Symphytum tuberosum complex in central Europe: cytogeography, morphology, ecology and taxonomy

***Symphytum tuberosum* ve střední Evropě – cytogeografie, morfologie, ekologie a taxonomie**

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The *Symphytum tuberosum* complex is a highly polyploid and taxonomically intriguing group. At least eight ploidy levels were recorded previously within this complex. Based on flow cytometric screening of 271 central-European populations, two dominant ploidy levels were revealed: tetraploid (2n = 4x = 32) and widespread dodecaploid (2n = 12x = 96). The tetraploid cytotype is mainly distributed along the southern and south-western margins of the West Carpathians where they abut the Pannonian basin, and found only in Slovakia, the Czech Republic (south-eastern Moravia) and Hungary; our findings represent the first records of this ploidy level for the latter two countries. In contrast, the dodecaploid cytotype occurs throughout the whole area studied. In addition to their geographic distributions, differences between the cytotypes in morphology and habitat requirements were detected using a multivariate morphometric analysis and analysis of a phytosociological database, respectively. Based on this information and taking certain overlaps in morphological traits and habitat requirements into account, we propose treating the dominant cytotypes as subspecies: *S. tuberosum* subsp. *tuberosum* (dodecaploids) and *S. tuberosum* subsp. *angustifolium* (tetraploids). In some populations, aneuploids and several minority ploidy levels were also detected, including DNA-hexaploids (only within populations of tetraploids), DNA-decaploids and DNA-tetradecaploids (both only within populations of dodecaploids).

K e y w o r d s: cytotype distribution, ecology, flow cytometry, morphology, multivariate morphometrics, polyploidy, *Symphytum tuberosum* subsp. *angustifolium*, taxonomy

Introduction

The family *Boraginaceae* Juss. is known for its considerable chromosome variation, which is a consequence of various cytological processes, such as chromosome fusion or fragmentation, polyploidy or aneuploidy (Britton 1951, Coppi et al. 2006). These processes seem to be common in the family and play a crucial role in the evolution of many genera, such as *Borago* L. (Selvi et al. 2006), *Cerinthe* L. (Selvi et al. 2009), *Myosotis* L. (Štěpánková 2001, 2006), *Nonea* Medik. (Selvi et al. 2002, Bigazzi & Selvi 2003), *Onosma* L. (Mártonfi et al. 2008), *Omphalodes* Mill. (Grau 1967) and *Pulmonaria* L. (Sauer 1975). In addition, the occurrence of B chromosomes is quite common (Gadella 1972, Sauer 1975, Bigazzi & Selvi 2003, Bedini et al. 2012). All these processes are also

important for genome evolution in the genus *Symphytum* L. (Grau 1968, 1971, Gadella & Kliphuis 1978, Murín & Májovský 1982). Gadella & Kliphuis (1978) report a high frequency of polyploids in comparison with other genera of *Boraginaceae* with the occurrence of polyploidy, as in *Onosma*, *Myosotis* and *Pulmonaria*. This phenomenon is well illustrated by the four ploidy levels reported for the *Symphytum officinale* complex (e.g. Markowa & Iwanowa 1970, Gadella & Kliphuis 1978) or, even more surprisingly, the eight ploidy levels reported in the *Symphytum tuberosum* complex (Murín & Májovský 1982), which range from presumably diploid (2n = 2x =18) up to octodecaploid cytotypes (2n = 18x = 144).

The Old World genus *Symphytum* L. belongs to the tribe *Boragineae* Bercht. et J. Presl, a major monophyletic group within the family *Boraginaceae* (Hilger et al. 2004). With approximately 40 species, it is one of the largest genera in this tribe (Bucknall 1913, Sandbrink et al. 1990). It includes perennial, roughly hirsute plants, which are morphologically well characterized by creeping, mostly fleshy rhizomes, alternate leaves, double scorpioid cymes (= boragoids) with tubular flowers and five corolla appendages (= fornices) inside the flower. The geographical range of the genus covers almost the whole of Europe and Asia Minor, as well as part of Western Asia and Siberia (Bucknall 1913). The centre of its diversity is situated in the Pontic area and in the western parts of the Irano-Turanian region, primarily in the mountain ranges around the Black Sea (Gadella & Kliphuis 1978, Davis 1988).

In central Europe, the following native species are recognized: *S. cordatum* Waldst. et Kit., the *S. officinale* complex (including *S. bohemicum* F. W. Schmidt, *S. officinale* s. str. and *S. tanaicense* Steven) and the *S. tuberosum* complex (including *S. angustifolium* A. Kern.; Kerner 1863, Murín & Májovský 1982, Marhold & Hindák 1998; and *S. tuberosum* L.; Pawłowski 1963, Gams 1966, Smejkal 1978, Májovský & Hegedüšová 1993, Slavík 2000, Danihelka et al. 2012). Five additional non-native species originating mostly from eastern Europe, the eastern Mediterranean and the Caucasus are also reported: *S. asperum* Lepech., *S. bulbosum* K. F. Schimp., *S. caucasicum* M. Bieb., *S. orientale* L. and *S. tauricum* Willd. (Pawłowski 1963, Gams 1966, Smejkal 1978, Danihelka et al. 2012, Bomble & Schmitz 2013).

Traditionally, the genus is divided into 2–9 sections, based on various infrageneric classifications (Boissier 1879, Kuznetsov 1910, Bucknall 1913, Pawłowski 1961, Wickens 1969, Sandbrink et al. 1990). The *S. tuberosum* complex belongs to the widely accepted section *Tuberosa* Buckn., which is characterized by (i) mostly tuberous rhizomes; (ii) triangular, densely papillose fornices that do not protrude from corollas; and (iii) yellow flowers (Bucknall 1913, Pawłowski 1961, Wickens 1969, Sandbrink et al. 1990). Species in this section occur almost all over the European continent and adjacent Anatolia, except for the cold regions of northern Europe (Bucknall 1913, Murín & Májovský 1982). The section is a taxonomically difficult and still unresolved group with high-level polyploids and considerable morphological variation (Gadella & Kliphuis 1978, Murín & Májovský 1982).

In total, there are up to 10 taxa described within the *S. tuberosum* complex (in chronological order): *S. tuberosum* L. s. str., *S. mediterraneum* W. D. J. Koch, *S. angustifolium* A. Kern., *S. nodosum* Schur, *S. foliosum* Rehmann, *S. gussonei* F. W. Schultz, *S. floribundum* Shuttlew. ex Nyman, *S. leonhardtianum* Pugsley, *S. besseri* Zaver. and *S. popovii* Dobrocz. (Koch 1837, Kerner 1863, Schur 1866, Rehmann 1868, Schultz 1872, 1875,

Nyman 1884, Pugsley 1931, Zaverucha 1962, Dobroczajeva 1968). However, the treatments of *Symphytum* in Flora Europaea and the Euro+Med Checklist recognize only two species: the Sicilian endemic *S. gussonei* and the widespread *S. tuberosum*, the latter comprising the western European subsp. *tuberosum* and central- and eastern-European subsp. *angustifolium/nodosum* (Pawłowski 1972, Valdés 2011). There is no clear relationship between the known karyological variation of *S. tuberosum* and its two subspecies on a European scale, and thus, only one broadly defined species, *S. tuberosum*, without any infra-specific units is usually recognized in recent floras (Mirek et al. 2002, Fischer et al. 2008, Jäger 2009, Király et al. 2011, Danihelka et al. 2012). The only exception is Slovakia, where the two known cytotypes are treated as separate species: *S. tuberosum* (dodecaploids) and *S. angustifolium* (tetraploids; Májovský & Hegedüšová 1993, Marhold & Hindák 1998).

In addition to the unresolved taxonomy, there is also controversy over the basic chromosome number of the *S. tuberosum* complex and the whole genus. The authors of the first karyological study suggest x = 18 as the basic chromosome number of *Symphytum* (Strey 1931). With the increasing number of counts, other basic numbers were proposed (x = 8, 9, 10, 12, 14, 15; e.g. Britton 1951, Markowa & Iwanowa 1970, Gadella & Kliphuis 1978, Luque 1989), and x = 12 is widely considered to be the basic chromosome number of this genus (Grau 1971, Murín & Májovský 1982, Slavík 2000). However, Murín & Májovský (1982) show that x = 8 best fits their comprehensive karyological survey of the *S. tuberosum* complex in the eastern part of central Europe.

The aim of this study was to examine the karyological variation and cytogeography of the *S. tuberosum* complex in central Europe and their relationship to morphological variation and habitat preferences. Based on the results, a revised taxonomic treatment of this complex in this region is proposed.

Materials and methods

Field sampling

Plant material was collected in the Czech Republic (207 populations), Slovakia (24 populations), Austria (24 populations), Hungary (9 populations), Poland (4 populations) and Germany (3 populations; Appendix 1) between 2011 and 2014. A total of 1693 plants from 271 populations (1–26 plant per population, depending on population size) were collected for DNA ploidy level estimation by flow cytometry. For the morphometric analyses, only well-developed plants with at least five flowers were selected. The collected plants were transplanted into the experimental garden of Palacký University (Olomouc, Czech Republic) or processed as standard herbarium vouchers and deposited in the Herbarium of the Palacký University in Olomouc (OL).

Estimation of DNA ploidy level

The DNA ploidy level was determined using a Partec CyFlow ML flow cytometer (Partec GmbH, Münster, Germany) equipped with a diode-pumped solid state green laser (532 nm, 100 mW, Cobolt Samba; Cobolt AB, Stockholm, Sweden), a BD Accuri C6 flow cytometer (BD Biosciences, San Jose, California) equipped with a blue laser

(488 nm, 20 mW, BD Accuri™; BD Biosciences, San Jose) or a Partec PA II flow cytometer (Partec GmbH., Münster, Germany) equipped with a mercury arc lamp. In the first two instruments, propidium iodide (PI) was used as a stain; in the last instrument, DAPI was used for staining. However, note that even the measurements with PI should be considered only as estimates of the ploidy level, as we have not fulfilled (and did not intend to fulfill) all the requirements for an exact estimate of genome size (e.g. repeated measurements on different days; all samples were measured only once; Doležel et al. 2007). Data calibration was done using measurements of the same individuals cultivated in a greenhouse using different instruments.

The samples stained with PI were prepared using a simplified protocol with LB01 isolation buffer (Doležel et al. 2007). Approximately 0.5 cm^2 of fresh leaf tissue (from cultivated or plants growing in the field stored in plastic bags for a maximum of few days in a refrigerator) was chopped together with an appropriate amount of the internal reference standard with a razor blade in a Petri dish containing 1 ml of the isolation buffer. *Pisum sativum* L. 'Ctirad' (2C = 9.09 pg; Doležel et al. 1998) was used as the primary standard. However, due to a peak overlap of tetraploids with *Pisum sativum*, we used *Zea mays* 'CE-777' (2C = 5.92 pg, value calibrated against *Pisum sativum* 'Ctirad') as the secondary standard. The isolation buffer was supplemented with PVP-40 (20 mg/ml) to suppress the phenolic compounds interfering with DNA staining (Doležel & Bartoš 2005). The solution was filtered through a 42-μm nylon mesh and incubated for ~1 min. at room temperature. Then, a flow-through fraction was stained with fluorochrome PI (50 μg/ml). Samples were run on the flow cytometer immediately after staining and the relative fluorescence intensity of at least 3000 particles was recorded. Each individual was analysed separately.

For samples stained with DAPI, a simplified two-step protocol (Doležel et al. 2007) was used. Fresh leaves were chopped with *Bellis perennis* L. leaf tissue as the internal standard (the ratio of mean relative fluorescence compared to *Pisum sativum* 'Ctirad' is 0.429) in a Petri dish containing 0.5 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween-20). The suspension was filtered through a 42-μm nylon mesh and then incubated for ~3 min. at room temperature. After incubation, 1 ml of the Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12H_2O$) supplemented with 2-mercaptoethanol (2 μl/ml) and DAPI (4 μg/ml) was added. Samples were run after ~1 min. of staining, and the fluorescence intensity of 3000–5000 particles was recorded. Usually, 3–5 individuals from the same population were analysed together; if the occurrence of more ploidy levels or genome size variation in the bulked sample was suspected, each individual was re-analysed separately. The ploidy level of each sample was determined by the position of its G_0/G_1 peak relative to the G_0/G_1 peak of the internal standard. Generally, for measurements using both PI and DAPI, histograms with coefficients of variation (CV) for the G_0/G_1 peaks of the analysed sample and the standard less than 5% were accepted.

Chromosome counts

To calibrate the results of the flow cytometry measurements, the chromosome numbers of two tetraploid (populations 11 and 84) and three dodecaploid (populations 5, 18B and 33; Appendix 1) plants were determined. Chromosomes in the metaphase cells of the root meristems of cultivated plants were counted. The root tips were pre-treated with

α-bromonaphthalene for 4 hours at room temperature, fixed in cold acetic acid : ethanol (1 : 3) overnight and stored in 70% ethanol at 4°C. Then, samples were macerated for 5 min. in 1 M hydrochloric acid at 60°C (Krahulcová & Krahulec 1999). The apical part of the root tip was cut and squashed in lacto-propionic orcein. Metaphases were observed under 1000× overall magnification.

Morphometric analyses

Only flowering plants with at least five flowers and known ploidy levels were used for morphometric analysis. Plants of minority cytotypes could not be included because they were either poorly developed or were only recorded by cytometric screening after anthesis. In total, 522 individuals (196 tetraploids and 326 dodecaploids) from 40 populations (at least 10 individuals per population) were analysed (Appendix 1). For each individual, 19 vegetative and generative characters were measured, and four ratios were calculated (Table 1). All quantitative characters were measured on fresh material using a digital calliper except for characters inside the corolla, which were measured on dried flowers under a binocular microscope. For generative characters, five flowers per plant were measured and median values used in all analyses.

The morphological dataset was analysed using NCSS 2007 (Hintze 2008) and CANOCO for Windows 4.5 (ter Braak & Šmilauer 2002) software. Initially, univariate analyses were used. Spearman correlation coefficients were computed to reveal pairs of highly correlated characters. One-way ANOVA was used to compare the mean values of characters between cytotypes. Because multiple tests were performed, Bonferroni correction of the significance values was applied. Principal component analysis (PCA; Sneath & Sokal 1973) based on the correlation matrix was used to display the overall pattern of variation. Canonical discriminant analysis (CDA; Legendre & Legendre 1998) was used to determine the extent of morphological separation between the cytotypes. Parametric classificatory discriminant analysis was used to estimate the percentage of plants correctly assigned to the predetermined groups (cytotypes) based on the morphological characters measured. The character 'branching of stem', due to its qualitative nature, was separately analysed using a contingency table and hence not included in the above-described statistical procedures. All analyses were performed with individual plants as objects.

Ecological differences among cytotypes

To understand the ecological differentiation and phytosociological affinity of the cytotypes in the Czech Republic, phytosociological relevés comprising *Symphytum tuberosum* were analysed. Relevés were obtained from the Czech National Phytosociological Database (ČNFD; Chytrý & Rafajová 2003), and 18 additional relevés were recorded. As only the species *S. tuberosum* is included in the database taxonomic list, all records must be considered as potentially referring to either cytotype. We therefore selected only relevés from localities that we screened using flow cytometry; for dodecaploids, relevés from Bohemia and northern Moravia, where tetraploids are unlikely to occur, were also included. In total, 520 phytosociological relevés were analysed (162 and 358 relevés related to tetraploids and dodecaploids, respectively).

Table 1. – Descriptive statistics (arithmetic mean with standard deviation, minimum and maximum) of the morphological characters of the major cytotypes of the *Symphytum tuberosum* complex in central Europe. Differences among cytotypes were tested by one-way ANOVA (DF = 1; 521), P shows significant differences after Bonferroni correction (n.s. = not significant). Abbreviations of characters given in parentheses correspond to those used in Fig. 7.

Morphological character	4x (n = 196)			12x (n = 326)			F	P
	Mean (SD)	Min	Max	Mean (SD)	Min	Max		
Height of plant (cm; height)	33.1 (7.2)	15.6	52.0	30.2 (7.9)	8.5	51.7	18.12	< 0.001
Length of uppermost leaf (cm; lleafU)	5.2 (1.6)	1.9	10.5	6.4 (2.1)	2.0	13.6	38.27	< 0.001
Width of uppermost leaf (cm; wleafU)	1.7 (0.7)	0.5	3.8	2.6 (1.1)	0.7	8.5	115.23	< 0.001
Length to width ratio of uppermost leaf (rleafU)	3.4 (0.7)	1.2	5.5	2.6 (0.5)	0.4	4.7	185.98	< 0.001
Length of middle leaf (cm; lleafL)	9.7 (2.3)	4.2	19.1	11.8 (2.9)	5.2	19.3	72.80	< 0.001
Width of middle leaf (cm; wleafM)	2.6 (0.7)	1.3	4.5	4.2 (1.2)	2.0	8.4	281.46	< 0.001
Length to width ratio of middle leaf (rleafM)	3.9 (0.8)	1.7	6.1	2.9 (0.5)	1.4	4.7	335.78	< 0.001
Length of lowermost leaf (cm; lleafL)	10.0 (2.6)	2.8	17.9	10.5 (3.7)	2,0	22.1	3.08	n.s.
Width of lowermost leaf (cm; wleafL)	2.50 (0.8)	0.7	5.3	4.0 (1.2)	1.5	8.7	251.10	< 0.001
Length to width ratio of lowermost leaf (rleafL)	4.2 (1.0)	1.3	8.7	2.6 (0.7)	1.0	5.5	411.29	< 0.001
Length of pedicel (mm; flstalk)	8.35 (1.94)	4.16	14.13	8.46 (2.01)	3.99	14.97	0.33	n.s.
Length of calyx (mm; calyx)	7.46 (0.96)	5.37	9.46	7.68 (1.02)	5.24	11.27	5.93	n.s.
Length of corolla (mm; corrolla)	15.24 (1.11)	12.32	17.59	16.03 (1.19)	12.34	18.82	57.97	< 0.001
Length of narrow part of corolla tube (mm; cortube)	7.60 (0.68)	5.52	9.66	8.37 (0.85)	5.71	10.76	117.00	< 0.001
Ratio of length of corolla to narrow part of corolla (rcorct)	2.01 (0.12)	1.72	2.45	1.92 (0.13)	1.66	2.37	54.58	< 0.001
Length of style (mm; style)	15.98 (1.78)	10.15	19.37	17.79 (1.58)	10.67	21.83	145.30	< 0.001
Length of filament (mm; lfil)	0.86 (0.09)	0.57	1.10	0.96 (0.12)	0.53	1.30	71.60	< 0.001
Length of free part of filament (mm; freefil)	0.05 (0.01)	0.03	0.78	0.06 (0.01)	0.03	0.08	18.70	< 0.001
Width of filament (mm; wfil)	0.21 (0.04)	0.14	0.29	0.23 (0.05)	0.09	0.34	26.51	< 0.001
Length of fornice (mm; corsca)	1.23 (0.11)	0.89	1.51	1.29 (0.14)	0.93	1.65	31.46	< 0.001
Length of anther (mm; lthec)	0.33 (0.04)	0.23	0.46	0.34 (0.04)	0.25	0.44	30.16	< 0.001
Width of anther (mm; wthec)	0.07 (0.01)	0.05	0.08	0.08 (0.01)	0.05	0.09	225.43	< 0.001

The relevés were classified by an expert system using the Cocktail method (Kočí et al. 2003, Chytrý 2007) in JUICE 7.0 software (Tichý 2002); this expert system allows classification of the rank of association following the Vegetation of the Czech Republic series (Chytrý 2007, 2009, 2013). Average Ellenberg's indicator values (light, temperature, continentality, moisture, soil reaction and nutrients; Ellenberg et al. 1992) were calculated for individual relevés using JUICE 7.0. Differences in the average Ellenberg's indicator values between the two dominant cytotypes were analysed using one-way ANOVA with the permutation significance test instead of the parametric method; presence/absence data and the R function summary.aov.iv (Zelený & Schaffers 2012) with 999 permutations were used, and the Bonferroni correction of the P-values was applied. The computation was performed in R 3.1.2 (R Core Team 2014).

