'38.2"N, 16°42'22.0"E

G060 – 4x (9) [0.504]^k 1/1 CZ, Jihomoravský kraj: Brno-Slatina, open *Sesleria*-grassland on north-facing slope of Stránská skála hill (310 m), open *Sesleria*-grassland on north-facing slope, limestone, 301 m a.s.l., coll. F. Kolář, M. Dortová, 4. 8. 2010, 49°11'28.4"N, 16°40'35.8"E

G106 – 4x (10) [0.508]^k 1/1 AT, Niederösterreich: Kollmitzgraben, pine forest next to the ruin, pine forest, silicate, 426 m a.s.l., coll. M. Kubešová, J. Suda, 25. 7. 2010, 48°49'21.8"N, 15°31'54.1"E

G116 – 4x (5) 1/1 CZ, Jihomoravský kraj: Tišnov, steppes on the slopes of Květnice hill, steppe, limestone, 350 m a.s.l., coll. P. Koutecký, 30. 5. 2011, 49°21'09"N, 16°25'01"E

G126 – 4x (1) CZ, Jihomoravský kraj: Bílovice nad Svitavou, road ditch 0.4 km ENE of the railway station, ditch along side of forest road, silicate, 272 m a.s.l., coll. T. Koutecký, 20. 5. 2011, 49°14'39.5"N, 16°40'44.0"E

G127 – 4x (5) CZ, Jihočeský kraj: Hodonice, slopes above Židova strouha brook, 1 km W of the village, open forest, rocks, silicate, 395 m a.s.l., coll. L. Ekrt, 20. 5. 2011, 49°16'11.7"N, 14°28'27.4"E

G134 – 4x (10) [0.502]^k 1/1 PL, Woj. Małopolskie: Zarogów (distr. Miechów), old quarry 40 m NE of the village, rocks in an old quarry, chalk, 228 m a.s.l., coll. F. Kolář, J. Chrtek, 14. 6. 2011, 50°20'09.0"N, 20°06'59.2"E

G150 – 4x (10) [0.510]^k CZ, Ústecký kraj: Boreč, screes on the NNE slope, scree, basalt, 390 m a.s.l., coll. M. Kubešová, J. Suda, 28. 6. 2011, 50°30'56.7"N, 13°59'19.0"E

G151 – 4x (15) [0.504]^k CZ, Karlovarský kraj: Velichov, S slope of the Thebisberg hill, W of the village, scree, basalt, 368 m a.s.l., coll. M. Kubešová, J. Suda, 28. 6. 2011, 50°16'55.8"N, 12°59'50.5"E

G213 – 4x (10) [0.498]^k CZ, Středočeský kraj: Nižbor, 2.1 km N from railway station, Vůznice national nature reserve, outcrop 500 m S from Vůznice water reservoir, rocky outcrop in forest, silicate, 320 m a.s.l., coll. M. Lučanová, 25. 8. 2011, 50°01'16.53"N, 13°59'30.16"E

G243 – 4x (9) CZ, Olomoucký kraj: Slatinice, forest 1 km N of Velký Kosíř hill (442 m), forest, silicate, 400 m a.s.l., coll. P. Koutecký, 11. 5. 2012, 49°33'31.9"N, 17°03'47.5"E

G244 – 4x (25) 1/- PL, Woj. Małopolskie: district Miechów: Kalina Lisiniec, slopes ca 0.3 km N of the northern part of the village, cretaceous steppic slopes (*Inuletum ensifoliae*), chalk, 350 m a.s.l., coll. J. Chrtek, Z. Szęlag, 24. 5. 2012, 50°21'46"N, 20°09'33"E

G247 – 4x (10) [0.501]^k D, Sachsen-Anhalt: Treseburg, rocky crest 1.1 km NNW of the village, open forest and rocks, silicate, 359 m a.s.l., coll. F. Kolář, 5. 6. 2012, 51°43'08.6"N, 10°57'32.2"E

G267 – 4x (10) PL, Woj. Małopolskie: Racławice, Wyżyna Miechowska Upland – open south-facing xerophilous grassland in "Wały" reserve N of the village, xerothermic grasslands of the *Inuletum ensifoliae* association, chalk, 302 m a.s.l., coll. P. Kwiatkowski, 2. 6. 2012, 50°20'24.2"N, 20°13'43.1"E

G271 – 4x (3) CZ, Jihomoravský kraj: Lelekovice, forest at the hill 442 m a.s.l., 1.2 km NE of the village, open forest, silicate, 440 m a.s.l., coll. J. Suda, R. Sudová, 4. 7. 2012, 49°17'57.8"N, 16°35'31.0"E

G272 – 4x (49) 1/- CZ, Jihomoravský kraj: Železné, W slope of the hill 347 m, N of the village, mosaic of open forest and steppe grassland, silicate, 305 m a.s.l., coll. J. Suda, R. Sudová, 4. 7. 2012, 49°21'52.5"N, 16°26'46.2"E

G273 – 4x (36) 1/- CZ, Jihomoravský kraj: Tišnov, S slopes of Květnice hill, steppe, limestone, 356 m a.s.l., coll. J. Suda, R. Sudová, 4. 7. 2012, 49°21'11.9"N, 16°24'57.7"E

