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Taxonomic status and typification of a neglected name *Symphytum leonhardtianum* from the *Symphytum tuberosum* complex (Boraginaceae)

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Abstract

Symphytum leonhardtianum, a member of the *S. tuberosum* complex, is investigated. This taxon was described by Pugsley in 1931, from the vicinity of Vienna, Austria. Nevertheless, it is generally not accepted in European floras. In this study, we conducted an evaluation of this taxon using flow cytometry, karyology and morphological analysis. Flow cytometric and karyological investigations of plants from the type locality of *S. leonhardtianum* revealed only dodecaploids ($2n$ = 12x = 96), a ploidy level corresponding to the *S. tuberosum* subsp. *tuberosum*. The chromosome number of the *S. tuberosum* from Austria is here recorded for the first time. Morphological comparison of Central European populations of *S. tuberosum* complex showed that *S. leonhardtianum* did not differ significantly from *S. tuberosum* subsp. *tuberosum*. Based on our findings, we propose treating the name *S. leonhardtianum* as a heterotypic synonym of *S. tuberosum* subsp. *tuberosum*. The lectotype of *S. leonhardtianum* is designated.

Key words: Central Europe, flow cytometry, karyology, lectotypification, morphology, polyploidy

Introduction

The *Symphytum tuberosum* complex belongs to one of the most complicated groups within the genus *Symphytum* Linnaeus (1753: 136) in Europe, mainly due to an occurrence of polyploidy and associated extensive morphological variability (Gadella & Kliphuis 1978, Murín & Májovský 1982, Kobrlová *et al.* 2016). Despite current progress, the taxonomy of *S. tuberosum* is still not satisfactorily resolved. The members of this complex are distributed across Europe and Asia Minor (Bucknall 1913, Murín & Májovský 1982, Kobrlová *et al.* 2016) and a total of ten taxa have been described within this complex, three of them from Central Europe: *Symphytum tuberosum* Linnaeus (1753: 136), *Symphytum angustifolium* A.Kerner (1863: 227) and *Symphytum leonhardtianum* Pugsley (1931: 95).

Symphytum tuberosum is one of the three species of *Symphytum* distinguished by Linnaeus. The original description is based on plant material apparently originating from southern Germany (Linnaeus 1753). It is traditionally accepted as a wide-ranging European species. Plants from southern Germany were shown to have a dodecaploid cytotype ($2n$ = 96; Kobrlová *et al.* 2016).

Symphytum angustifolium was described from the plant material collected in the Pilis Mountains in northern Hungary as a narrow-leaved morph of *S. tuberosum* (Kerner 1863). Later, it was also discovered in Slovakia and in the south-eastern part of the Czech Republic. It has been shown to have a tetraploid chromosome number ($2n$ = 32; Murín & Májovský 1982, Kobrlová *et al.* 2016). Nevertheless, there has been much confusion surrounding this name, and it has been often synonymised with *S. nodosum* Schur (1866: 468) or applied to all populations of the *S. tuberosum* complex from East and Central Europe (cf. Pawłowski 1972, Smejkal 1978, Valdés 2011).

Symphytum leonhardtianum was described from specimens collected in Haltertal near Vienna, Lower Austria and was originally differentiated from *S. tuberosum* s. str. by its slender rhizomes, shorter and less branched stems, fewer and broader leaves, shorter and more strongly ciliate calyx lobes, brightly coloured corollas and smaller and paler mericarpids (Pugsley 1931). According to Pugsley (1931) the species is mainly confined to Central Europe, with its range extending from the French Alps and Pyrenees to Russia and Balkan Peninsula. However, *S. leonhardtianum* has

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been neglected in most European floras and only the Soviet and Ukrainian floras (Popov 1953, Dobroczajeva 1957) and some Ukrainian studies (Zaverucha 1962, Dobroczajeva 1968) recognize it.

Kobrlová *et al.* (2016) recently showed that two members of the *S. tuberosum* complex should be recognized in Central Europe: the widespread dodecaploid (2*n* = 12x = 96) and broad-leaved taxon corresponding to *S. tuberosum* subsp. *tuberosum* (thereafter *S. *tuberosum*) and the tetraploid (2*n* = 4x = 32) narrow-leaved taxon corresponding to *S. tuberosum* subsp. *angustifolium* (A.Kern.) Nyman (1881: 510; thereafter *S. *angustifolium*), which shows an affinity to the northern regions of the Pannonian Basin (Kaplan *et al.* 2016, Kobrlová *et al.* 2016). Unfortunately, the name *S. leonhardtianum* was omitted from their study and its analysis is therefore provided here.

The aims of the present study are (i) to determine DNA-ploidy level, the number of chromosomes and morphological variation of the populations from the locus classicus of *S. leonhardtianum* and its close vicinity and (ii) to infer the relationship of these populations within the *S. tuberosum* complex in Central Europe.