The main trends in the composition of the vegetation with *S. tuberosum* cytotypes were analysed using two approaches. First, fidelity (i.e. species concentration in vegetation units; Chytrý et al. 2002) was calculated in JUICE 7.0 using Fisher's exact test with a significance level $P < 0.01$ (Chytrý et al. 2002). Second, multivariate ordination techniques were employed using Canoco for Windows 4.5 (ter Braak & Šmilauer 2002). Data were exported from TURBOVEG using the van der Maarel transformation of species abundances from Braun-Blanquet to the ordinal scale (Kočí et al. 2003). The length of the gradient was tested in DCA. Because the length of the gradient was greater than 4 SD units, unimodal techniques were used (ter Braak & Šmilauer 2002). Canonical correspondence analysis (CCA; ter Braak 1986) was done using individual relevés as objects and the ploidy level as the sole explanatory variable. A Monte Carlo permutation test with 499 permutations was used to assess the significance of the explanatory variable (Lepš & Šmilauer 2003).

Results

Chromosome numbers, cytotype diversity and distribution

Two dominant cytotypes were found in the 271 populations sampled, corresponding to previously published tetraploid and dodecaploid chromosome counts and confirmed by our own chromosome counts (Figs 1 & 5, Tables 2 & 3). Tetraploids ($2n = 4x = 32$) were detected in central and southern Moravia, southern and south-western Slovakia and northern Hungary. For the Czech Republic and Hungary, this ploidy level is reported for the first time. Dodecaploids ($2n = 12x = 96$) occurred in all countries included in this study (Fig. 2).

The dodecaploid cytotype was more frequent and occurred at 70.8% (192 populations) of the localities sampled, while the tetraploid cytotype occurred at 27.7% of the localities (75 populations). Mixed populations were rare, occurring in only four cases (1.5% of all localities): three adjacent populations in south-eastern Moravia (the northern White Carpathians Mts) and one population in Slovakia (the Tríbeč hills). However, in some other areas at the northern limit of the tetraploids' distribution, both cytotypes occurred in close proximity (less than 1 km; Fig. 3). In addition to the dominant cytotypes, three minority ploidy levels were detected using FCM in certain populations (Tables 2 & 3). As the exact chromosome numbers of these cytotypes were not established, we refer to them henceforth as DNA-hexaploids (≈6x), DNA-decaploids (≈10x)

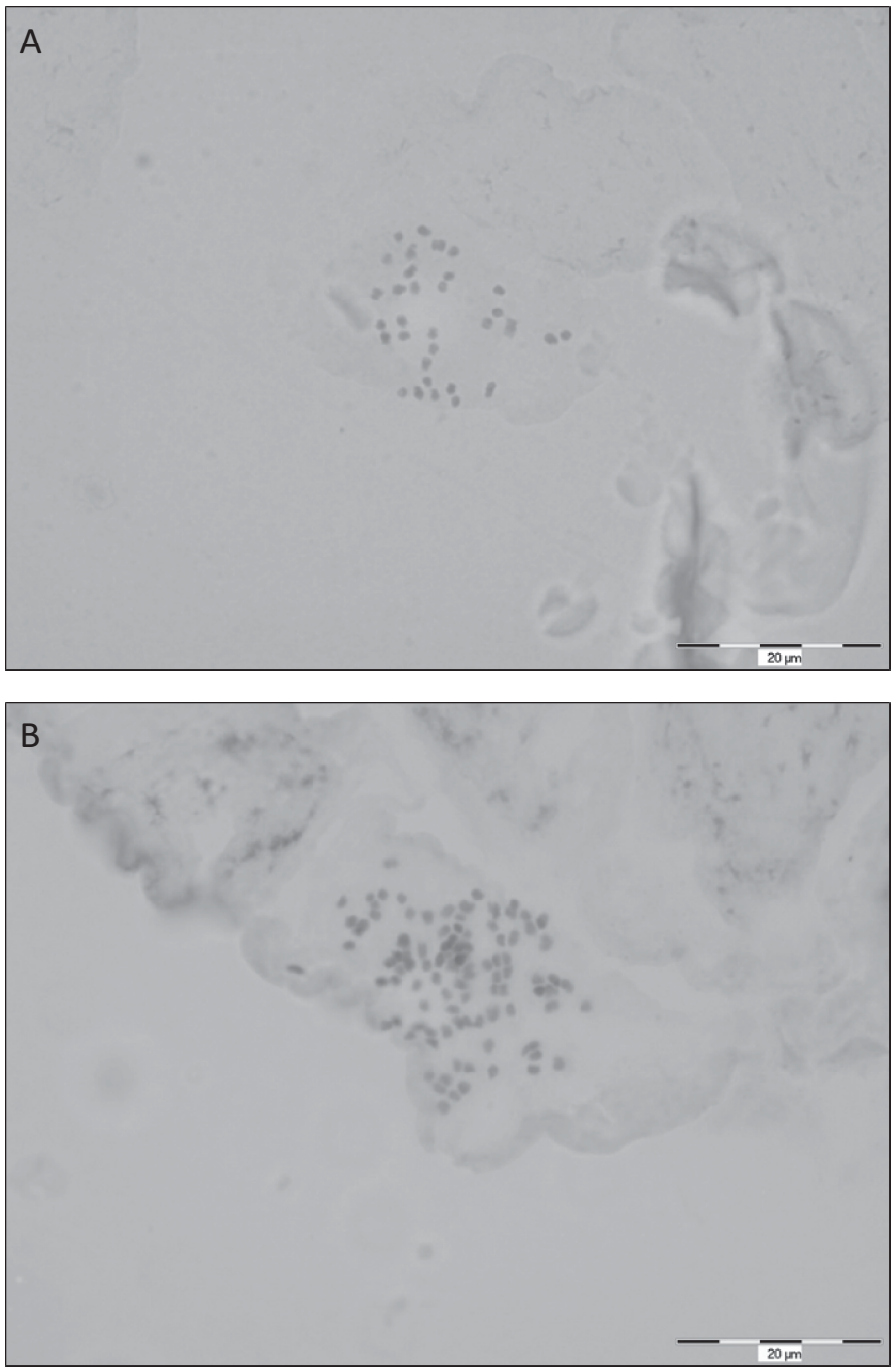

Fig. 1. – Mitotic metaphase chromosomes of (A) *Symphytum tuberosum* subsp. *angustifolium* (2n = 4x = 32; population 84, Velká nad Veličkou) and (B) *S. tuberosum* subsp. *tuberosum* (2n = 12x = 96; population 18, Tavíkovice).

Table 2. – Relative DNA content of the individual cytotypes of the *Symphytum tuberosum* complex in central Europe assessed using flow cytometry; the stain was PI. All values are calculated relative to the internal standard *Pisum sativum* 'Ctirad'. Recalculation to *Zea mays* 'CE-777' based on reciprocal calibration of the two standards using PI as a stain is also provided. Note that tetraploids and hexaploids were analysed with *Zea mays*; the result was then recalculated to *Pisum sativum*. N = number of samples analysed; SE = standard error of mean. Variation is calculated as the difference between the most extreme values expressed in % of the mean value.

DNA ploidy level	N	Standard = *Pisum sativum*			Standard = *Zea mays*
		Mean ratio to the standard ± SE	Range	Variation (%)	Calculated mean ratio to the standard
4x	381	0.246±0.010	0.221–0.278	22.9	0.373
6x	6	0.384±0.012	0.365–0.404	10.2	0.582
10x	13	0.584±0.019	0.569–0.598	12.7	0.889
12x	739	0.663±0.017	0.628–0.698	10.6	1.005
14x	17	0.762±0.010	0.748–0.783	4.5	1.155

Table 3. – Relative DNA content of the individual cytotypes of the *Symphytum tuberosum* complex in central Europe assessed using flow cytometry; DAPI was the stain. All values are calculated relative to the internal standard *Bellis perennis* (2C = 3.62 pg), which is given the unit value. Recalculation to *Pisum sativum* 'Ctirad' based on reciprocal calibration of the two standards using DAPI as the stain is also provided. Note that hexaploids overlap with *Bellis perennis* and were analysed with *Pisum sativum*; the result was then recalculated to *Bellis perennis*. N = number of samples analysed (note that the number of individuals measured is higher due to use of bulked samples); SE = standard error of mean. Variation is calculated as the difference between the most extreme values expressed in % of the mean value.

DNA ploidy level	N	Standard = *Bellis perennis*			Standard = *Pisum sativum*
		Mean ratio to the standard ± SE	Range	Variation (%)	Calculated mean ratio to the standard
4x	36	0.650 ± 0.003	0.623–0.702	12.2	0.279
6x	1	0.982			0.421
10x	1	1.539			0.660
12x	129	1.842 ± 0.003	1.746–1.954	11.3	0.790

and DNA-tetradecaploids (≈14x; Fig. 4; Tables 2 & 3). DNA-hexaploids were found admixed in populations of the tetraploid cytotype, whilst DNA-decaploids and DNA-tetradecaploids occurred within the dodecaploid populations. The minority cytotypes were rare (2.6% of all analysed individuals analysed). We also detected aneuploidy. An aneuploid chromosome number was successfully established for a plant from a dodecaploid population 5 (2n = 94). Given the associated variation in the mean relative florescence both between and within populations of both dominant cytotypes (~23% for tetraploids and ~10% for dodecaploids), aneuploid plants may be quite frequent. In certain cases, the difference between individual plants was corroborated by bifurcated peaks in joint FCM analyses (Fig. 5).

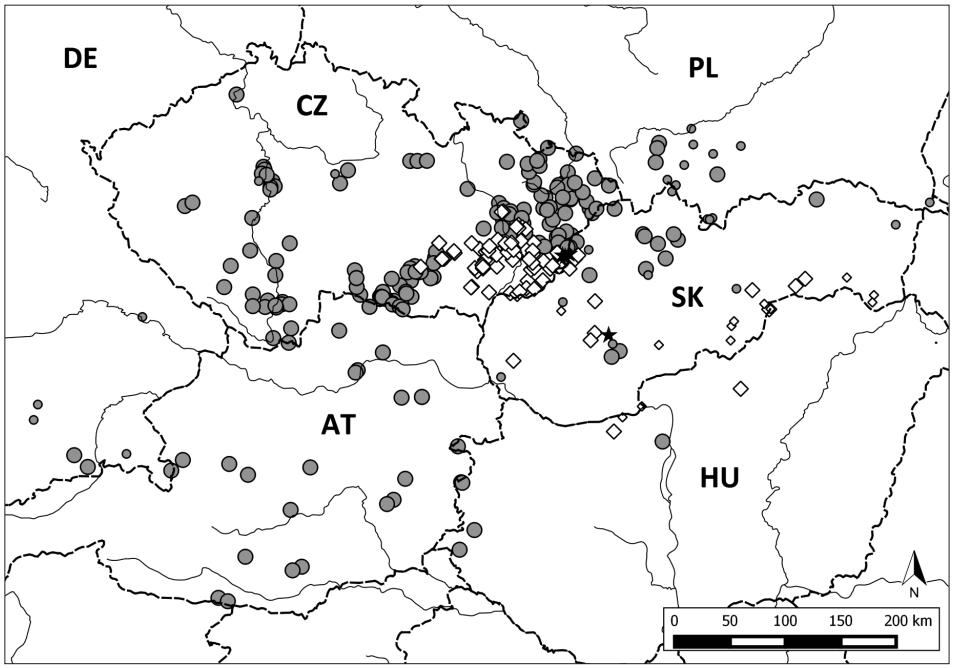

Fig. 2. – Map showing the locations of the populations sampled; ◇ *Symphytum tuberosum* subsp. *angustifolium*, ● subsp. *tuberosum*, ★ mixed populations of both subspecies. Additional localities from previous karyological studies by Grau (1968), Wcisło (1972), Májovský (1976), Gadella & Kliphuis (1978), Murín & Májovský (1982), Javůrková-Jarolímová & Měsíček (1992) and Lippert (2006) are indicated by smaller symbols.

Morphometric analyses

In total, 522 plants from 40 populations were used for the morphological analyses. No pairs of highly correlated characters ($r > |0.95|$) were found, and thus, the entire dataset was used in the multivariate analyses. The two dominant cytotypes significantly differed in most of the morphological characters studied, except for the length of the lowermost leaf, length of pedicel and length of calyx (see Table 1, Fig. 6). However, most of the ranges in variation overlap and no single character can be used for unambiguous determination of the cytotypes.

The only qualitative character used in our morphometric study ('branching of stem') was separately analysed using a contingency table. The pattern of branching was significantly different between the two cytotypes (Pearson's $\chi^2 = 57.67$; DF = 3; $P < 0.01$), although all character states were found in both cytotypes and only their frequencies were somewhat different. Thus, this character can only be used as a supplement to quantitative characters. The two cytotypes differ in the proportion of unbranched individuals (23% of tetraploids, 53% of dodecaploids). The frequency of plants branched in the upper part is similar (9% tetraploids and 8% of dodecaploids), while the frequency of individuals that branched in the middle or lower part of the stem was higher in tetraploids (18% and 50%, respectively) than dodecaploids (4% and 35%, respectively).

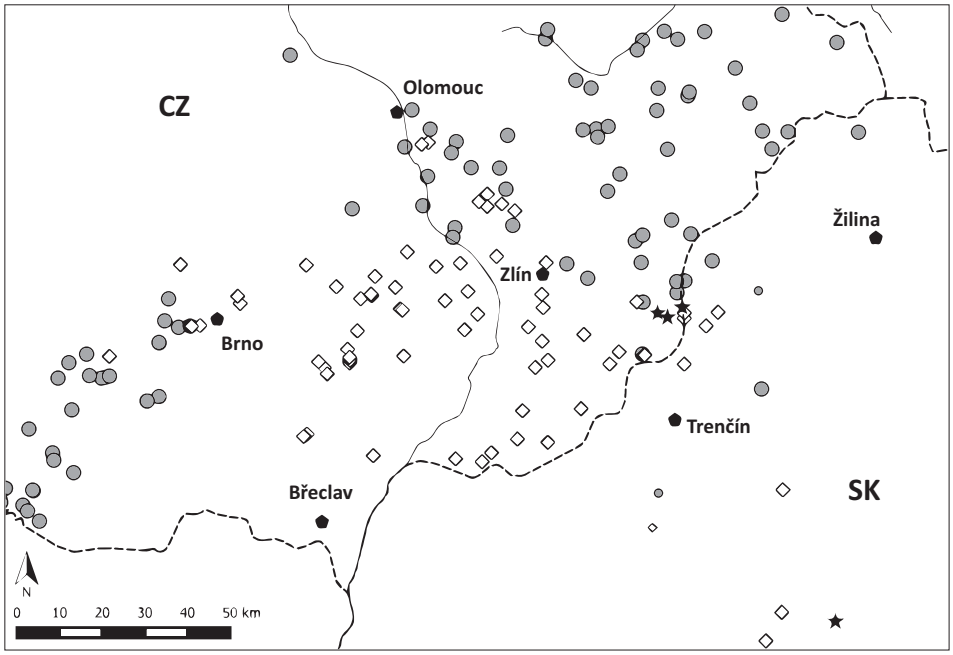

Fig. 3. – A detailed map of the contact zone between the two subspecies of *Symphytum tuberosum* in Moravia and western Slovakia; ◇ *Symphytum tuberosum* subsp. *angustifolium*, ● subsp. *tuberosum*, ★ mixed populations of both subspecies.

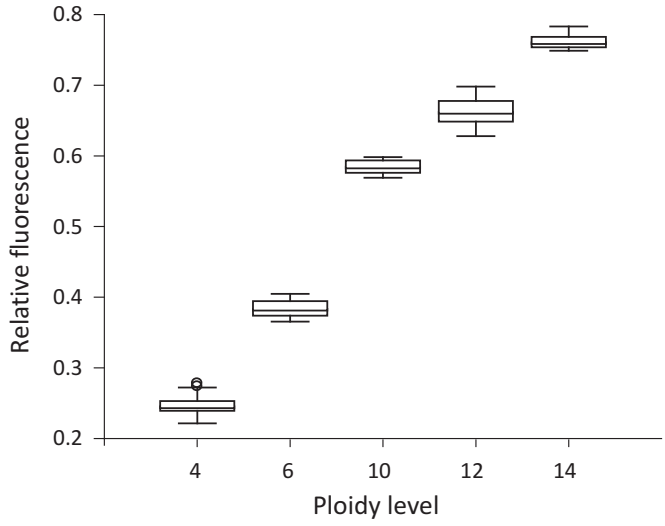

Fig. 4. – Relative fluorescence (ratio to the internal standard *Pisum sativum* 'Ctirad') for individual cytotypes of the *Symphytum tuberosum* complex in central Europe assessed using flow cytometry; the stain was PI (4x – 381 samples, 6x – 6 samples, 10x – 13 samples, 12x – 739 samples, 14x – 17 samples). Median, quartiles, non-outlier range and outliers are depicted.

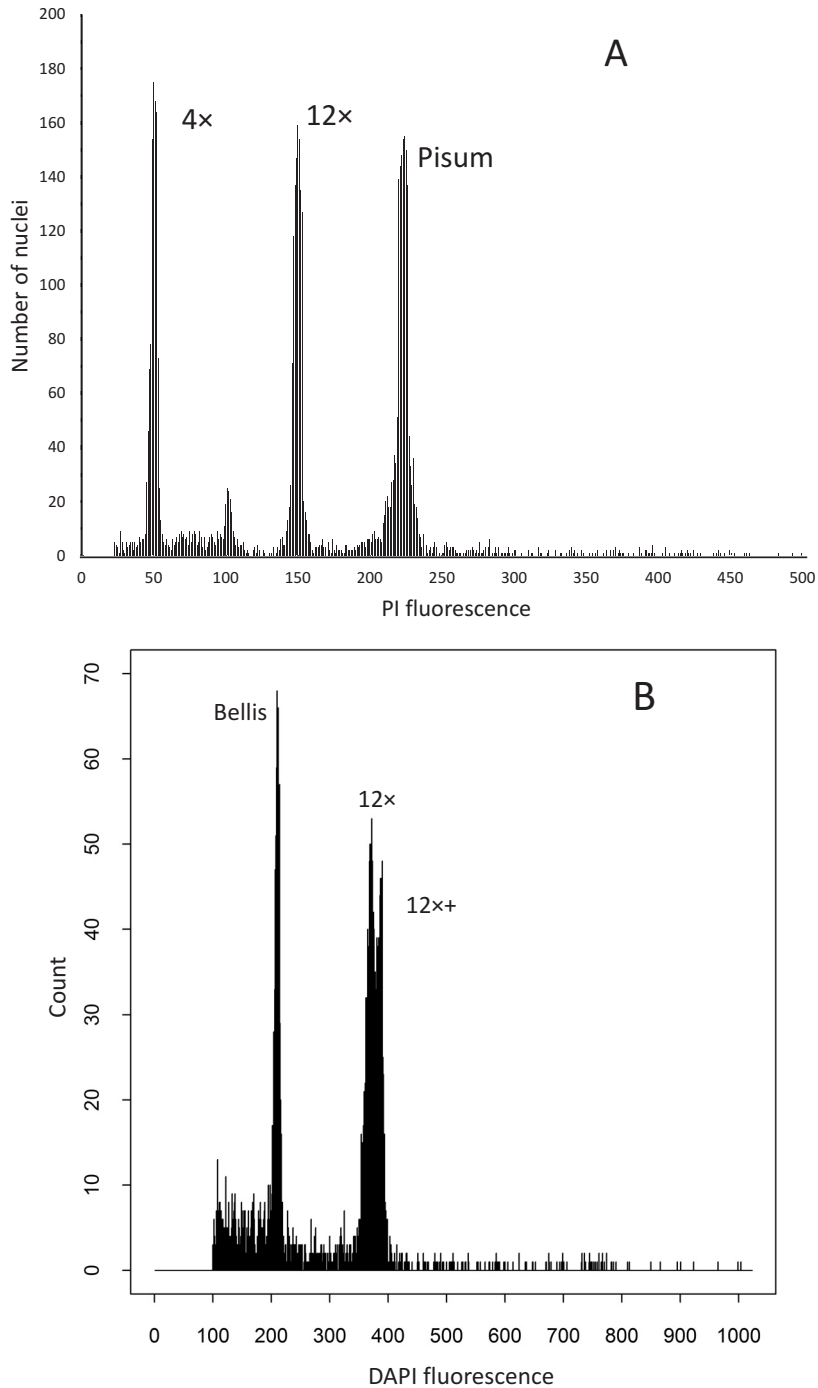

Fig. 5. – Flow cytometric histograms of the relative DNA content of: (A) simultaneous analysis of PI-stained nuclei isolated from tetraploid and dodecaploid plants of the *Symphytum tuberosum* complex, with the internal standard *Pisum sativum* 'Ctirad', (B) DAPI-stained nuclei showing variation in the relative fluorescence of two individuals of the dodecaploid ploidy level with *Bellis perennis* as the internal standard.