G289 – 4x (10) CZ, Plzeňský kraj: Svojšín, rocks above Mže river ca 600 m E of the village, shaded spilite rocks, silicate, 400 m a.s.l., coll. M. Hanzl, 16. 6. 2013, 49°46'09.2"N, 12°55'07.7"E

G294 – 4x (7) CZ, Liberecký kraj: Hradčany, sandstone rock 1.2 km W of the village, crevices and ledges of lime-enriched sandstone rock, neutral-basic sandstone, 327 m a.s.l., coll. F. Kolář, 26. 6. 2013, 50°36'59"N, 14°41'19.1"E

G295 – 4x (10) CZ, Liberecký kraj: Hradčany, sandstone rock 0.5 km SW of the village, crevices and ledges of lime-enriched sandstone rock, neutral-basic sandstone, 329 m a.s.l., coll. F. Kolář, 26. 6. 2013, 50°36'51.6"N, 14°42'03.9"E

G305 – 4x (3) CZ, Jihočeský kraj: Vráž, edge of the Otava river canyon, in Žlíbky reserve, 1.2 km E of the chateau in the village, open forest, silicate, 400 m a.s.l., coll. P. Koutecký, 5. 5. 2013, 49°22'40.6"N, 14°08'36.8"E

Western Bohemian serpentine populations traditionally referred to as *G. sudeticum* but most likely conspecific with *G. valdepilosum*

G032 – 4x (1) [0.511]^{k} 1/1 CZ, Karlovarský kraj: Mnichov, pine forest 100 m SW of the small serpentine quarries, 1.5 km W of the church in the village, rocks in open pine forest, serpentine, 740 m a.s.l., coll. F. Kolář, 27. 6. 2010, 50°02'16.1"N, 12°45'59.0"E

G136 – 4x (10) [0.503]^{k} 1/1 CZ, Karlovarský kraj: Prameny, Vlčí Hřbet hill 1.9 km S of the village, open pine forest, serpentine, 850 m a.s.l., coll. F. Kolář, 21. 6. 2011, 50°01'59.0"N, 12°44'04.2"E

G277 – 4x (10) [0.501]^{n} CZ, Karlovarský kraj: Prameny, isolated rocky outcrop 1.2 km N of the village, rocky outcrop, serpentine, 790 m a.s.l., coll. A. Knotek, M. Hanzl, 1. 9. 2012, 50°03'41.22"N, 12°43'54"E

G278 – 4x (8) [0.505]^{n} CZ, Karlovarský kraj: Nová Ves, Dominova skalka rock, 1.6 km SSE of the village, rocky outcrop, serpentine, 750 m a.s.l., coll. A. Knotek, M. Hanzl, 1. 9. 2012, 50°04'17"N, 12°47'10"E

Subalpine *Galium sudeticum* Tausch

G171 – 4x (15) [0.526]^{k} 2/1 CZ, Královéhradecký kraj: Pec pod Sněžkou, Čertova zahrádka, 3,6 km N of the town, rocks and screes, erlan, 1050 m a.s.l., coll. F. Kolář, A. Knotek, M. Hanzl, 13. 7. 2011, 50°43'37.8"N, 15°43'27.3"E

G172 – 4x (20) [0.534]^{k} CZ, Královéhradecký kraj: Horní Mísečky, ridge between Malá and Velká Kotelná jáma glacial cirque, subalpine grassland, erlan, 1381 m a.s.l., coll. F. Kolář, A. Knotek, M. Hanzl, 13. 7. 2011, 50°45'08.7"N, 15°31'56.7"E

G212 – 4x (2) [0.528]^{n} 1/- CZ, Královéhradecký kraj: Pec pod Sněžkou, Rudník, 3.8 km N of the town, scree, erlan, 1100 m a.s.l., coll. A. Knotek, M. Hanzl, 13. 8. 2011, 50°43'50.2"N, 15°43'53.1"E

G260 – 4x (12) [0.515]^{n} 1/- PL, Woj. Dolnośląnskie: Szklarska Poręba, basaltic outcrop in Mały Śnieżny Kocioł glacial cirque, rocks and talus slope, basalt, 1254 m a.s.l., coll. F. Kolář, A. Knotek, T. Urfus, 18. 7. 2012, 50°46'58.4"N, 15°33'24.7"E

G261 – 4x (5) [0.537]^{n} PL, Woj. Dolnośląnskie: Szklarska Poręba, upper edge of the Wielki Śnieżny Kocioł glacial cirque in Krysztalowy żleb, open gravely soil, silicate, 1483 m a.s.l., coll. F. Kolář, A. Knotek, T. Urfus, 18. 7. 2012, 50°46'45.8"N, 15°33'27.3"E

***Galium pusillum* agg. with unclear assignment**

G135 – 4x (10) [0.500]^{n} 1/1 PL, Woj. Dolnośląnskie: Kletno by Stronie Sląskie, limestone rocks opposite (N of) Jaskynia Niedzwiedzia, scree, rocks, limestone, 890 m a.s.l., coll. F. Kolář, J. Chrtek, 15. 6. 2011, 50°14'19.0"N, 16°50'33.2"E