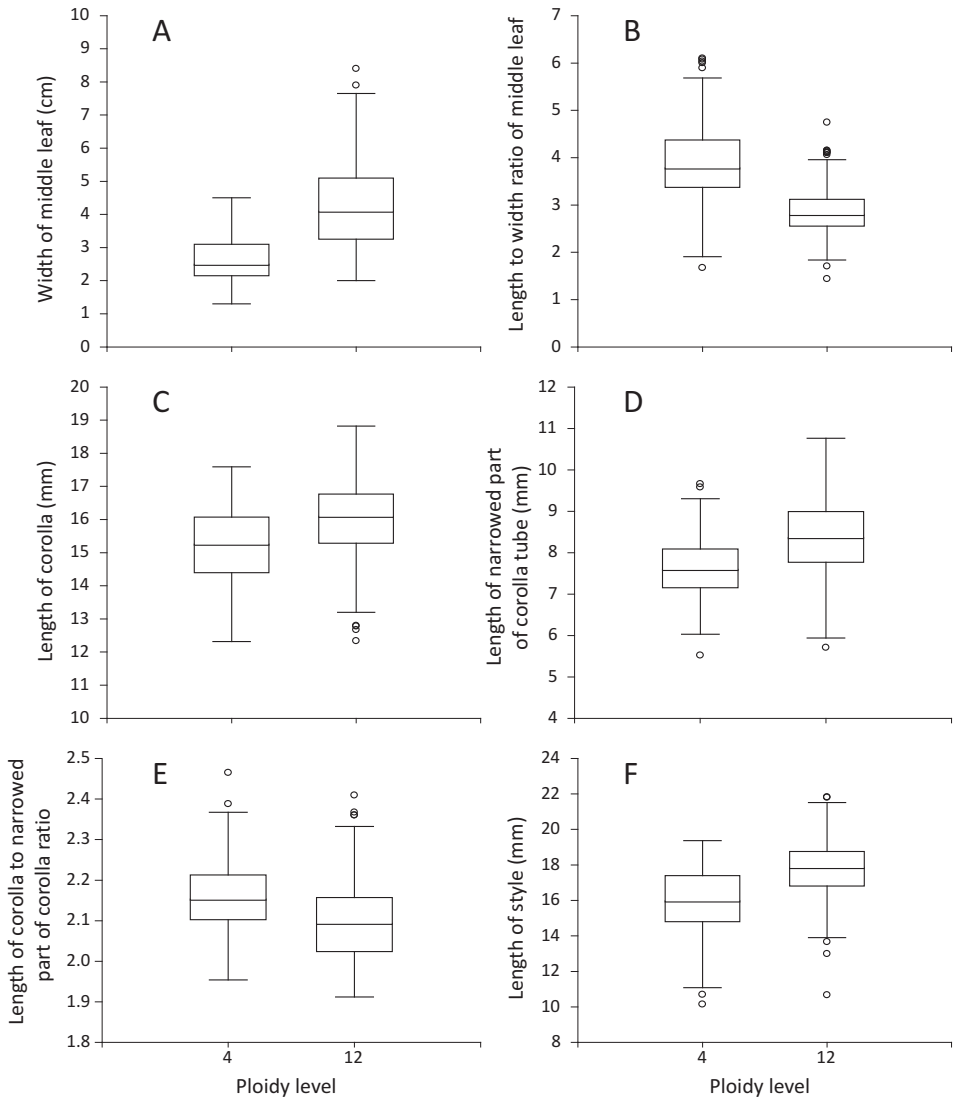

Fig. 6. – Variation in the quantitative characters of tetraploid and dodecaploids cytotypes of the *Symphytum tuberosum* complex in central Europe: (A) width of the middle stem leaf, (B) length to width ratio of the middle stem leaf, (C) length of the corolla, (D) length of narrow part of the corolla tube, (E) ratio of the length of corolla to that of the narrow part of the corolla and (F) length of the style.

PCA based on individuals (Fig. 7) revealed partial separation of the dominant cytotypes. The characters most correlated with the first component were the widths of the uppermost, middle and lowermost leaves and lengths of the uppermost and middle leaves, whereas the ratios of the length of the corolla to the narrow part of the corolla, the length to width of the lowermost leaves and the height of the plant were mainly correlated with the second component. Canonical discriminant analysis (CDA) based on individual

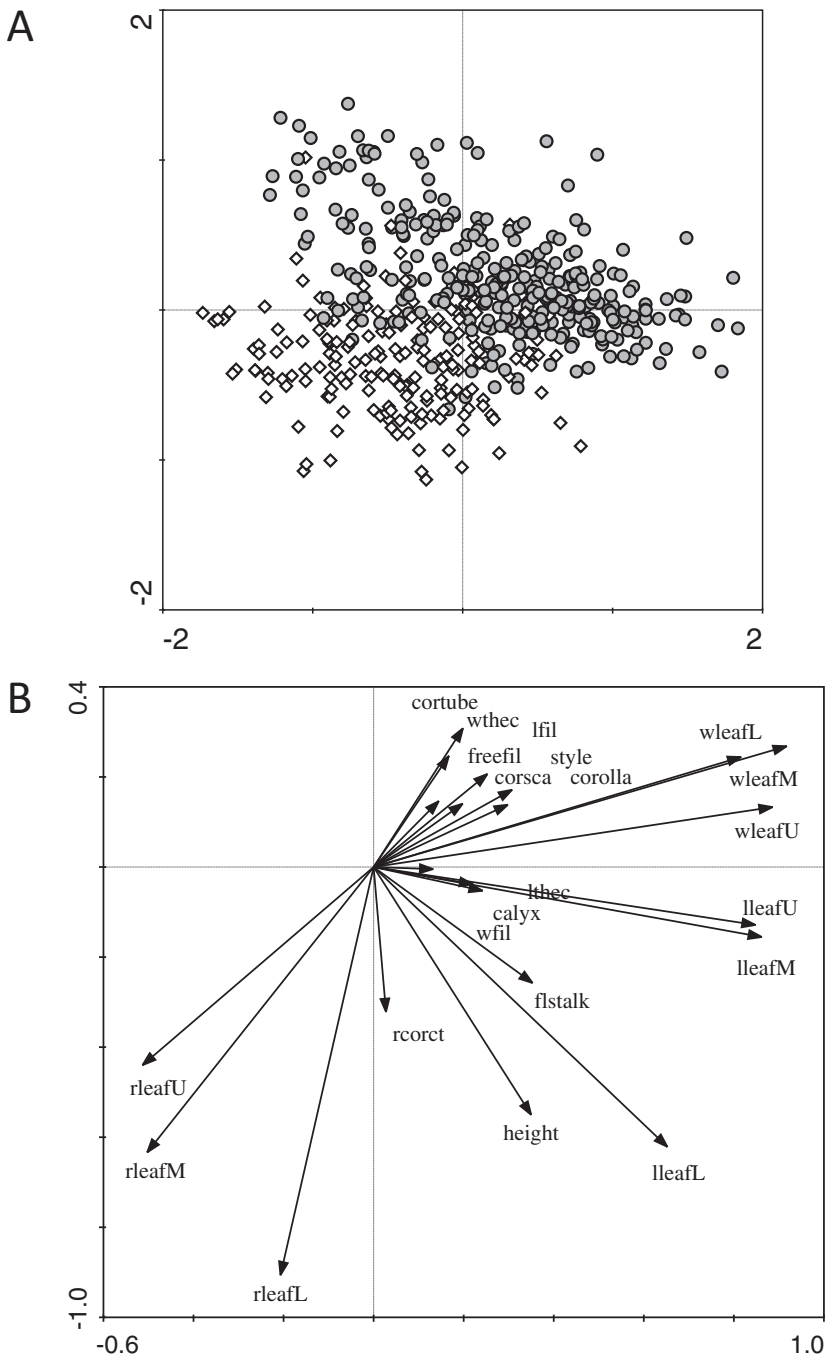

Fig. 7. – PCA of morphological characters of the two cytotypes of *Symphytum tuberosum*: (A) distribution of individuals in ordination space (◇ 4x, ● 12x), (B) fit of the 18 morphological characters and four ratios studied to the ordination axes (abbreviations of morphological characters explained in Table 1). The first and the second ordination axis explain 48% and 20% of the variation, respectively.

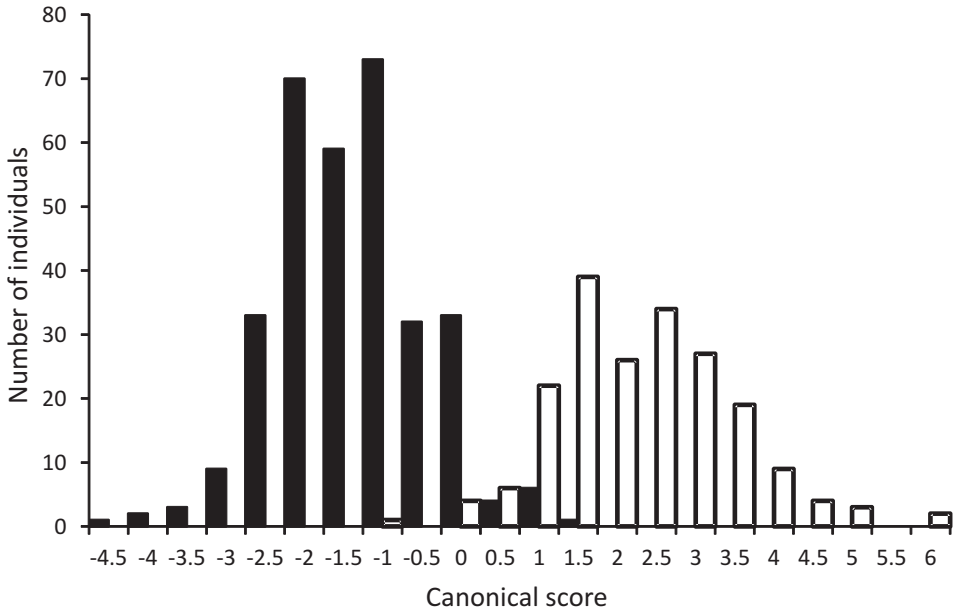

Fig. 8. – Histogram of canonical scores of linear discriminant analysis of individuals (n = 522) of the *Symphytum tuberosum* complex in central Europe (□ tetraploids; ■ dodecaploids). All quantitative characters were used in this analysis.

plants resulted in a clear morphological separation between the two cytotypes (F = 76; num. DF 22; denum. DF 499; P < 0.01; Fig. 8). The greatest weight included the length of the corolla to the narrow part of the corolla ratio, the length of the corolla and the width of the filament. The parametric method of classificatory discriminant analysis based on probability models resulted in a high number of plants being correctly classified to the cytotype (97.0% of tetraploids and 97.2% of dodecaploids).

Ecological differences of cytotypes

Altogether, 520 phytosociological relevés (162 with tetraploids and 358 with dodecaploids) were analysed. All relevés were successfully classified by the expert system. Tetraploids were present mostly in oak-hornbeam forests, thermophilous and acidophilous oak forests and beech forests of the *Carici pilosae-Fagetum sylvaticae* association (in contrast to dodecaploids; see Table 4) and in semi-dry grasslands and herbaceous communities along forest edges. Dodecaploids were present mostly in humid, broad-leaved floodplain forests, ravine and cliff forests, mesic and nutrient-rich beech and coniferous forests. They also occurred in the vegetation along river banks, on gravel deposits and alluvial sediments. In addition, they pervaded ruderal vegetation and disturbed forest sites. In Bohemia, dodecaploids were also common in thermophilous oak and oak-hornbeam woodlands (particularly *Galio sylvatici-Carpinetum betuli*; Table 4).

The two sets of relevés with individual cytotypes significantly differ in Ellenberg's indicator values for temperature (P = 0.030 after Bonferroni correction), moisture (P = 0.042) and nutrients (P = 0.006); other indicator values would be insignificant even

Table 4. – The most frequent vegetation units with subspecies of *Symphytum tuberosum* in the Czech Republic based on the classification of 520 phytosociological relevés (162 with tetraploids and 358 with dodecaploids). The percentage of both subspecies in each unit is displayed.

Vegetation unit	*S. tuberosum* subsp. *angustifolium*	*S. tuberosum* subsp. *tuberosum*
Alnion incanae	1.2%	19.0%
Carpinion betuli	55.9%	17.9%
Fagion sylvaticae		
as. *Carici pilosae-Fagetum sylvaticae*	13.7%	1.1%
other associations	–	18.2%
Tilio platyphylli-Acerion	0.6%	16.5%
Quercion petraeae	9.9%	–
Quercion roboris	1.7%	4.3%
Petasition hybridi	–	4.5%
Aegopodion podagrariae	–	8.9%
Fragarion vescae	–	2.5%
Bromion erecti	5.6%	0.3%
Trifolion medii	2.5%	0.3%

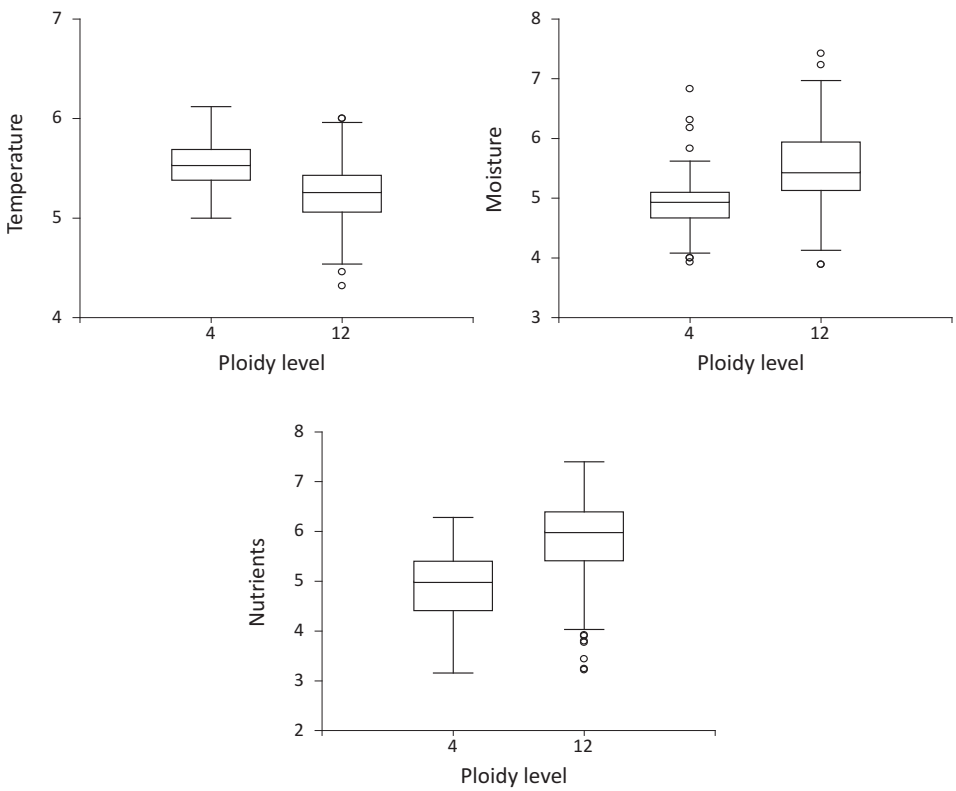

Fig. 9. – Selected average Ellenberg's indicator values of vegetation plots from the Czech Republic containing *Symphytum tuberosum* subspecies/cytotypes.

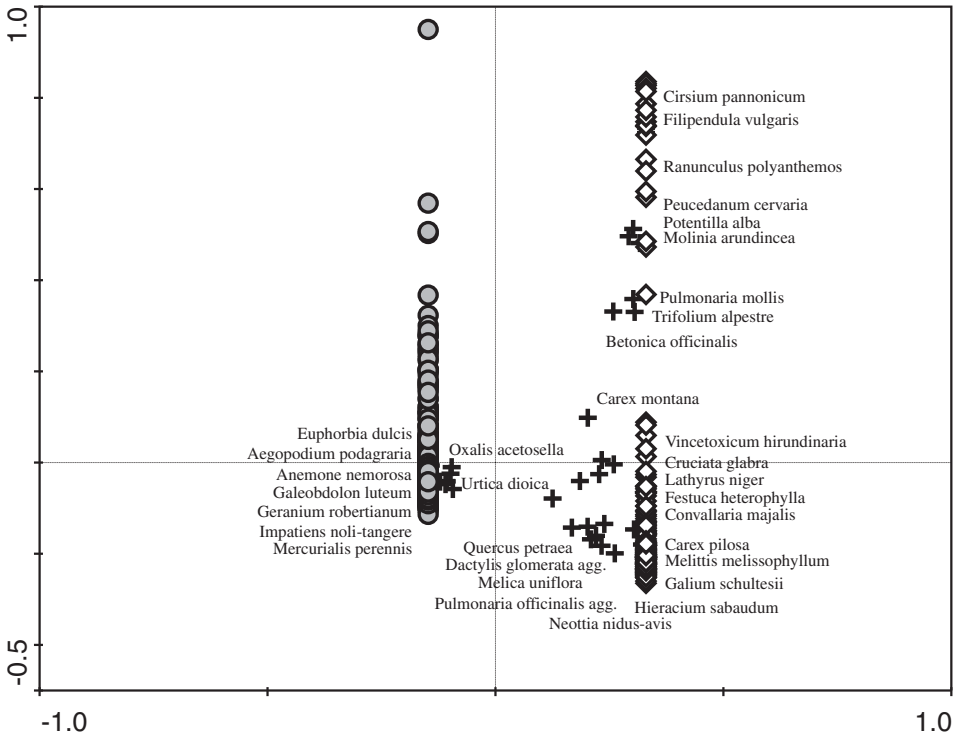

Fig. 10. – Canonical correspondence analysis (CCA) of phytosociological relevés from the Czech Republic (● relevés with 12x cytotype, ◇ relevés with 4x cytotype) with 33 most important species displayed (i.e. species with best fit to the first axis, depicted by black crosses).

without the application of the Bonferroni correction. Tetraploids grew at sites with, on average, higher temperature and lower moisture and nutrients, than the dodecaploids (Fig. 9).

The plants with the highest fidelity (> 45) to relevés with tetraploids were *Carex pilosa*, *Quercus petraea* agg. and *Lathyrus niger*, and the highest fidelity of those to relevés with dodecaploids included *Urtica dioica* (see Electronic Appendix 1 for more species). The main differences in the composition of the vegetation with *S. tuberosum* cytotypes were also confirmed by the CCA (P = 0.002, F = 8.31; Fig. 10). Nitrophilous, hygrophilous and sciophylous species, such as *Urtica dioica*, *Aegopodium podagraria*, *Geranium robertianum*, *Galeobdolon luteum* agg. and *Anemone nemorosa*, correlated most strongly with the presence of dodecaploids. Among relevés associated with tetraploids, two groups of species were identified, the first are species of thermophilous woodlands (*Lathyrus niger*, *Melittis melissophyllum* and *Carex montana*), and the second those of slightly thermophilous grasslands (e.g. *Betonica officinalis*, *Potentilla alba* and *Filipendula vulgaris*).

Discussion

Cytotype distribution

A detailed screening of the cytotype diversity of the *Symphytum tuberosum* complex in central Europe revealed two dominant (tetraploid and dodecaploid) and three minority cytotypes (DNA-hexaploids, DNA-decaploids and DNA-tetradecaploids). The dominant cytotypes correspond well with the previously published chromosome counts (Wcisło 1972, Murín & Májovský 1982, Javůrková-Jarolímová & Měsíček 1992). Overall, the cytotype diversity of the *S. tuberosum* complex is similar to other thoroughly studied polyploid complexes in the central-European flora, such as *Senecio carniolicus* (Suda et al. 2007) and *Gymnadenia conopsea* (Trávníček et al. 2012), both with five cytotypes, or *Allium oleraceum* (Duchoslav et al. 2013), with four cytotypes.

The most frequent cytotype of the *S. tuberosum* complex in central Europe is dodecaploid, which occurs throughout the whole area. Apparently, it is the prevalent cytotype throughout the whole distribution range of the complex (e.g. Grau 1968, Wcisło 1972, Gadella & Kliphuis 1978, Murín & Májovský 1982, Luque 1989). In contrast, tetraploids are only recorded in Slovakia (Murín & Májovský 1982). In this paper, we record them for the first time from two other adjacent countries (Czech Republic, Hungary). Based on published chromosome counts for the whole *S. tuberosum* complex and our own unpublished data from different parts of Europe, it seems that tetraploids might be a very restricted central-European element, with a possible overlap into the Balkans.

Within the area studied, the distribution of the cytotypes is parapatric and mirrors their different habitat preferences. Dodecaploids do not occur in the warmest and driest part of the area studied, which is at the periphery of the Pannonian Lowlands in southern Moravia (south-eastern part of the Czech Republic) and south-western and southern Slovakia. Nevertheless, the rare occurrence of dodecaploids in the Pannonian Lowlands cannot be completely ruled out, as there are reports of *S. tuberosum* s.l. from alluvial forests along large rivers (e.g. Májovský & Hegedüšová 1993) that might represent dodecaploid plants dispersed by floods from higher altitudes; during this study, however, we failed to find any of these small and probably temporary populations. In contrast, tetraploids mainly occur in hilly (not flat) landscapes at the northern border of the Pannonian Lowlands and the lower parts of the Western Carpathians and from a phytogeographic perspective may be considered to be a Matrian-Praecarpathian floristic element (similar to e.g. *Chamaecytisus virescens*, *Dorycnium pentaphyllum* s.l., *Glechoma hirsuta*, *Iris graminea* or *Pseudolysimachion orchideum*). Due to sparse sampling, the exact southern and especially eastern limits of the tetraploids' distribution require further study.

In the narrow contact zone, the two dominant cytotypes are spatially intermixed, but only four mixed populations (three in the Czech Republic and one in Slovakia) were discovered, despite intensive sampling. This result may be due to the different habitat requirements of the cytotypes (see below). In mixed populations, no intermediate (octoploid) individuals were detected, which might indicate limited or no gene flow between the two cytotypes. A similar parapatric distribution due to (at least partly) different habitat requirements with only rare mixed populations and breeding barriers between the cytotypes is reported for other polyploid complexes: for example, in central Europe,

the *Aster amellus* complex (Münzbergová et al. 2013), the *Centaurea phrygia* group (Koutecký et al. 2012) or *Galium valdepilosum* (Kolář et al. 2014).

Differences in the ecology of the cytotypes

Numerous studies also report that cytotypes in a wide range of polyploid complexes differ ecologically (e.g. Rothera & Davy 1986, Hülber et al. 2009, Kolář et al. 2014). Cytogeographic data (see above) and our detailed analysis of phytosociological relevés from the Czech Republic indicate there is also a clear differentiation in the *S. tuberosum* complex. The latter is consistent with the results of the study by Murín & Májovský (1982) in Slovakia, which indicates that the tetraploid cytotype is a typical element of thermophilous oak and oak-hornbeam woodlands, whereas the dodecaploid cytotype is a sub-montane or montane element of beech forests with only occasional occurrence in oak woodlands. The four mixed populations all occurred in the contact zones between semi-dry meadows (three cases) or oak forests (one case; typical habitat of tetraploids) and more mesophilous vegetation along small streams (marginal habitat of dodecaploids). Finally, there is a habitat differentiation in the altitudinal distributions of the cytotypes. Tetraploids occur in warmer areas and only rarely occur at higher altitudes (in this study the recorded maximum is 710 m a.s.l. in the Mátra Mts), whereas dodecaploids frequently grow in colder areas and at high altitudes; in the Slovakian Carpathians, Májovský & Hegedüšová (1993) record a maximum of ~1650 m a.s.l., in this study, the locality Turracher Höhe in Austria is at 1725 m a.s.l., and some populations in the Alps may occur at even higher altitudes.

Minority cytotypes and aneuploidy

Three minority cytotypes were rarely detected in populations of the two dominant cytotypes. In four out of the 75 tetraploid populations studied (see Appendix 1), DNA-hexaploid plants were discovered. The origin of DNA-hexaploids can be explained by the fusion of an unreduced and a reduced gamete produced by tetraploids. An analogous scheme seems to occur quite frequently in plants and is assumed, for example, in mixed 4x + 6x populations of *Allium oleraceum* (Šafářová & Duchoslav 2010), *Hypericum perforatum* (Qu et al. 2010) and *Molinia caerulea* (Dančák et al. 2012). Within dodecaploid populations, DNA-decaploid (in seven populations) and DNA-tetradecaploid (in 10 out of 192 populations) plants were detected. There is no simple explanation of their origin as in the case of DNA-hexaploids, and either the presence of other undiscovered cytotypes or aneuploidy/dysploidy must be hypothesized. Dysploidy is expected to be important in the karyotype evolution of several genera of *Boraginaceae*, such as *Nonea* (Selvi & Bigazzi 2002) and *Pulmonaria* (Sauer 1987). Moreover, we recorded significant variation in relative fluorescence reflecting genome size variation in both dominant cytotypes. This finding could be explained by the occurrence of aneuploidy or the presence of B chromosomes, both of which are recorded in *Symphytum* (Grau 1971, Gadella & Kliphuis 1978). Indeed, aneuploidy in dodecaploids was directly confirmed by the chromosome count of 2n = 94, recorded for one divergent individual. In general, this is more likely to occur in high-level polyploids because they possess multiple gene copies, and the gain/loss of some of them may not have a serious effect on individual viability (Leitch & Leitch 2008).

Morphological differences between cytotypes

Polyploidy is a common source of taxonomical problems, mainly due to the formation of difficult polyploid complexes/series and/or the origin of taxa by reticulate evolution (Rieseberg 1991, Marhold & Lihová 2006, Soltis et al. 2009). In many cases, cytotypes of polyploid complexes do not clearly differ morphologically, such as in *Allium oleraceum* (Fialová et al. 2014), *Juncus bufonius* (Rooks et al. 2011) and *Pseudolysimachion maritimum* (Trávníček et al. 2004). In such cases, individual cytotypes are usually not recognized as autonomous taxa. Even if there is some morphological differentiation among cytotypes, there may be no correlation between quantitative and other characters, and the ploidy level (e.g. the *Centaurea phrygia* group, Koutecký et al. 2012). In contrast, there is a clear trend of enlarging organs with increasing ploidy level in some polyploid complexes (e.g. the *Cerastium pumilum* group, Letz et al. 2012; the *Molinia caerulea* complex, Dančák et al. 2012). Our results indicate that the *S. tuberosum* complex also fits the latter case: tetraploids have narrower leaves, smaller corollas, shorter styles, and so on (Table 1), although a certain overlap occurs, mostly because of the greater morphological variability of the dodecaploid cytotype. In general, our results are similar to those of Májovský & Hegedüšová (1993) for generative characters, but the vegetative characters are more variable in both cytotypes than previously reported; this can be explained by the more extensive sampling used in this study.

Taxonomic treatment of Symphytum tuberosum in central Europe

Symphytum tuberosum s.l. is a morphologically and karyologically variable complex (Gadella & Kliphuis 1978, Murín & Májovský 1982). In addition to the ploidy levels revealed in this study, other cytotypes exist in south-eastern and southern Europe (e.g. Grau 1968, Markowa & Iwanowa 1970, Jaarsma et al. 1990, Bottega et al. 2001). There seems to be no clear relation between karyology and taxonomic classification on a European scale; instead, western-European plants are classified as *S. tuberosum* subsp. *tuberosum* and central- and eastern-European plants as *S. tuberosum* subsp. *angustifolium/nodosum* (Pawłowski 1972, Bottega & Garbari 2003, Valdés 2011). In contrast, both the study of Murín & Májovský (1982) and this study clearly show that there are two elements in central Europe that merit taxonomic classification, which correspond to the tetraploid and dodecaploid cytotypes. Thus, the current taxonomic treatment of the whole complex is challenged and needs to be revised.

Two dominant cytotypes of the *Symphytum tuberosum* complex in central Europe can be recognized based on morphology and differ in their habitat requirements and geographic distribution. Their distribution is parapatric, with a narrow contact zone. Mixed populations are rare, and no intermediate (hybrid) cytotypes were discovered. However, because the ranges in the variation of most of the morphological characters of the different cytotypes overlap and their habitat requirements are not completely distinct, we propose treating the cytotypes as subspecies.

Murín & Májovský (1982) suggest that the widespread dodecaploid cytotype is *S. tuberosum* L., 1753, s. str., i.e. the type subspecies in our treatment. This view is consistent with the original location in the protologue ("Germania australi") for which dodecaploid chromosome counts are known (surroundings of München, Grau 1968; however, more chromosome counts from this area are needed) and also with the morphology of the

lectotype (LINN 185.3; but note that plants could have originated from cultivation; Pugsley 1931). For tetraploids, Murín & Májovský (1982) propose the name *S. angustifolium* A. Kern., 1863 [*S. tuberosum* subsp. *angustifolium* (A. Kern.) Nyman, 1881], of which the type material (WU 69896–69899, high-resolution images available at http://herbarium.univie.ac.at) collected by Anton Kerner in the Pilis Mts (northern Hungary) corresponds well with the tetraploids. The name *S. angustifolium* is sometimes synonymized with *S. nodosum* Schur, 1866 [*S. tuberosum* subsp. *nodosum* (Schur) Soó, 1941], based on plants from southern Romania. The latter name, however, probably belongs to a different ploidy level and cannot be applied to central-European tetraploids. Moreover, even if it belonged to the same taxon, the names *S. angustifolium* and *S. tuberosum* subsp. *angustifolium* have priority in their respective ranks.

Identification key and taxonomic treatment of the *Symphytum tuberosum* group in central Europe

The central-European subspecies of *S. tuberosum* can be identified using the following key. If possible, several plants from a population should be studied and the average values used.

1a Rhizome stout; stem fleshy, thick; leaves elliptic, broadly ovate to ovate lanceolate, obtuse to acute; middle stem leaves 8–15.5 cm long and 2.5–5 cm wide, 2.3–3.5× long as wide; corolla yellow to dark yellow, somewhat robust, with lower narrowed part of the tube 7.3–9.5 mm long; style 15.8–19.8 mm long ..... ***S. tuberosum* subsp. *tuberosum***

1b Rhizome rather slender; stem rather thin; leaves ovate lanceolate to narrowly lanceolate, acuminate; middle stem leaves 7–13 cm long and 1.6–3.6 cm wide, 3–4.8× long as wide; corolla pale yellow, smaller, with lower narrowed part of the tube 6.7–8.4 mm long; style 13.5–18.2 mm long ..... ***S. tuberosum* subsp. *angustifolium***

Symphytum tuberosum Linnaeus, Sp. Pl. 136, 1753.

Typus: LINN 185.3 (lectotypus Stearn 1985: 177).

Symphytum tuberosum L. subsp. *tuberosum* (Fig. 11A–C)

Description: Perennial plants. Rhizomes stout, creeping, horizontal to oblique, tuberous. Stem 20–41 (–52) cm tall, erect, fleshy, roughly hairy, simple or branched. Lower leaves petiolate, upper leaves sessile. Leaf blade of middle stem leaves elliptic, broadly ovate to ovate lanceolate, (5.1–) 8.0–15.5 (–19.3) cm long, (2.0–) 2.5–5 (–8.4) cm wide, 2.3–3.5 × long as wide, obtuse to acute, densely hairy. Corollas dark yellow, (12.3–) 14.5–17.5 (–18.8) mm long, with lower narrowed part of the tube (5.7–) 7.3–9.5 (–10.8) mm long. Style (10.7–) 15.8–19.8 (–21.8) mm long. Filaments (0.5–) 0.8–1.1 (–1.3) mm long, anthers (0.25–) 0.30–0.38 (–0.44) mm long. Fornices triangular, (0.9–) 1.1–1.5 (–1.7) mm long. Mericarpids dark brown, densely verrucose. Flowers from late April to early June. 2n = 12x = 96; DNA-decaploid and DNA-tetradecaploid individuals are rarely detected within populations using FCM.

Distribution: Europe except for Scandinavia, rarely in western Mediterranean regions (southern border is unclear due to unresolved taxonomy of the other ploidy levels). In central Europe, it is widespread in Austria, the Czech Republic, southern Poland, northern Slovakia and western Hungary, but rare or absent in the warmest and driest areas, such as the Pannonian Lowlands.

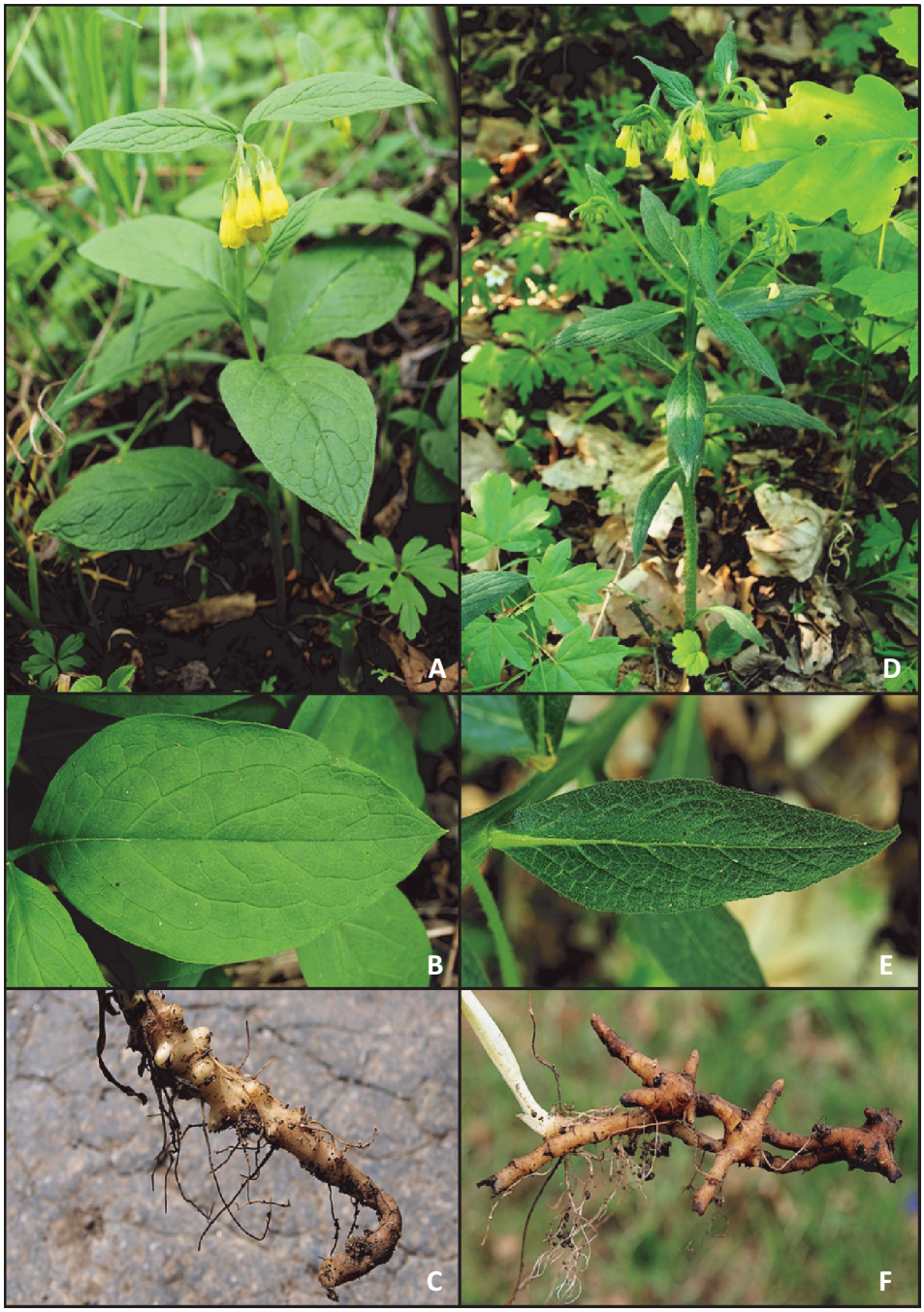

Fig. 11. – Typical plants of *Symphytum tuberosum* subsp. *tuberosum* (A – general habit, B – middle stem leaf, C – rhizome) and subsp. *angustifolium* (D – general habit, E – middle stem leaf, F – rhizome).

E c o l o g y: Mesic deciduous woodlands, usually in shady places with humid soils, also along streams and on river alluvia or in ruderal vegetation.

Symphytum tuberosum subsp. *angustifolium* (A. Kern.) Nyman, Consp. Fl. Eur. 510, 1881. (Fig. 11 D–F)

Typus: Hungary, Pilis, Slanitzka bei Csaba, Kerner, s.d., WU 0069897 (lectotypus Bottega & Garbari 2003: 247).
≡ *Symphytum angustifolium* A. Kern., Österr. Bot. Z. 13: 227, 1863.
= *Symphytum tuberosum* auct. medioeur. pro parte

D e s c r i p t i o n: Perennial plants. Rhizomes slender, creeping, oblique, interruptedly tuberous. Stem 24–43 (–52) cm tall, erect, with short appressed rough hairs throughout, simple or branched from the middle or from the base. Lower leaves long petiolate, upper almost sessile. Petioles narrowly winged and shortly descending to the stem. Leaf blade of middle stem leaves ovate lanceolate to narrowly lanceolate, (4.2–) 7–13 (–19) cm long, (1.3–) 1.6–3.6 (–4.5) cm wide, 3.0–4.8 × long as wide, acuminate, sparsely to densely roughly hairy. Peduncles hairy, hairs often with bulbous base. Corollas pale yellow, (12.3–) 13.9–16.8 (–17.6) mm long, with lower narrowed part of the tube (5.5–) 6.7–8.4 (–9.7) mm long. Style (10.2–) 13.5–18.2 (–19.4) mm long. Filaments (0.6–) 0.7–1.0 (–1.1) mm long, anthers (0.23–) 0.29–0.37 (–0.46) mm long. Fornices narrowly triangular, (0.9–) 1.1–1.4 (–1.5) mm long. Mericarpids light brown, shiny, finely wrinkled. Flowers from early May to early June. 2n = 4x = 32; DNA-hexaploid individuals are rarely detected within populations using FCM.

D i s t r i b u t i o n: Northern margin of the Pannonian Basin and adjacent part of the Western Carpathians; known from the Czech Republic (central and south-eastern Moravia), southern Slovakia and northern Hungary. For detailed information on the distribution in Slovakia, see Murín & Májovský (1982).

E c o l o g y: It is more thermophilous than the type subspecies, grows in deciduous forests (especially oak-hornbeam or Carpathian beech forests with *Carex pilosa*), at their fringes and in semi-dry grasslands, on intermittently wet soils.

See www.preslia.cz for Electronic Appendix 1

Acknowledgements

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Souhrn

Symphytum tuberosum agg. představuje vysoce polyploidní, taxonomicky poměrně komplikovanou skupinu. V rámci této skupiny bylo doposud publikováno nejméně 8 různých ploidních stupňů. Ze střední Evropy jsou uváděny pouze dvě ploidní úrovně, a to dodekaploidní (známa z celého území, většinou pod jménem *S. tuberosum* L.) a tetraploidní (uváděna pouze ze Slovenska pod jménem *S. angustifolium* A. Kern.). V rámci naší studie jsme pomocí průtokové cytometrie stanovili ploidní úroveň vzorků z 271 středoevropských populací z okruhu *S. tuberosum*. Celkem byly identifikovány dva hlavní ploidní stupně, a to tetraploidní (2n = 4x = 32)

a dodekaploidní (2n = 12x = 96). Tetraploidní cytotyp byl potvrzen na Slovensku a nově zaznamenán v České republice (jihovýchodní Morava) a v Maďarsku. Tetraploidní populace se nacházejí na jižním a jihozápadním okraji Západních Karpat a přilehlých partiích panonské pánve. Naopak dodekaploidní cytotyp byl výrazně častější a jeho výskyt byl prokázán ve všech studovaných státech. Většina populací byla karyologicky homogenní, podařilo se nalézt pouze čtyři smíšené populace. Studium morfologické variability a ekologických preferencí prokázalo signifikantní rozdíl mezi cytotypy. Na základě výsledků morfometrických analýz a při zohlednění ekologických nároků a rozšíření lze oba cytotypy hodnotit jako samostatné taxony. Z důvodu patrných morfologických a ekologických překryvů navrhujeme jejich klasifikaci na úrovni poddruhů: převládající dodekaploidní (2n = 96) širokolistý poddruh *S. tuberosum* L. subsp. *tuberosum*, a tetraploidní (2n = 32) matransko-předkarpatský taxon s užšími listy *S. tuberosum* subsp. *angustifolium* (A. Kern.) Nyman. V některých populacích byly navíc detekovány další minoritní (zřejmě opakovaně na místě vznikající) ploidní stupně, a to DNA-hexaploidi (v tetraploidních populacích), DNA-dekaploidi a DNA-tetradekaploidi (obojí v dodekaploidních populacích).

Středoevropské poddruhy kostivalu hlíznatého (*S. tuberosum*) lze určit dle následujícího klíče:

1a Oddenek tlustý, masitý; lodyha dužnatá; listy eliptické, široce vejčité až vejčitě kopinaté, tupé až lehce zašpičatělé; střední listy 8–15,5 cm dlouhé a 2,5–5 cm široké, 2,3–3,5 × delší než široké; koruna žlutá až tmavě žlutá, poněkud robustnější, zúžená část korunní trubky 7,3–9,5 mm dlouhá; čnělka 15,8–19,8 mm dlouhá ..... *S. tuberosum* L. subsp. *tuberosum*

1b Oddenek spíše tenký; lodyha tenká, méně dužnatá; listy vejčité až úzce kopinaté, dlouze zašpičatělé; střední listy 7–13 cm dlouhé a 1,6–3,6 cm široké, 3–4,8 × delší než široké; koruna světle žlutá, útlejší, zúžená část korunní trubky 6,7–8,4 mm dlouhá; čnělka 13,5–18,2 mm dlouhá .... *S. tuberosum* subsp. *angustifolium* (Kern.) Nyman

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Appendix 1. – List of populations sampled. The format of the entries is as follows: Locality number: country; number of phytogeographical district (for CZ and SK; Futák 1984, Skalický 1988); town (district), localization; coordinates (WGS 84); altitude; collector/s; collection date; number of analysed samples (all individuals were analysed using flow cytometry, morphologically analysed populations are denoted by *). Abbreviations of countries: AT – Austria, CZ – Czech Republic, DE – Germany, HU – Hungary, PL – Poland, SK – Slovakia. Abbreviations of collectors: BT – B. Trávníček, LK – L. Kobrlová, MH – M. Hroneš, PK – Petr Koutecký, MŠ – Milan Štech.

***Symphytum tuberosum* subsp. *angustifolium*: 2:** CZ; 77c; Koryčany (distr. Kroměříž), Moravanské lúky Nature Reserve, forest edge in the central part; 49°05'13.8"N, 17°11'38.6"E; 472 m a.s.l.; LK; 2. 5. 2011; 15*. – **6:** SK; 3; Jasov (distr. Košice-okolie), Jasovské dubiny Nature Reserve, dry broadleaved woodland near Jasovské rybníky ponds; 48°40'44.9"N, 20°57'38.9"E; 289 m a.s.l.; LK; 12. 5. 2011; 6*. – **7a:** SK; 3; Turňa nad Bodvou, (distr. Košice-okolie), Zádielská plošina plateau, grasslands near the blue touristic trail; 48°37'07.6"N, 20°51'04.9"E; 572 m a.s.l.; LK; 13. 5. 2011; 7*. – **7b:** SK; 3; Turňa nad Bodvou, (distr. Košice-okolie), Zádielská plošina plateau, open broadleaved woodland near the blue touristic trail near the gamekeeper's lodge; 48°37'57.4"N, 20°50'00.0"E; 582 m a.s.l.; LK; 24. 6. 2011; 6. – **8:** CZ; 78; Březová (distr. Uherské Hradiště), edge of the forest near the S border of the Kalábová Nature Reserve, ca 1 km N of the village; 48°56'22.7"N, 17°44'37.1"E; 538 m a.s.l.; LK & MH; 17. 5. 2011; 15*. – **9:** CZ; 78; Luhačovice (distr. Zlín), edge of the forest and ditches near the crossroads Solné, ca 1 km NE of the town; 49°05'34.4"N, 17°46'40.7"E; 410 m a.s.l.; LK & MH; 17. 5. 2011; 15*. – **11:** CZ; 68; Brno-Hády (distr. Brno), edges of the forest's path and the undergrowth of the open broadleaved woodland; 49°13'43.6"N, 16°41'10.3"E; 433 m a.s.l.; LK & MH; 19. 5. 2011 & 3. 5. 2012; 19*. – **12:** HU; -; Mátraháza (distr. Észak-Magyarország), forest under Kekés peak; 47°52'31.7"N, 19°59'18.2"E; 709 m a.s.l.; LK; 23. 6. 2011; 3. – **13:** SK; 2; Kečovo (distr. Rožňava), Domica cave, light forest edge under the grass-steppe, ca 400 m of the car parking; 48°28'40.8"N, 20°28'08.4"E; 354 m a.s.l.; LK; 24. 6. 2011; 3. – **16:** HU; -; Agostyán (distr. Central Transdanubia), the Gerecse Mts, forest on the left of the road to the village Tardos; 47°39'46.0"N, 18°24'06.9"E; 227 m a.s.l.; BT; 27. 7. 2010; 1. – **17:** CZ; 79; Pitín (distr. Uherské Hradiště), Pitínský vrch hill; 49°01'32.9"N, 17°51'01.0"E; 500 m a.s.l.; BT; 4. 7. 2010; 3. – **25:** CZ; 19; Hluk (distr. Uherské Hradiště), Horní louky meadows, undergrowth of the broadleaved woodland's enclave in the middle of grasslands; 48°56'53.9"N, 17°33'22.3"E; 327 m a.s.l.; LK; 14. 4. 2012; 5. – **26:** CZ; 21a; Beňov (distr. Přerov), E part of the small forest enclave ESE of the village; 49°24'19.1"N, 17°30'56.5"E; 307 m a.s.l.; LK; 20. 4. 2012; 5. – **32:** CZ; 77a; Těšanka, bus station (distr. Hodonín), oak-hornbeam forest near the crossroads of the road from Slavkov to Žarošice; 49°04'44.6"N 16°56'10.1"E; 411 m a.s.l.; LK; 3. 5. 2012; 5*. – **38:** SK; 12; Zlatno (distr. Zlaté Moravce); 48°28'15.9"N, 18°18'29.5"E; 380 m a.s.l.; PK; 1. 7. 2012; 2. – **40:** SK; 6; Kostolany pod Tribečom (distr. Zlaté Moravce), edge of the road near the abandoned vineyards towards to the Kostoľanské lúky Nature Reserve; 48°24'57.1"N, 18°14'53.8"E; 274 m a.s.l.; LK; 4. 7. 2012; 4. – **42:** CZ; 78; Štítná nad Vláří (distr. Zlín), forest under Javorník peak in the valley of the Zelenský potok rivulet; 49°02'15.6"N, 17°57'52.8"E; 513 m a.s.l.; MH; 17. 6. 2012; 3. – **43:** CZ; 78; Štítná nad Vláří (distr. Zlín), forest under Javorník peak in the valley of the Zelenský potok rivulet; 49°02'10.8"N, 17°57'52.6"E; 510 m a.s.l.; MH; 17. 6. 2012; 1. – **46:** CZ; 78; Návojná-Nedašov (distr. Zlín), Kaňoury Nature Reserve; 49°06'52.4"N, 18°06'24.6"E; 628 m a.s.l.; MH; 17. 6. 2012; 1. – **47:** SK; 27a; Vršatské Podhradie (distr. Ilava), edge of the broadleaved woodland; 49°04'57.1"N, 18°10'18.4"E; 555 m a.s.l.; LK; 20. 7. 2012; 1. – **84:** CZ; 78; Velká nad Veličkou (distr. Hodonín), shrubs, edges of the forest and the grasslands (alliance *Bromion*); 48°53'25.8"N, 17°31'50.3"E; 325 m a.s.l.; LK & MH; 1. 5. 2013; 15*. – **85:** CZ; 78; Radějov (distr. Hodonín), Žerotín hill, open, sparse broadleaved woodland and meadow's hillsides under the chapel; 48°51'46.1"N, 17°19'38.4"E; 313 m a.s.l.; LK & MH; 1. 5. 2013; 15*. – **86:** CZ; 77c; Dolina (distr. Uherské Hradiště), Kudlovická dolina valley, S edge of the forest on the N border of the village; 49°09'30.0"N, 17°26'40.9"E; 235 m a.s.l.; LK & MH; 1. 5. 2013; 5*. – **101:** CZ; 20a; Kunkovice (distr. Kroměříž), ditches of the forest near the road to Litenčice; 49°11'07.9"N, 17°11'52.3"E; 400 m a.s.l.; M. Dančák & M. Duchoslav; 15. 5. 2013; 4. – **112:** CZ; 19; Radějov (distr. Hodonín), Čertoryje Nature Reserve, grasslands; 48°51'03.8"N, 17°24'38.0"E; 347 m a.s.l.; MH; 25. 5. 2013; 4. – **124:** CZ; 79; Bohuslavice u Zlína-Doubravy (distr. Zlín), edge of the Oskorušný les forest between villages Bohuslavice and Doubravy; 49°09'27.8"N, 17°39'24.4"E; 394 m a.s.l.; LK; 19. 3. 2014; 3. – **126:** SK; 10; Vinosady (distr. Pezinok), S foothill of Kamenica peak; 48°19'04.0"N, 17°16'54.0"E; 226 m a.s.l.; BT; 22. 3. 2014; 3. – **141:** CZ; 19; Malá Vrbka (distr. Hodonín), edge of the forest under Výzkum hill; 48°52'4.7"N, 17°26'36.0"E; 397 m a.s.l.; LK & MH; 29. 3. 2014; 4. – **147:** CZ; 21a; Pacetluky (distr. Kroměříž), edges of the path in the light oak-hornbeam forest above the village; 49°22'55.3"N, 17°33'31.8"E; 328 m a.s.l.; LK & MH; 3. 4. 2014 & 19. 4. 2014; 20*. – **148:** CZ; 76a; Tučapy (distr. Kroměříž), former Kruhy u Tučap Nature Reserve,

Robinia grove and the adjacent shrubs; 49°21'52.0"N, 17°35'55.2"E; 333 m a.s.l.; LK; 3. 4. 2014; 5. – **154:** CZ; 77a; Uhřice u Dambořic (distr. Vyškov), edge of the oak-hornbeam forest near the road to Slavkov; 49°03'55.4"N, 16°56'38.7"E; 337 m a.s.l.; LK; 9. 4. 2014; 5. – **155:** CZ; 77a; Kobeřice u Brna (distr. Vyškov), settlement Bílý Vlk, edge of the oak-hornbeam forest near the rest area, ca 800 m ESE of the settlement; 49°05'32.3"N, 16°55'12.2"E; 385 m a.s.l.; LK; 9. 4. 2014; 5. – **157:** CZ; 77a; Ždánice (distr. Vyškov), edge of the oak-hornbeam forest U silnice; 49°05'00.9"N, 17°01'09.1"E; 353 m a.s.l.; LK; 9. 4. 2014; 5. – **181:** CZ; 21a; Kostelec u Holešova (distr. Přerov), SE edge of the Dubina forest under Holý kopec hill; 49°22'47.2"N, 17°30'39.7"E; 283 m a.s.l.; LK & MH; 19. 4. 2014; 15*. – **182:** CZ; 21a; Přestavlky (distr. Přerov), Přestavlcký les forest and adjacent grassland SE of the village; 49°23'30.0"N, 17°29'11.2"E; 306 m a.s.l.; LK & MH; 19. 4. 2014; 15*. – **183:** CZ; 76b; Krčmaň (distr. Olomouc), S part of the Chlum forest above pond by the road; 49°31'30.4"N, 17°20'33.7"E; 238 m a.s.l.; LK & MH; 19. 4. 2014; 5*. – **187:** CZ; 21a; Hlubočany (distr. Vyškov), Hlubočanský háj forest, oak-hornbeam woodland on the W edge; 49°14'40.6"N, 17°00'01.2"E; 392 m a.s.l.; LK; 22. 4. 2014; 5*. – **188:** CZ; 77b; Pavlovice (distr. Vyškov), oak-hornbeam forest by the blue touristic trail, ca 1 km NE of the village; 49°12'53.8"N, 17°04'28.7"E; 392 m a.s.l.; LK; 22. 4. 2014; 5*. – **189:** CZ; 77b; Orlovice-Hvězdlice (distr. Vyškov), Úpaly forest, forest edge by the crossroads of the road and the green touristic trail between the villages Orlovice and Hvězdlice; 49°13'05.2"N, 17°06'38.4"E; 429 m a.s.l.; LK; 22. 4. 2014; 15*. – **190:** CZ; 77b; Litenčice (distr. Vyškov); edge of the road in the forest between villages Litenčice and Morkovice-Slížany, ca 900 m S of the settlement Skavsko; 49°13'52.7"N, 17°11'22.0"E; 351 m a.s.l.; LK; 22. 4. 2014; 5*. – **191:** CZ; 20a; Lísky (distr. Kroměříž), edge of the Bažantnice forest near the road between villages Litenčice and Lísky, ca 1.4 km N of the village Lísky; 49°10'57.4"N, 17°12'16.5"E; 342 m a.s.l.; LK; 22. 4. 2014; 5*. – **192:** CZ; 77c; Velehrad (distr. Uherské Hradiště), forest edge near the road between the village Modrá and the settlement Horní Dubiny, ca 1.2 km N of the observation tower Modrá; 49°07'43.2"N, 17°23'52.7"E; 298 m a.s.l.; LK; 22. 4. 2014; 5*. – **193:** CZ; 77c; Zdounky (distr. Kroměříž), forest edge under Na Kopě peak, ca 4.7 km S of the village; 49°11'36.0"N, 17°20'39.9"E; 421 m a.s.l.; LK; 22. 4. 2014; 5*. – **194:** CZ; 77c; Nová Dědina (distr. Kroměříž), crossroads Tabarky, U Sv. Jana forest, 2 km E of the village; 49°12'27.7"N, 17°25'15.3"E; 354 m a.s.l.; LK; 22. 4. 2014; 5*. – **195:** CZ; 20a; Vitčice (distr. Prostějov), Vitčický les forest, oak-hornbeam woodland above the observation tower; 49°18'08.6"N, 17°14'20.5"E; 313 m a.s.l.; LK; 22. 4. 2014; 5*. – **215:** CZ; 79; Vlachova Lhota (distr. Zlín), ditches by the road in the N part of the village; 49°08'54.3"N, 17°57'32.2"E; 434 m a.s.l.; LK & MH; 26. 4. 2014; 5*. – **217:** CZ; 79; Zlín (distr. Zlín), Kocanda, Zdražilova studánka forest, hillsides under the road ca 600 m N of the crossroads with the road to Fryšták; 49°15'01.2"N, 17°40'53.9"E; 268 m a.s.l.; LK & MH; 26. 4. 2014; 5*. – **218:** CZ; 19; Uherský Brod, Havřice (distr. Uherský Brod), grasslands under the E edge of the Obora forest; 49°02'07.4"N, 17°36'42.1"E; 234 m a.s.l.; LK & MH; 26. 4. 2014; 16*. – **219:** CZ; 19; Uherský Brod (distr. Uherský Brod), crossroad Loučka, ditches around the road to the Maršov; 49°02'52.1"N, 17°39'15.9"E; 347 m a.s.l.; LK & MH; 27. 4. 2014; 5. – **220:** CZ; 78; Pašovice (distr. Uherské Hradiště), ditches near the road under the S edge of the Zálovčí forest; 49°05'17.9"N, 17°38'31.3"E; 324 m a.s.l.; LK & MH; 27. 4. 2014; 5. – **222:** CZ; 79; Březnice (distr. Zlín), ditches near the road in the village; 49°11'07.5"N, 17°39'25.0"E; 297 m a.s.l.; LK & MH; 27. 4. 2014; 5. – **223:** CZ; 20a; Kroměříž (distr. Kroměříž), E edge of the forest complex above the village Rataje; 49°15'57.5"N, 17°19'36.8"E; 320 m a.s.l.; LK & MH; 1. 5. 2014; 4. – **225:** CZ; 21a; Kurovice (distr. Kroměříž), Kurovický lom Nature Reserve, NE edge of the reserve and ditches near the road to Kotojedy; 49°16'27.3"N, 17°31'28.5"E; 266 m a.s.l.; LK & MH; 1. 5. 2014; 8. – **230:** CZ; 20a; Nevojnice (distr. Vyškov), Malhotky Nature Reserve, NW edge of the reserve by the edge of the forest; 49°08'55.2"N, 17°03'14.0"E; 282 m a.s.l.; MH; 5. 5. 2014; 3. – **236:** SK, 27a; Horné Srnie (distr. Trenčín), grassy ditch near the road; 49°00'30.9"N 18°05'17.3"E; 265 m a.s.l.; BT; 4. 5. 2014; 2. – **252:** CZ; 78; Hostětín (distr. Uherské Hradiště), edge of the abandoned orchard above the village, 0.3 km E of the church in the village; 49°02'56.0"N 17°53'03.0"E; 410 m a.s.l.; T. Koutecký; 22. 5. 2011; 6. – **253:** CZ; 68; Bílovice nad Svitavou (distr. Brno-venkov), edge of the path in the hornbeam woodland, 0.5 km NE from the railway station; 49°14'39.0"N 16°40'49.0"E; 265 m a.s.l.; T. Koutecký; 16. 5. 2011; 5. – **254:** CZ; 16; Brno-Kohoutovice (distr. Brno-město), light oak forest on the top of the ridge, 0.3 km NW from the hotel Myslivna; 49°11'26.0"N 16°33'04.0"E; 362 m a.s.l.; T. Koutecký; 18. 5. 2011; 11. – **255:** CZ; 77b; Orlovice (distr. Vyškov), hornbeam woodland near the road to the village Hvězdlice, ca 2.2 km SSE of the middle of the village; 49°13'20.0"N, 17°06'33.0"E; 440 m a.s.l.; M. Popelářová; 5. 5. 2011; 2. – **256:** CZ; 20a; Švábenice (distr. Vyškov), hornbeam woodland ca 1.7 km S of the church in the village; 49°15'29.0"N, 17°07'40.0"E; 350 m a.s.l.; M. Popelářová; 5. 5. 2011; 2. – **257:** CZ; 16; Malhostovice (distr. Brno-venkov), Zlobice Nature Reserve, oak forest ca 1.45 km S of the church in the village; 49°19'15.0"N, 16°30'21.0"E; 340 m a.s.l.; PK & MŠ; 30. 4. 2011; 10. – **259:** CZ; 77a; Ždánice (distr. Hodonín), hornbeam woodland on the S hillside near the road to the village Bučovice, ca 2.5 km NNW of the church in the village; 49°05'22.0"N, 17°01'13.0"E; 380 m a.s.l.; PK; 19. 5. 2011; 4. – **260:**

CZ; 20b; Kobylí (distr. Břeclav), Panský les forest, ca 1.8 km NW of the railway station by the blue touristic trail; 48°56'39.0"N, 16°51'39.0"E; 220 m a.s.l.; MŠ; 27. 4. 2013; 2. – **261:** CZ; 20b; Němčičky (distr. Břeclav), forest near the E border of the Nosperk Nature Reserve, ca 2.4 km NE of the church in the village; 48°56'26.0"N, 16°50'59.0"E; MŠ; 27. 4. 2013; 5. – **262:** CZ; 78; Vápenky (distr. Hodonín), shrubs in the grassland's complex, ca 0.5 km NW of the village; 48°52'39.0"N, 17°37'34.0"E; 510 m a.s.l.; J. Těšitel; 1. 6. 2013; 2. – **263:** CZ; 68; Ketkovice (distr. Brno-venkov), oak woodland on the loessial drifts on the SE hillside above the meadow of the Oslava river, ca 2 km SW of the church; 49°08'35.0"N, 16°15'04.0"E; 300 m a.s.l.; L. Ekrt; 1. – **264:** SK; 27a; Lednica (distr. Púchov); 49°06'30.0"N, 18°12'53.0"E; 397 m a.s.l.; F. Kolář; 11. 6. 2011; 1. – **303a:** CZ; 16; Brno-Kohoutovice (distr. Brno-město), N oriented peak of the broad ridge by the green touristic trail, 0.5 km W of water tank in the village; 49°11'30.1"N 16°31'06.2"E; 390 m a.s.l.; T. Koutecký; 17. 4. 2014; 2. – **303b:** CZ; 16; Brno-Kohoutovice (distr. Brno-město), W hillside of the flat valley with the cover of oak and lime trees, 0.3 km SW of water tank in the village; 49°11'25.7"N 16°31'26.2"E; 385 m a.s.l.; T. Koutecký; 17. 4. 2014; 2. – **304:** CZ; 18b; Hodonín (distr. Hodonín), Dúbrava forest, degraded oak forest with *Robinia pseudoacacia* NW of the town, ca 150 m SE of the gamekeeper's lodge Zbrod; 48°53'11.0"N 17°03'57.0"E; 170 m a.s.l.; PK; 8. 6. 2014; 4. – **305:** CZ; 71c; Ježkovice (distr. Vyškov), Na Zemánkách forest, oak-hornbeam woodland ca 1.35 km SE of the middle of the village; 49°17'43.2"N 16°54'35.2"E; 395 m a.s.l.; PK; 21. 4. 2014; 5. – **339:** SK; 6/11; Ochtiná (distr. Rožňava), saddle Hrádok, beech forest and road edges; 48°39'02.4"N, 20°17'49.0"E; 625 m a.s.l.; F. Kolář; 19. 6. 2013; 3. – **358:** HU; -; Mátraháza (distr. Észak-Magyarország), beech forest 0.4 km SW of the village; 47°52'01,0"N, 19°58'31.0"E; 710 m a.s.l.; PK; 28. 7. 2014; 1.

***Symphytum tuberosum* subsp. *tuberosum*: 1:** CZ; 76a; Přerov (distr. Přerov), edge of the alder carr Žebračka near the garden colony; 49°27'49.5"N, 17°28'19.9"E; 216 m a.s.l.; LK & MH; 25. 4. 2011; 15*. – **14:** CZ; 37m; Rožmberk nad Vltavou (distr. Český Krumlov), forest near the castle Rožmberk; 48°39'21.6"N, 14°21'55.3"E; 570 m a.s.l.; M. Dančák; 28. 4. 2012; 6. – **18:** CZ; 68; Tavíkovice (distr. Znojmo), edge of the Knížecí seč forest; 49°02'19.4"N, 16°06'57.7"E; 334 m a.s.l.; LK; 7. 7. 2011; 9. – **24:** CZ; 16; Znojmo (distr. Znojmo), Tvořihrádský les forest complex, N part of the forest Vlčí jámy near the yellow touristic trail between crossroads Ruda and the gamekeeper's lodge Svatý Hubert; 48°54'28.5"N, 16°06'17.7"E; 321 m a.s.l.; LK; 1. 4. 2012; 2. – **27** HU; -; Köszeg (distr. Nyugat-Dunántúl), forest edges near the crossroads above the slope; 47°22'56.0"N, 16°30'41.0"E; 459 m a.s.l.; BT; 22. 4. 2012; 15*. – **28:** HU; -; Körmend-Katafa (distr. Nyugat-Dunántúl), broadleaved woodland near the road between the towns Körmend and Katafa; 46°59'17.9"N, 16°37'05.0"E; 197 m a.s.l.; BT; 21. 4. 2012; 7*. – **29:** HU; -; Köszeg (distr. Nyugat-Dunántúl), shrubs near the road; 47°22'36.9"N, 16°31'30.0"E; 372 m a.s.l.; BT; 22. 4. 2012; 2. – **30:** HU; -; Sopron-Göberhalom (distr. Nyugat-Dunántúl), broadleaved woodland near the rivulet between the towns Sopron and Göberhalom; 47°40'23.9"N, 16°30'40.0"E; 315 m a.s.l.; BT; 20. 4. 2012; 3. – **36:** SK; 21c; Blatnica (distr. Martin), Dedošová dolina valley, path edge on the edge of the beech forest; 48°56'03.9"N, 19°01'44.0"E; 763 m a.s.l.; LK; 30. 6. 2012; 5. – **39:** SK; 6; Rybnik (distr. Zlaté Moravce), abandoned spring in the middle of the fields; 48°18'07.8"N, 18°35'9"E; 200 m a.s.l.; LK; 2. 7. 2012; 4. – **41:** SK; 6; Malé Kozmálovce (distr. Levice), damp part of the forest above the vineyards; 48°15'48.7"N, 18°28'50.1"E; 218 m a.s.l.; LK; 5. 7. 2012; 3. – **44:** CZ; 78; Štítná nad Vláří (distr. Zlín), forest in the valley of the Zelenský potok rivulet near the settlement Kochavec; 49°02'21.1"N, 17°57'29.7"E; 481 m a.s.l.; MH; 17. 6. 2012; 3. – **82:** CZ; 63c; Choceň (distr. Ústí nad Orlicí), Peliny Nature Reserve, valley of the Orlice river; 50°00'02.0"N, 16°14'01.3"E; 298 m a.s.l.; LK & MH; 29. 4. 2013; 15*. – **83:** HU;-; Őriszentpéter (distr. Nyugat-Dunántúl), bosk near the car parking; 46°50'27.0"N, 16°25'18.0"E; 226 m a.s.l.; BT; 26. 4. 2013; 7. – **87:** CZ; 63c; Sudislav nad Orlicí, Bezpráví (distr. Ústí nad Orlicí), valley of the Orlice river; 49°59'44.6"N, 16°19'27.3"E; 329 m a.s.l.; M. Jandová; 28. 4. 2013; 15*. – **89:** AT;-; Stangau (distr. Mödling), Wienerwald, glade near the road; 48°05'44.0"N, 16°07'44.0"E; 438 m a.s.l.; BT; 3. 5. 2013; 15*. – **90:** AT;-; Mauerbach (distr. Wien-Umgebung), Wienerwald; 48°15'36.0"N, 16°10'14.0"E; 303 m a.s.l.; BT; 4. 5. 2013; 10*. – **91:** AT;-; Baden (distr. Baden), shrubs near the Schwechat rivulet on the NW edge of the town; 48°00'40.0"N, 16°12'13.0"E; 244 m a.s.l.; BT; 3. 5. 2013; 3. – **92:** AT;-; Wöllersdorf (distr. Wiener Neustadt-Land), shrubs near the road (Laabnerstrasse); 48°06'12.0"N, 15°52'52.0"E; 363 m a.s.l.; BT; 4. 5. 2013; 3. – **93:** CZ; 79; Hvozdná (distr. Zlín), shrubs near the road; 49°14'36.0"N, 17°44'51.0"E; 290 m a.s.l.; BT; 7. 5. 2013; 15*. – **94:** CZ; 84a; Třinec, Karpentná (distr. Frýdek-Místek), valley of the Liderov stream; 49°38'17.7"N, 18°41'53.5"E; 345 m a.s.l.; LK & MH; 8. 5. 2013; 4. – **95:** SK; 28; Raková (distr. Čadca), alluvium of the Kysuca river; 49°26'48.9"N, 18°43'58.6"E; 428 m a.s.l.; LK & MH; 8. 5. 2013; 15*. – **97:** CZ; 99a; Bílá (distr. Frýdek-Místek), alluvial tall-forb vegetation dominated by *Petasites* in the valley of the Smradlava rivulet; 49°26'01.2"N, 18°26'52.7"E; 578 m a.s.l.; LK & MH; 8. 5. 2013; 3. – **99:** CZ; 76a; Hustopeče nad Bečvou (distr. Přerov), edge of the canal flowing to the Velký lesní rybník pond, near the turning to the settlement Valcha; 49°30'59.7"N, 17°53'21.8"E; 270 m a.s.l.; LK & MH; 8. 5. 2013; 5. – **100a:** CZ; 16; Moravský Krumlov (distr. Znojmo), Moravský Krumlov deer hunting park, edge

of the road ca 1 km of the settlement Stavení; 49°03'02.1"N 16°23'55.5"E; 388 m a.s.l.; LK & MH; 8. 5. 2013; 4. – **100b:** CZ; 16; Moravský Krumlov (distr. Znojmo), Moravský Krumlov deer hunting park, edge of the road near the gamekeeper's lodge Hubertus; 49°02'37.0"N, 16°21'33.7"E; 391 m a.s.l.; LK & MH; 8. 5. 2013; 5. – **102:** CZ; 99a; Ostravice (distr. Frýdek-Místek), shrubs near the Ostravice rivulet in the SE part of the village; 49°32'02.0"N, 18°23'39.0"E; 425 m a.s.l.; BT; 14. 5. 2013; 4. – **103:** PL; -; Niedźwiedź (distr. Ostrzeszów), park in the village; 49°36'55.0"N, 20°04'03.0"E; 510 m a.s.l.; BT; 17. 5. 2013; 5. – **114:** DE; -; Bayrischzell (distr. Miesbach), grassy hillside near the road; 47°41'00.0"N, 12°02'13.0"E; 1097 m a.s.l.; BT; 22. 5. 2013; 5. – **115:** DE; -; Berchtesgaden (distr. Berchtesgadener Land), shrubs near the road; 47°37'50.0"N, 13°02'30.0"E; 948 m a.s.l.; BT; 22. 5. 2013; 3. – **116:** AT; -; Krispl (distr. Hallein), shrubs near the pasture; 47°42'42.0"N, 13°11'13.0"E; 876 m a.s.l.; BT; 24. 5. 2013; 4. – **117:** CZ; 76a; Hustopeče nad Bečvou (distr. Přerov), settlement Pod Doubravou, the forest edge; 49°31'02.1"N, 17°50'46.7"E; 277 m a.s.l.; MH; 25. 5. 2012; 2. – **118:** HU;-; Budapest, Hűvösvölgy (II. Kerület; distr. Közép-Magyarország), forest above the road (Csibor utca); 47°32'08.3"N, 18°58'11.8"E; 244 m a.s.l.; BT, LK & MH; 8. 3. 2014; 4. – **119:** CZ; 21b; Citov (distr. Přerov), Citovský háj forest, path edge in the N part of the alder carr, on the right-hand shore of the Morávka millrace; 49°27'17.2"N, 17°19'44.1"E; 208 m a.s.l.; LK; 13. 3. 2014; 4. – **122:** CZ; 21b; Chropyně (distr. Kroměříž), Břestský les forest, alder carr near the road in direction to the village Skaštice; 49°20'33.8"N, 17°24'01.0"E; 196 m a.s.l.; LK; 19. 3. 2014; 4. – **123:** CZ, 21b; Kroměříž (distr. Kroměříž), crossroads Kaplanova, alder carr Horní les in the valley of the Morava river, near the road between Chorynĕ and Kroměříž; 49°19'23.2"N, 17°23'25.7"E; 200 m a.s.l.; LK; 13. 3. 2014; 4. – **127:** CZ; 76a; Hrabětice (distr. Nový Jičín), Hrabětický les grove, forest edge and an alluvium of the left-hand tributary of the Luha river; 49°36'08.6"N, 17°53'12.0"E; 275 m a.s.l.; LK; 26. 3. 2014; 1. – **128:** CZ; 76a; Hynčice-Vražné (distr. Nový Jičín), valley of the Vraženský potok rivulet, the shore of the rivulet near the rest area; 49°37'17.8"N, 17°50'24.8"E; 291 m a.s.l.; LK; 26. 3. 2014; 3. – **129:** CZ; 75; Heřmánky (distr. Nový Jičín), alluvium of the Čermná river near the confluence with the Odra river, ca 200 m from the railway station; 49°42'52.6"N, 17°45'23.6"E; 331 m a.s.l.; LK; 26. 3. 2014; 3. – **131:** CZ; 75; Zálužné (distr. Opava), path edge and hillsides under the Moravice river by the green touristic trail to the PP Nové Těchanovice Nature Reserve; 49°49'15.9"N, 17°42'58.3"E; 392 m a.s.l.; LK; 26. 3. 2014; 6. – **132:** CZ; 75; Mladecko (distr. Opava), left shore of the left-handed tributary of the Jordán rivulet, near the road; 49°53'11.9"N, 17°42'34.4"E; 373 m a.s.l.; LK; 26. 3. 2014; 2. – **133:** CZ; 74b; Otice (distr. Opava), shore of the Hvozdnice rivulet behind the football pitch (Sokolská street); 49°54'57.5"N, 17°51'51.7"E; 267 m a.s.l.; LK; 26. 3. 2014; 2. – **135:** CZ; 83; Studénka-Nová Horka (distr. Nový Jičín), alluvium of the Sedlnice rivulet under the road in the village; 49°41'22.8"N, 18°04'13.0"E; 240 m a.s.l.; LK; 27. 3. 2014; 4. – **136:** CZ; 76a; Veřovice (distr. Nový Jičín), alluvium of the Jičínka rivulet on the W edge of the village; 49°32'26.8"N, 18°05'28.0"E; 376 m a.s.l.; LK; 27. 3. 2014; 5. – **137:** CZ; 84a; Tichá (distr. Nový Jičín), shores of the Tichávka rivulet in the village; 49°34'16.7"N, 18°12'09.4"E; 346 m a.s.l.; LK; 27. 3. 2014; 4. – **138:** CZ; 84a; Pržno (distr. Frýdek-Místek), alluvium of the Ostravice river near the football pitch; 49°36'36.2"N, 18°21'36.2"E; 339 m a.s.l.; LK; 27. 3. 2014; 4. – **139:** CZ; 84a; Dolní Třanovice (distr. Frýdek-Místek), right-hand shore of the Stonávka rivulet in the village, ca 100 m of the church of Sv. Bartoloměj; 49°42'36.1"N, 18°31'53.8"E; 308 m a.s.l.; LK; 27. 3. 2014; 3. – **140:** CZ; 83; Dolní Polanka (distr. Ostrava-město), S edge of the alder carr Dolní Polanský les; 49°47'00.7"N, 18°12'07.3"E; 222 m a.s.l.; LK; 27. 3. 2014; 3. – **142:** CZ; 76b; Velký Týnec (distr. Olomouc), alluvium of the Týnečka rivulet; 49°33'08.2"N, 17°21'10.8"E; 238 m a.s.l.; LK; 31. 3. 2014; 5. – **143:** CZ; 76b; Penčice (distr. Přerov), valley of the rivulet flowing from the forest Zátěš; 49°31'15.1"N, 17°25'58.1"E; 254 m a.s.l.; LK; 31. 3. 2014; 4. – **144:** CZ; 76b; Lhotka u Přerova (distr. Přerov), alluvium of the rivulet under the PP Lhotka u Přerova Nature Reserve; 49°29'55.0"N, 17°24'49.4"E; 241 m a.s.l.; LK; 31. 3. 2014; 3. – **145:** CZ; 76a; Turovice (distr. Přerov), humid *Robinia* grove near the nameless brooklet on the N edge of the PP Kamenice Nature Reserve; 49°24'41.9"N, 17°34'38.0"E; 267 m a.s.l.; LK; 31. 3. 2014; 5. – **146:** CZ; 76a; Hradčany (distr. Přerov), S edge of the forest near the road to the village Pavlovice u Přerova; 49°27'24.4"N, 17°33'49.9"E; 299 m a.s.l.; LK; 31. 3. 2014; 3. – **149:** CZ; 80a; Kateřinice (distr. Vsetín), humid shores of the Březinka rivulet on the N edge of the village; 49°23'04.4"N, 17°54'14.3"E; 385 m a.s.l.; LK; 3. 4. 2014; 5. – **150:** CZ; 80a; Mikulůvka (distr. Vsetín), shores of the Bečva river; 49°25'03.2"N, 17°56'58.1"E; 312 m a.s.l.; LK; 3. 4. 2014; 4. – **151:** CZ; 76a; Lešná (distr. Vsetín), garden of the castle Lešná; 49°31'07.4"N, 17°55'42.4"E; 282 m a.s.l.; LK; 3. 4. 2014; 5. – **152:** CZ; 71a; Loštice (distr. Šumperk), valley of the Třebůvka rivulet, shores behind the football pitch on the edge of the village; 49°44'04.3"N, 16°55'22.5"E; 271 m a.s.l.; LK; 7. 4. 2014; 3. – **158a:** CZ; 68; Citonice (distr. Znojmo), oak-hornbeam woodland and *Robinia* grove by the blue touristic trail close to the Gránický potok rivulet, ca 200 m from the sewage disposal plant; 48°52'38.3"N, 15°58'17.6"E; 375 m a.s.l.; LK & MH; 12. 4. 2014; 10*. – **158b:** CZ; 68; Citonice (distr. Znojmo), forest edge behind the bridge over the Gránický potok rivulet, near the sewage disposal plant; 48°52'43.6"N, 15°58'10.7"E; 375 m a.s.l.; LK & MH; 12. 4. 2014; 10*. – **159:** CZ; 68; Podmolí (distr. Znojmo), shore of the left-hand tribu-

tary of the Žlebský potok rivulet, 350 m SW of the village; 48°50'56.4"N, 15°56'04.3"E; 375 m a.s.l.; LK & MH; 12. 4. 2014; 5. – **160a:** CZ; 68; Horní Břečkov (distr. Znojmo), alluvium of the Klaperův potok rivulet on the right side of the road between villages Horní Břečkov and Čížov; 48°53'13.9"N, 15°53'00.4"E; 375 m a.s.l.; LK & MH; 12. 4. 2014; 4. – **160b:** CZ; 68; Čížov (distr. Znojmo) forest edge and forest's paths ca 1.4 km SSW of the chapel in the village; 48°52'07.0"N, 15°51'41.0"E; 440 m a.s.l.; M. & P. Lepší; 8. 5. 2011; 5. – **160c:** CZ; 68; Čížov (distr. Znojmo), forest near the road to the Hardegg outlook, SSW of the village; 48°51'31.0"N, 15°51'53.0"E; 405 m a.s.l.; Z. Chlumská; 15. 6. 2013; 5. – **161:** CZ; 68; Čížov (distr. Znojmo), oak-hornbeam woodland and adjacent edges and ditches around the road from the village Čížov to the Hardegg outlook; 48°51'51.2"N, 15°51'47.6"E; 375 m a.s.l.; LK & MH; 12. 4. 2014; 15*. – **162:** CZ; 68; Vranov nad Dyjí (distr. Znojmo), forest edge on the right side of the road in direction to the village Lančov; 48°54'22.6"N, 15°47'37.5"E; 375 m a.s.l.; LK & MH; 12. 4. 2014; 5. – **163:** CZ; 68; Lančov (distr. Znojmo), road edge to the bay of the Vranovská přehrada dam; 48°54'36.3"N, 15°46'26.3"E; 375 m a.s.l.; LK & MH; 12. 4. 2014; 5. – **164:** CZ; 68; Bítov (distr. Znojmo), forest near the road above the ruins of the caste Cornštejn; 48°55'49.8N, 15°43'00.1"E; 375 m a.s.l.; LK & MH; 12. 4. 2014; 2. – **165:** CZ; 68; Chvalatice (distr. Znojmo), road edges near the crossroad Chvalatice-Zálesí; 48°57'57.7"N, 15°45'23.0"E; 375 m a.s.l.; LK & MH; 12. 4. 2014; 10*. – **166:** CZ; 68; Střelice (distr. Znojmo), oak woodland Střelice forest, ca 200 m N of the gamekeeper's lodge Střelice; 49°00'23.2"N, 15°58'25.7"E; 375 m a.s.l.; LK & MH; 12. 4. 2014; 4. – **167:** CZ; 68; Libeř (distr. Praha-západ), valley of the Zabořanský potok rivulet in the village; 49°55'10.9"N, 14°28'32.5"E; 375 m a.s.l.; LK & MH; 15. 4. 2014; 7. – **168:** CZ; 68; Jílové u Prahy (distr. Praha-západ), hornbeam woodland near the railway station Jílové u Prahy; 49°52'39.2"N, 14°29'56.3"E; 375 m a.s.l.; LK & MH; 15. 4. 2014; 7. – **169:** CZ; 68; Pikovice (okr. Praha-západ), Medník, path edge to the PP Medník Nature Reserve; 49°52'25.2"N, 14°26'42.3"E; 375 m a.s.l.; LK & MH; 15. 4. 2014; 5/-. – **171:** CZ; 68; Písečné (distr. Jindřichův Hradec), humid shores of the Moravská Dyje river; 48°57'24.1"N, 15°28'23.9"E; 375 m a.s.l.; LK, MH, MŠ & PK; 16. 4. 2014; 6. – **172:** CZ; 68; Primmersdorf (distr. Waidhofen an der Thaya), valley of the Dyje river near the Thayatal Straße; 48°51'17.4"N, 15°34'38.9"E; 375 m a.s.l.; LK, MH, MŠ & PK; 16. 4. 2014; 15*. – **173:** CZ; 68; Rosenburg (distr. Horn), valley of the Taffa river, path edge above the river near the Kamptal Straße; 48°38'00.6"N, 15°39'38.4"E; 375 m a.s.l.; LK, MH, MŠ & PK; 16. 4. 2014; 5. – **174:** CZ; 68; Langenlois (distr. Krems-Land), valley of the Kampa river, road edges along the canal of the Kampa river on the NE edge of the village; 48°28'44.9"N, 15°41'50.6"E; 375 m a.s.l.; LK, MH, MŠ & PK; 16. 4. 2014; 8. – **175:** CZ; 68; Gut am Steg (distr. Krems-Land), shores of the nameless tributary of the Spitzer Bach rivulet, near the river in direction to the village Oberndorf am Jauerling; 48°21'06.6"N, 15°22'22.9"E; 375 m a.s.l.; LK, MH, MŠ & PK; 16. 4. 2014; 10. – **177:** CZ; 68; Kleinotten (distr. Zwettl), surroundings of the Rotbach rivulet in the undergrowth of the spruce forest; 48°40'51.7"N, 15°10'55.1"E; 375 m a.s.l.; LK, MH, MŠ & PK; 16. 4. 2014; 5. – **178:** CZ; 68; Chedrbí (distr. Kutná Hora), Dolní Dubina, valley of the Paběnický potok rivulet near the road in direction to the village Hraběšín; 49°51'54.4"N, 15°19'31.1"E; 375 m a.s.l.; LK & MH; 17. 4. 2014; 4. – **179:** CZ; 68; Žehušice (distr. Kutná Hora), Bojmany, remaining fragment of the alder carr in the valley of the Doubrava river on the edge of the Žehušice deer hunting park; 49°57'57.4"N, 15°26'16.5"E; 375 m a.s.l.; LK & MH; 17. 4. 2014; 5. – **180:** CZ; 68; Blatnice (distr. Třebíč), 49°03'57.5"N, 15°52'47.3"E; 424 m a.s.l.; L. Ekrt; 16. 4. 2014; 11. – **184:** PL; -; Gierałtowice (distr. Gliwice), ditches around the road behind Staw Podwieśnik pond, ca 100 m S of the bridge over the Wieprzówka river; 49°55'48.8"N, 19°23'29.9"E; 257 m a.s.l.; LK & MH; 20. 4. 2014; 15*. – **185:** PL; -; Wieprz (distr. Gliwice), valley of the Wieprzówka river, surroundings of the river under the bridge; 49°53'22.7"N, 19°21'37.8"E; 289 m a.s.l.; LK & MH; 20. 4. 2014; 5. – **186:** PL; -; Targanice (distr. Wadowicki), edge of the road under the hotel Kocierz; 49°46'38.3"N, 19°19'05.3"E; 739 m a.s.l.; LK & MH; 20. 4. 2014; 5. – **196:** AT; -; Anger (distr. Weiz), valley of the Feistritz river; 47°17'13.0"N, 15°41'14.0"E; 485 m a.s.l.; BT; 20. 4. 2014; 3. – **197:** AT; -; Waldbach (distr. Hartberg-Fürstenfeld), near the confluence of the Weißenbach rivulet and the Lafnitz river; 47°26'54.0"N, 15°50'47.0"E; 385 m a.s.l.; BT; 20. 4. 2014; 6. – **198:** AT; -; Vorderberg (distr. Hermagor), forest edge near the power station; 46°35'12.0"N, 13°32'05.0"E; 580 m a.s.l.; BT; 19. 4. 2014; 5. – **200:** AT; -; Naas (distr. Weiz); 47°15'24.0"N, 15°36'17.0"E; 580 m a.s.l.; BT; 20. 4. 2014; 6. – **201:** CZ; 80a; Huslenky (distr. Vsetín), shores of the Losový rivulet; 49°18'36.8"N, 18°05'57.5"E; 423 m a.s.l.; MH; 23. 4. 2014; 5. – **202:** CZ; 84a; Trnávka (distr. Nový Jičín), near the pond in the village; 49°41'00.3"N, 18°11'03.5"E; 254 m a.s.l.; BT; 23. 4. 2014; 2. – **203:** CZ; 76a; Bartošovice (distr. Nový Jičín), park in the village; 49°40'15.3"N, 18°02'59.0"E; 245 m a.s.l.; BT; 23. 4. 2014; 5. – **204:** CZ; 21b; Holešov (distr. Kroměříž), park in the town; 49°20'05.7"N, 17°35'13.1"E; 240 m a.s.l.; BT; 23. 4. 2014; 5. – **205:** CZ; 76a; Štramberk (distr. Nový Jičín), near the pond in the W part of the town; 49°35'11.4"N, 18°06'13.6"E; 309 m a.s.l.; BT; 23. 4. 2014; 5. – **206:** CZ; 80a; Leskovec (distr. Vsetín), valley of the Senice rivulet and grasslands behind the railway bridge, ca 50 m S of the railway station Leskovec; 49°17'08.8"N, 18°00'04.5"E; 378 m a.s.l.; LK & MH; 26. 4. 2014; 5. – **207:** CZ; 79; Pozděchov (distr. Vsetín), road edge in

direction to the castle Trubiska; 49°13'45.2"N, 17°59'11.3"E; 491 m a.s.l.; LK & MH; 26. 4. 2014; 5. – **208:** SK; 27a; Lazy pod Makytou (distr. Púchov), ravine forest above the road; 49°12'58.2"N, 18°12'53.6"E; 403 m a.s.l.; LK & MH; 26. 4. 2014; 5. – **209:** CZ; 82; Střelná (distr. Vsetín), hillside above the car parking on the border crossing with Slovakia; 49°10'51.9"N, 18°07'12.4"E; 477 m a.s.l.; LK & MH; 26. 4. 2014; 5. – **210:** CZ; 82; Študlov (distr. Vsetín), valley of the rivulet under the PP Hrádek Nature Reserve; 49°09'29.7"N, 18°05'26.4"E; 570 m a.s.l.; LK & MH; 26. 4. 2014; 4. – **214:** CZ; 79; Valašské Klobouky-Mirošov (distr. Zlín), glade near the road in the valley of the Smolinka rivulet; 49°08'48.4"N, 17°58'42.7"E; 387 m a.s.l.; LK & MH; 26. 4. 2014; 7. – **216:** CZ; 79; Horní Trávníky (distr. Zlín), valley of the Horský potok rivulet; 49°12'31.2"N, 17°48'34.4"E; 288 m a.s.l.; LK & MH; 26. 4. 2014; 5. – **226:** CZ; 8; Hlubočepy (distr. Hlavní město Praha), Prokopské údolí Nature Reserve, Dalejský háj grove, oak woodland near the crossroads of the forest's paths 50 m of the gamekeeper's lodge; 50°02'24.4"N, 14°22'19.1"E; 282 m a.s.l.; J. Kocián; 1. 5. 2014; 6. – **227:** CZ; 41; Zbraslav (distr. Hlavní město Praha), broadleaved woodland by the green and yellow touristic trails, 140 m E of the railway station Zbraslav; 49°58'14.2"N, 14°24'05.1"E; 212 m a.s.l.; J. Kocián; 1. 5. 2014; 5. – **228:** CZ; 41 Zbraslav (distr. Hlavní město Praha), oak-hornbeam woodland between crossroads of the forest's paths with the arbour and the fortress Závist; 49°57'50.0"N, 14°24'21.0"E; 356 m a.s.l.; J. Kocián; 1. 5. 2014; 5. – **229:** CZ; 21a; Vranovice-Kelčice (distr. Prostějov), PR Blátka Nature Reserve, oak-hornbeam woodland in the valley of the Kelčický potok rivulet; 49°24'12.0"N, 17°04'32.2"E; 292 m a.s.l.; LK & MH; 4. 5. 2014; 5. – **231:** SK; 30c; Bardejov (distr. Bardejov), alluvium of the Topľa river; 49°17'51.2"N, 21°15'6.6"E; 276 m a.s.l.; Ľ. Majeský; 4. 5. 2014; 4. – **232:** SK; 26a; Bukovina (distr. Liptovský Mikuláš), edge of the forest; 49°08'08.0"N, 19°28'04.0"E; 611 m a.s.l.; BT; 2. 5. 2014; 5. – **233:** SK; 21c/22; Liptovská Osada (distr. Ružomberok), crossroads Podsuchá, valley of the Revúca river; 48°59'54.0"N, 19°17'16.0"E; 555 m a.s.l.; BT; 30. 4. 2014; 3. – **234:** SK; 21d; Malatiná (distr. Dolný Kubín); 49°11'01.6"N, 19°25'33.6"E; 796 m a.s.l.; BT; 2. 5. 2014; 3. – **235:** SK; 21d; Švošov (distr. Ružomberok); 49°07'26.0"N, 19°12'58.0"E; 472 m a.s.l.; BT; 30. 4. 2014; 4. – **237:** CZ; 21b; Charváty (distr. Olomouc), fragment of the alder carr near the arm of the Morava river; 49°31'14.2"N, 17°15'53.6"E; 205 m a.s.l.; M. Dančák; 8. 5. 2014; 5. – **239:** AT; -; Bad Mitterndorf (distr. Liezen), edge of the forest; 47°33'59.0"N, 13°57'46.0"E; 985 m a.s.l.; BT; 8. 5. 2014; 5. – **240:** AT; -; Hieflau (distr. Leoben), Hochleiten; 47°35'44.0"N, 14°43'03.0"E; 532 m a.s.l.; BT; 9. 5. 2014; 2. – **241:** AT; -; Ramsau am Dachstein (distr. Liezen); 47°39'42.0"N, 13°44'38.0"E; 1084 m a.s.l.; BT; 8. 5. 2014; 3. – **244:** CZ; 82; Střelná (distr. Vsetín), beech forest N of the village; 49°10'52.9"N, 18°05'37.2"E; 649 m a.s.l.; MH; 15. 6. 2014; 3. – **246:** SK; 21b; Terchová (distr. Žilina), Vrátna dolina valley, edge of the forest's path under the cabin Na Grúni; 49°12'55.9"N, 19°02'48.3"E; 946 m a.s.l.; M. Dančák; 17. 6. 2014; 4. – **265:** CZ; 99a; Bílá (distr. Frýdek-Místek), humid shores of the Černá Ostravice rivulet, ca 3 km ENE of the confluence with the Bílá Ostravice rivulet; 49°27'55.0"N, 18°30'23.0"E; 570 m a.s.l.; V. Koutecká; 24. 5. 2011; 5. – **266:** CZ; 37p; Doubravice (distr. České Budějovice), forest edge ca 0.75 km NNW of the pond in the village; 48°56'32.0"N, 14°30'26.0"E; 430 m a.s.l.; A. Jírová; 9. 5. 2011; 3. – **267:** CZ; 8; Praha-Velká Chuchle (distr. Hlavní město Praha), oak-hornbeam woodland Chuchelský háj grove, ca 350 m W of the church of Sv. Jan Nepomucký, N of the village; 50°01'01.0"N, 14°22'57.0"E; 270 m a.s.l.; MŠ; 5. 5. 2011; 5. – **268:** CZ; 38; Kvítkovice (distr. České Budějovice), shrubs and forest near the road, ca 540 m NW of the chapel in the village; 48°57'38.0"N, 14°19'31.0"E; 450 m a.s.l.; MŠ; 2. 5. 2011; 3. – **269:** CZ; 37i; Hrbov-Lhenice (distr. Prachatice), shores of the valley of the Melhutka rivulet, ca 1.5 km WSW of the chapel in the village; 49°01'03.0"N, 14°08'40.0"E; 480 m a.s.l.; MŠ; 22. 5. 2011; 8. – **270:** CZ; 68; Kladeruby nad Oslavou (distr. Třebíč), shores of the Oslava river, ca 1 km NNE of the middle of the village; 49°09'07.0"N, 16°10'43.0"E; 290 m a.s.l.; J. Těšitel; 8. 5. 2011; 3. – **271:** CZ; 68; Ostrovačice (distr. Brno-venkov), grassy edge of the oak forest near the crossroads of the roads on the W part of the racetrack, ca 2.25 km SE of the church in the village; 49°12'25.0"N, 16°26'20.0"E; 430 m a.s.l.; PK; 22. 5. 2014; 5. – **272:** CZ; 41; Tábor-Staré Horky (distr. Tábor), forest park Pintovka in the valley of the Lužnice river, ca 1.5 km NNE of the chapel in the village; 49°24'40.0"N, 14°38'33.0"E; 490 m a.s.l.; P. Drahník; 8. 5. 2011; 3. – **273:** CZ; 80a; Rožnov pod Radhoštěm (distr. Vsetín), shore of the Rožnovská Bečva river, ca 2.3 km W of the square in the town; 49°27'28.0"N, 18°06'42.0"E; 360 m a.s.l.; M. Popelářová; 11. 5. 2011; 3. – **274:** CZ; 68; Staré Hobzí (distr. Jindřichův Hradec), steep ravine forest under the Moravská Dyje river, ca 1.5 km NW of the centre of the village; 49°01'17.0"N, 15°26'13.0"E; 475 m a.s.l.; L. Ekrt; 16. 5. 2011; 6. – **275:** CZ; 75; Valšov (distr. Bruntál), shores of the Moravice river, ca 1.8 km SE of the railway station across the quarry; 49°55'14.0"N, 17°27'06.0"E; 500 m a.s.l.; V. Koutecká; 22. 5. 2011; 3. – **276:** CZ; 37p; Vidov (distr. České Budějovice), broadleaved woodland (oak-hornbeam forest and the carr) in the valley of the rivulet on the S edge of the village; 48°55'26.0"N, 14°29'49.0"E; 420 m a.s.l.; L. Ekrt; 5. 5. 2011; 5. – **277:** CZ; 41; Nuzice (distr. České Budějovice), oak-hornbeam woodland in the valley of the rivulet in the Židova strouha Nature Reserve, ca 580 m NE of the church in the village; 49°16'33.0"N, 14°27'37.0"E; 415 m a.s.l.; L. Ekrt; 14. 5. 2011; 6. – **278:** CZ; 37p; Ledenice (distr. České Budějovice), oak forest on the hillside over the riverbed

of the Zborovský potok rivulet, ca 2.6 km SW of the square in the town; 48°55'14.0"N, 14°35'23.0"E; 495 m a.s.l.; PK; 21. 4. 2012; 3. – **279:** CZ; 45a; Ústí nad Labem-Nová Ves (distr. Ústí nad Labem), beech-hornbeam forest SE of the village, ca 200 m SE of peak Vysoký Ostrý; 50°38'06.0"N, 14°04'56.0"E; 500 m a.s.l.; PK & MŠ; 30. 4. 2012; 12. – **280:** CZ; 37l; Kamenný Újezd (distr. České Budějovice), shores of the rivulet ca 1.7 km W of the church in the village; 48°54'03.0"N, 14°25'28.0"E; 440 m a.s.l.; PK; 20. 5. 2012; 15. – **281:** CZ; 68; Brno-Žebětín (distr. Brno-město), oak forest on the base of the hillside, 1.5 km S of the church in the village Žebětín; 49°11'27.0"N, 16°28'53.0"E; 310 m a.s.l.; T. Koutecký; 25. 6. 2012; 8. – **282:** CZ; 83; Bohumín (distr. Karviná), Hraniční meandry Odry Nature Reserve, alder carr 0.5 km N of the church in the village Starý Bohumín; 49°55'31.0"N, 18°19'59.0"E; 200 m a.s.l.; T. Koutecký; 26. 6. 2012; 5. – **283:** CZ; 68; Vevčice (distr. Znojmo), thermophilous oak forest under the confluence of the Jevišovka river and the Hluboký potok rivulet, ca 1 km S to SSW of the church in the village; 48°57'10.0"N, 16°02'35.0"E; 260 m a.s.l.; PK & MŠ; 26. 4. 2013; 26. – **284:** CZ; 68; Kramolín (distr. Třebíč), oak forest ca 0.9 km ENE of the village; 49°08'14.0"N, 16°07'10.0"E; 450 m a.s.l.; PK & MŠ; 28. 4. 2013; 16. – **285:** CZ; 37d; Sudslavice (distr. Prachatice), shores of the Volyňka river, W of Opolenec peak; 49°05'20.0"N, 13°47'40.0"E; 550 m a.s.l.; PK; 1. 5. 2013; 10. – **286:** CZ; 41; Vráž u Písku (distr. Písek), Žlíbky Nature Reserve, ravine forest on the hillside of the valley of the Otava river; 49°22'20.0"N, 14°08'36.0"E; 370 m a.s.l.; PK; 5. 5. 2013; 15. – **287:** CZ; 37e; Strakonice-Kalvárie (distr. Strakonice), forest on the hillside under the right-hand shore of the Oslava river, W edge of the town ca 1.9 km W of the railway station; 49°15'26.0"N, 13°53'24.0"E; 410 m a.s.l.; R. Paulič; 6. 5. 2013; 5. – **288:** CZ; 37k; Křemže (distr. Český Krumlov), oak-hornbeam woodland in the alluvium of the Křemžský potok rivulet; 48°54'38.0"N, 14°17'16.0"E; 515 m a.s.l.; M. Lepší; 8. 5. 2013; 10. – **289:** CZ; 40a; Litoradlice (distr. České Budějovice), forest ca 1.8 km NE of the village; 49°09'42.0"N, 14°26'16.0"E; 470 m a.s.l.; PK; 8. 5. 2013; 10. – **290:** CZ; 68; Rancířov (distr. Jindřichův Hradec), oak-hornbeam woodland in the Kysibl Nature Reserve, ca 2.5 km ESE of the church of the village; 48°55'27.0"N, 15°33'30.0"E; 470 m a.s.l.; L. Ekrt; 11. 5. 2013; 5. – **291:** CZ; 68; Bítov (distr. Znojmo), oak-hornbeam woodland on the right-hand shore of the Vranovská přehrada dam, ca 0.9 km NNE of the church in the village; 48°55'43.0"N, 15°44'04.0"E; 445 m a.s.l.; L. Ekrt; 12. 5. 2013; 5. – **292:** CZ; 37n; Cetviny (distr. Český Krumlov), shore of the Malše river ca 0.5 km NNE of the church in the former village; 48°36'39.0"N, 14°33'05.0"E; 650 m a.s.l.; PK; 12. 5. 2013; 12. – **293:** CZ; 68; Chvalatice (distr. Znojmo), oak-hornbeam woodland in the S part of the Babka peninsula, ca 2.7 km SSW of the church in the village; 48°55'30.0"N, 15°44'40.0"E; 360 m a.s.l.; L. Ekrt; 12. 5. 2013; 6. – **294a:** CZ; 68; Čučice (distr. Brno-venkov), thermophilous oak forest on the steep hillside under the Oslava river, ca 1.8 km NE of the church in the village; 49°08'51.0"N, 16°15'29.0"E; 350 m a.s.l.; L. Ekrt; 16. 5. 2013; 5. – **294b:** CZ; 68; Čučice (distr. Brno-venkov), forest path close to the edge of the oak-hornbeam woodland, ca 1.2 km NE of the church in the village; 49°08'39.0"N, 16°15'58.0"E; 375 m a.s.l.; L. Ekrt; 16. 5. 2013; 5. – **295:** CZ; 41; Solenice (distr. Příbram), horizontal forest path in the valley of the rivulet, ca 0.7 km NNE of the settlement Větrov, N of the village Solenice; 49°38'00.0"N, 14°11'31.0"E; 460 m a.s.l.; PK; 19. 5. 2013; 10. – **296:** CZ; 31a; Plzeň (distr. Plzeň-město), forest in the western part of the ZOO; 49°45'28.0"N, 13°21'09.0"E; 330 m a.s.l.; MŠ; 25. 5. 2013; 5. – **297:** CZ; 32; Plzeň-Bukovec (distr. Plzeň-město), Zábělá Nature Reserve, ash-maple forest on the scree slope in the mouth of the ravine, ca 1.4 km NE of the village; 49°46'59.0"N, 13°27'03.0"E; 300 m a.s.l.; PK; 2. 6. 2013; 10. – **298:** CZ; 37i; Ktiš (distr. Prachatice), mixed forest above the road to the village Smědče, ca 0.8 km NNE of the church; 48°55'23.0"N, 14°08'10.0"E; 780 m a.s.l.; PK & MŠ; 6. 6. 2013; 10. – **299:** CZ; 74a; Krnov-Chomýž (distr. Bruntál), shrubs under the former quarry on the N edge of the former army shooting range, ca 0.7 km SW of the village; 50°06'37.0"N, 17°38'28.0"E; 365 m a.s.l.; PK; 9. 6. 2013; 5. – **300:** CZ; 74b; Slavkov (distr. Opava), dam of Slavkovský rybník pond and the adjacent oak-hornbeam forest, ca 2.1 km S of the church; 49°54'12.0"N, 17°50'01.0"E; 280 m a.s.l.; PK; 11. 6. 2013; 4. –**301:** CZ; 68; Podmolí (distr. Znojmo), forest near the crossroad U Milíře, ca 1.7 km SSE of the middle of the village; 48°50'11.0"N, 15°56'52.0"E; 415 m a.s.l.; Z. Chlumská; 15. 6. 2013; 4. – **302:** SK; 13; Horná Poruba (distr. Ilava), peak Vápeč; 48°56'20.0"N, 18°19'32.0"E; 870 m a.s.l.; F. Kolář; 12. 6. 2011; 1. – **306:** CZ; 8; Praha-Radotín (distr. Hlavní město Praha), oak-hornbeam woodland ca 1.3 km W from the railway station; 49°59'10.0"N, 14°20'48.0"E; 310 m a.s.l.; PK & MŠ; 4. 5. 2014; 10. – **307:** CZ; 16; Brno-Kohoutovice (distr. Brno-město), N oriented part of the ridge covered by oaks, lindens and pine trees, near the green touristic trail, ca 0.5 km W of water tank in the village Kohoutovice; 49°11'29.7"N, 16°31'07.4"E; 392 m a.s.l.; T. Koutecký; 17. 4. 2014; 6. – **308:** CZ; 16; Havraníky (distr. Znojmo), riparian forest by the Dyje river and the right-hand tributary near the former Baštův mlýn mill, ca 1.9 km W of the church; 48°48'47.0"N, 15°58'59.0"E; 250 m a.s.l.; L. Ekrt; 6. 4. 2014; 10. – **309:** CZ; 16; Moravský Krumlov (distr. Znojmo), dry oak forest ca 3.5 km ESE of the historic centre of the town, near the road from Moravský Krumlov to Jezeřany; 49°02'37.0"N, 16°21'36.0"E; 385 m a.s.l.; PK; 17. 4. 2014; 12. – **310:** CZ; 16; Lhánice (distr. Znojmo), light oak forest on the edge of the plateau over the valley of the Jihlava river, 1.1 km S of the municipal authority in the village

Lhánice; 49°05'58.0"N, 16°13'14.0"E; 330 m a.s.l.; T. Koutecký; 27. 4. 2014; 15. – **311:** CZ; 16; Lhánice (distr. Znojmo), forest edge on the E oriented part of the ridge with the former cover of the coppiced woodland of *Quercus petraea*, 2.3 km ESE of the municipal authority in Lhánice; 49°06'08.0'"N, 16°14'45.0"E; 365 m a.s.l.; T. Koutecký; 27. 4. 2014; 6. – **312:** CZ; 37l; Boršov nad Vltavou (distr. České Budějovice), riparian and ravine forests on the left-handed shore of the Vltava river, ca 1.35 km WSW of the church; 48°55'01.0"N, 14°25'01.0"E; 415 m a.s.l.; PK; 6. 4. 2014; 5. – **313:** CZ; 37l; Český Krumlov-Vyšný (distr. Český Krumlov), forest ca 0.4 km S of the middle of the village; 48°49'33.0"N, 14°18'00.0"E; 575 m a.s.l.; P. Drahník; 30. 4. 2014; 5. – **314:** CZ; 37p; Benešov nad Černou (distr. Český Krumlov), shores of the Černá river S of Kancléřský rybník pond, ca 2.6 km SW of the church; 48°43'31.0"N, 14°35'34.0"E; 595 m a.s.l.; PK; 25. 5. 2014; 5. – **315:** CZ; 41; Zbraslav (distr. Hlavní město Praha), hornbeam woodland on the N hillsise of peak Šance, 0.7 km of the railway station; 49°58'14.2"N, 14°24'05.1"E; 275 m a.s.l.; PK & MŠ; 2. 5. 2014; 5. – **316:** CZ; 41; Dobříš (distr. Příbram), fragment of the oak forest ca 0.9 km SSW of the castle; 49°46'24.0"N, 14°10'34.0"E; 370 m a.s.l.; PK; 1. 5. 2014; 5. – **317:** CZ; 41; Třebsín (distr. Praha-západ), dry oak forest 1.1 km WSW of the village; 49°51'14.0"N, 14°26'30.0"E; 410 m a.s.l.; PK & MŠ; 3. 5. 2014; 5. – **318:** CZ; 67; Kostelní Myslová (distr. Jihlava), valley of the Myslůvka rivulet ca 1 km N of the church; 49°09'27.0"N, 15°25'45.0"E; 495 m a.s.l.; L. Ekrt; 7. 4. 2014; 20. – **319:** CZ; 68; Veverská Bítýška (distr. Brno-město), riparian forest by the rivulet near the gamekeeper's lodge Prádelna, ca 50 m SSW of the castle Veveří; 49°15'07.0"N, 16°27'27.0"E; 250 m a.s.l.; MŠ & T. Štechová; 4. 4. 2014; 10. – **320:** CZ; 68; Hrotovice (distr. Třebíč), dry oak forest ca 2 km E of the church in the village; 49°06'25.7"N, 16°04'50.1"E; 400 m a.s.l.; PK; 17. 4. 2014; 5. – **321A:** CZ; 68; Tetčice (distr. Brno-venkov), S oriented part of peak 444 with oak forest (*Querces robur*) dominated by *Carex pilosa* and *Melica nutans*, 1.2 km SSE of the railway station in Tetčice; 49°09'44.0"N, 16°24'53.0"E; 440 m a.s.l.; T. Koutecký; 26. 4. 2014; 6. – **321B:** CZ; 68; Tetčice (distr. Brno-venkov), shallow valley of the ridge over peak Bučín, 1 km SSE of the railway station in Tetčice; 49°09'47.0"N, 16°24'51.0"E; 435 m a.s.l.; T. Koutecký; 26. 4. 2014; 9. – **322:** CZ; 68; Hluboké Mašůvky (distr. Znojmo), dry oak-hornbeam woodland ca 0.3 km NE of the Bábovec mlýn mill, ca 2.1 km NE of the church in the village; 48°56'14.0"N, 16°02'42.0"E; 300 m a.s.l.; PK & MŠ; 11. 3. 2014; 5. – **323:** CZ; 74b; Hněvošice (distr. Opava), Hněvošický háj grove, S edge of the oak forest, ca 2 km SW of the church; 49°59'49.0"N, 17°59'14.0"E; 305 m a.s.l.; PK; 28. 3. 2014; 5. – **324:** CZ; 84a; Staříč (okr. Frýdek-Místek), oak forest on the N hillside of peak Kamenná, ca 0.8 km N of the church; 49°41'35.0"N, 18°16'28.0"E; 360 m a.s.l.; PK; 28. 3. 2014; 8. – **340:** AT; -; Klosterneuburger Hütte (distr. Steiermark), light spruce forest along the asphalt road to Klosterneuburger Hütte; 47°15'48.7"N, 14°26'56.5"E; 1441 m a.s.l.; F. Kolář; June 2011; 5. – **341:** AT; -; Eberstein (distr. Sankt Veit an der Glan), forest path from the village St. Florian to nameless peak between villages St. Florian and Eberstein; 46°48'00.8"N, 14°32'21.5"E; 806 m a.s.l.; F. Kolář; June 2011; 3. – **342:** AT; -; St. Georgen im Lavanttal (distr. Wolfsberg), shady forest on the NW hillside of rocky peak Ortwinkogel; 46°46'32.2"N, 14°25'41.8"E; 614 m a.s.l.; F. Kolář; June 2011; 3. – **343:** AT; -; Turracher Höhe (distr. Steiermark/Kärnten), forest by the main road; 46°54'24.6"N, 13°52'43.5"E; 1726 m a.s.l.; F. Kolář; June 2011; 4. – **357:** AT; -; Eisenhüttl (distr. Güssing), NW edge of the forest Zickenwald, ca 1.3 km SE of the village; 47°05'44.0"N, 16°12'40.0"E; 240 m a.s.l.; PK; 27. 7. 2014; 2.

Populations with occurrence of tetraploid and DNA-hexaploid cytotypes of *Symphytum tuberosum* subsp. *angustifolium*: 10: CZ; 21a; Grygov (distr. Olomouc), forest Chrast, coppiced woodland ca 250 m NE of the monument near the path to the PP U strejčkova lomu Nature Reserve; 49°31'19.7"N, 17°19'14.2"E; 233 m a.s.l..; LK & MH; 19. 5. 2011; 15*(1 hexaploid individual). – **156:** CZ; 77a; Kloboučky u Bučovic (distr. Vyškov); oak-hornbeam woodland on the edge of the Louky u dubu forest; 49°06'44.5"N, 17°01'00.5"E; 348 m a.s.l.; LK; 9. 4. 2014; 6 (4 hexaploid individuals). – **221:** CZ; 79; Březolupy (distr. Uherské Hradiště), Svárov-paseky, bus station, ditch of the road to the village Březolupy; 49°07'15.2"N, 17°36'08.5"E; 250 m a.s.l.; LK & MH; 27. 4. 2014; 4+1/-. – **224:** CZ; 21a Těšnovice, (distr. Kroměříž), Obora Nature Reserve, N edge of the reservation near the shooting range and cynological club; 49°16'03.7"N, 17°24'20.3"E; 248 m a.s.l.; LK & MH; 1. 5. 2014; 4 (1 hexaploid individual). – **258:** CZ; 77a; Ždánice (distr. Hodonín), hornbeam-beech forest by the road to the village Bučovice, ca 3.1 km NNW of the church in the village; 49°05'44.0"N, 17°01'15.0"E; 430 m a.s.l.; PK; 19. 5. 2011; 5 (1 hexaploid individual).

Populations with occurrence of DNA-decaploid and dodecaploid cytotypes of *Symphytum tuberosum* subsp. *tuberosum*: 3: CZ; 74a; Dolní Povelice (distr. Bruntál), forest edge ca 0.5 km NNE of the village; 50°14'11.1"N, 17°41'07.9"E; 262 m a.s.l.; BT; 26. 4. 2011; 15* (3 decaploid individuals). – **4:** CZ; 68; Mohelno (distr. Třebíč), Mohelenská hadcová step Nature Reserve, forest's undergrowth by the blue touristic trail, ca 0.2 km N of the crossroads Papírna; 49°06'24.2"N, 16°10'55.9"E; 313 m a.s.l.; LK & MH; 5. 5. 2011; 15* (3 decaploid individuals). – **5:** CZ; 82; Huslenky (distr. Vsetín), valley of the Kychová rivulet, alluvial tall-forb vegetation dominated by *Petasites* near the road to the settlement U Dražků; 49°16'37.4"N, 18°09'21.3"E;

565 m a.s.l.; M. Dančák; 5. 5. 2011; 15* (1 decaploid individual). – **31:** CZ; 21b; Olomouc (distr. Olomouc), valley of the Bystřice river, ca 50 m W of the bridge over the river; 49°35'46.1"N, 17°18'00.8"E; 219 m a.s.l.; LK & MH; 2. 5. 2012; 15* (1 decaploid individual). – **170:** CZ; 68; Dačice (distr. Jindřichův Hradec), Dubová stráň Nature Reserve, S edge of the reservation ca 1.8 km NNE of the castle; 49°05'38.2"N, 15°26'33.3"E; 375 m a.s.l.; L. Ekrt; 17. 5. 2013; 20 (2 decaploids individuals). – **199:** AT; -; Neu-Draschitz (distr. Hermagor), grassland in the village; 46°33'21.0"N, 13°38'38.0"E; 655 m a.s.l.; BT; 19. 4. 2014; 3 (1 decaploid individual). – 247: SK; 21b; Terchová (distr. Žilina), alpine grasslands between Hromové and Poludňový Grúň peaks; 49°11'49.5"N, 19°03'48.7"E; 1523 m a.s.l.; M. Dančák; 17. 6. 2014; 3 (1 decaploid individual).

Populations with occurrence of dodecaploid and DNA-tetradecaploid cytotypes of *Symphytum tuberosum* subsp. *tuberosum*: 33: CZ; 76a; Lipník nad Bečvou (distr. Přerov), Škrabalka Nature Reserve, alder carr in the death arm of the Bečva river; 49°31'22.0"N, 17°36'02.9"E; 231 m a.s.l.; LK & MH; 6. 5. 2012; 15* (1 tetradecaploid individual). – **34:** CZ; 83; Petřvald (distr. Nový Jičín), valley of the Lubina brook; 49°42'11.0"N, 18°08'39.0"E; 237 m a.s.l.; E. Jahodářová & M. Motyka; 5. 5. 2012; 15* (1 tetradecaploid individual). – **35:** CZ; 80a; Vsetín (distr. Vsetín), Seninka; 49°16'31.0"N, 17°58'31.0"E; 430 m a.s.l.; BT; 8. 5. 2012; 15* (1 tetradecaploid individual). – **88:** CZ; 84a; Frenštát pod Radhoštěm (distr. Nový Jičín), forest edge in the valley of the Dlouhý potok rivulet; 49°33'49.5"N, 18°11'49.3"E; 371 m a.s.l.; J. Balarynová; 28. 4. 2013; 15* (1 tetradecaploid individual). – **98:** CZ; 76a; Choryně (distr. Vsetín), Choryňská stráž Nature Reserve; 49°29'56.7"N, 17°53'27.3"E; 349 m a.s.l.; LK & MH; 8. 5. 2013; 6 (1 tetradecaploid individual). – **113:** DE; -; Leitzach (distr. Miesbach), shrubs near the road; 47°46'51.0"N, 11°52'36.0"E; 684 m a.s.l.; BT; 22. 5. 2013; 3 (1 tetradecaploid individual). – **120:** CZ; 21b; Chrbov (distr. Přerov), alder carr in the alluvium of the Morava river near the edge of the Zástudánčí Nature Reserve; 49°23'42.2"N, 17°18'15.1"E; 196 m a.s.l.; LK; 13. 3. 2014; 3 (2 tetradecaploid individuals). – **130:** CZ; 75; Vítkov (distr. Opava), shores of the Čermná river under the road from the village Klokočůvky, ca 500 m S from the railway station Klokočov; 49°44'00.1"N, 17°46'00.0"E; 372 m a.s.l.; LK; 26. 3. 2014; 6 (1 tetradecaploid individual). – **134:** CZ; 74b; Žimrovice (distr. Opava), crossroads Pilanka, alder carr in the valley of the Moravice river, edge of the brooklet running from sewage disposal plant; 49°51'35.3"N, 17°51'24.0"E; 282 m a.s.l.; LK; 26. 3. 2014; 4 (2 tetradecaploid individuals). – **176:** CZ; 68; Oberndorf am Jauerling (distr. Krems-Land), shores of the nameless tributary of the Altmannsbach rivulet near the car parking under the ski slope; 48°20'02.8"N, 15°20'44.5"E; 375 m a.s.l.; LK, MH, MŠ & PK; 16. 4. 2014; 9 (2 tetradecaploid individuals).

Mixed populations of tetraploid cytotype of *Symphytum tuberosum* subsp. *angustifolium* and dodecaploid cytotype of subsp. *tuberosum*: 37: SK; 14a; Obyce (distr. Zlaté Moravce), forest edge near the rivulet; 48°26'21.8"N, 18°28'27.2"E; 312 m a.s.l.; LK; 1. 7. 2012; 4 (2 tetra- and 2 dodecaploid individuals). – **211:** CZ; 78; Nedašova Lhota, Podvysočí (distr. Zlín), grassy hillsides near the road in the valley of the rivulet; 49°07'36.4"N, 18°06'08.2"E; 521 m a.s.l.; LK & MH; 26. 4. 2014; 9 (4 tetra- and 5 dodecaploid individuals). – **212:** CZ; 78; Návojná (distr. Zlín), garden of house no. 185 and neighbouring garden; 49°06'34.0"N, 18°03'05.31E; 374 m a.s.l.; LK & MH; 26. 4. 2014; 6 (5 tetra- and 1 dodecaploid individuals). – **213:** CZ; 82; Valašské Klobouky, Vlčí potok (distr. Zlín), edge of the road under the railway viaduct and the forest under the Bílé potoky Nature Reserve, ca 200 m SW of the Vlčí potok; 49°07'14.5"N, 18°01'15.9"E; 400 m a.s.l.; LK & MH; 26. 4. 2014; 5 (3 tetra- and 2 dodecaploid individuals).