Material & Methods

Plant material and morphometric analyses

Plant material for *S. leonhardtianum* was collected in the locus classicus (i.e., Haltertal) and its vicinity in western surroundings of Vienna (Pugsley 1931). In total, five populations (37 individuals) were collected (see Appendix 1). Additional four populations (32 individuals) of *S. *angustifolium* (two from the locus classicus in Pilis Mts., northern Hungary and two from Moravia) were also collected. Voucher specimens are deposited in the Herbarium of the Palacký University in Olomouc (OL). A morphological investigation was conducted on 64 individuals from eight populations and added to the dataset used in Kobrlová *et al.* (2016). Altogether, 50 populations of the *S. tuberosum* complex from Central Europe were morphologically evaluated. For each individual, 19 vegetative and generative characters were studied (Table 1), i.e. the same set of morphological traits that was already used for differentiation of Central European populations of *S. tuberosum* (Kobrlová *et al.* 2016). Other characters, such as rhizome slenderness and colour of flowers and mericarpids were compared later in the herbaria and are not included in the analyses.

TABLE 1. List of the morphological characters analysed, and their codes used in the descriptive statistics (Fig. 3).

Morphological character (unit)	Code
Height of plant (cm)	height
Length to width ratio of uppermost leaf	shape_U
Length of uppermost leaf (cm)	
Width of uppermost leaf (cm)	
Length to width ratio of middle leaf	shape_M
Length of middle leaf (cm)	
Width of middle leaf (cm)	
Length to width ratio of lowermost leaf	shape_L
Length of lowermost leaf (cm)	
Width of lowermost leaf (cm)	
Length of pedicel (mm)	l_ped
Length of calyx (mm)	calyx
Length of corolla (mm)	corolla
Length of narrow part of corolla tube (mm)	cor_tube
Length of style (mm)	style
Length of filament (mm)	l_fill
Width of filament (mm)	w_fill
Length of free part of filament (mm)	l_ffill
Length of fornice (mm)	l_forn
Length of anther (mm)	l_anth
Width of anther (mm)	w_anth

Flow Cytometry (FCM)

DNA-ploidy amounts were estimated using a Partec PAS flow cytometer equipped with a green solid-state laser. Samples were prepared following the simplified protocol with LB01 isolation buffer and propidium iodide (Sigma-Aldrich, St Louis, MO, USA) staining (Doležel *et al.* 2007). Details for sample preparation are given in Kobrlová *et al.* (2016). *Pisum sativum* 'Ctirad' (2C = 9.09 pg; Doležel *et al.* 1998) and *Zea mays* 'CE-777' (2C = 5.92 pg, value calibrated against *Pisum sativum* 'Ctirad') were used as the internal standards. Each plant was analysed separately and the fluorescence intensity of at least 3,000 particles was recorded. The resulting values were determined by the position of its G0/G1 peak relative to the G0/G1 peak of the internal standard. Histograms with a coefficient of variation less than 5 % were accepted.

Chromosome counts

Actively growing, young roots were harvested from the cultivated plants, pre-treated with ice-cold water for 24 h, fixed in ethanol/acetic acid (3:1) fixative for 24 h at 4°C and stored at -20°C until further use. Selected root tips were rinsed in distilled water (twice for 5 min) and citrate buffer (10 mM sodium citrate, pH 4.8; twice for 5 min), and digested in 0.3% cellulase, cytohelicase and pectolyase (all Sigma-Aldrich, St Louis, MO, USA) in citrate buffer at 37°C for 90 min. After digestion, individual root tips were dissected on a microscope slide in approximately 10 µl acetic acid and covered with a cover slip. The cell material was then spread evenly using tapping, thumb pressing and gentle flame-heating. Finally, the slide was quick frozen in liquid nitrogen and the cover slip flicked off with a razor blade. Slides were fixed in ethanol/acetic acid (3:1) and air-dried. Chromosomes were counterstained with 2 µg/ml DAPI in Vectashield. Preparations were photographed using Zeiss Z2 epifluorescence microscope and CoolCube CCD camera.

Statistical analyses

All studied morphological characters were used except for the length and width of the leaves from which ratios were calculated. The morphological dataset therefore contained 12 measured morphological characters and three ratios (Table 1). The dataset was analysed using a set of R functions contained in MorphoTools version 1.01 (Koutecký 2015). Basic descriptive statistics (average, minimum, maximum) were calculated for each morphological character and studied taxon. Tukey-Kramer multiple comparison tests at $p \leq 0.01$ for all three putative taxa (*S. *angustifolium*, *S. leonhardtianum*, *S. *tuberosum*) were calculated to determine which characters show significant differences among groups. Population averages were calculated and used as operational taxonomic units (OTUs) for multivariate analyses. Logarithmic transformations of several characters were applied, i.e. natural logarithmic transformations (log) of the pedicel length and the fornice length and common logarithmic transformations (log10) for the style length and the anthers width. Correlations of morphological characters were tested using Pearson's correlation coefficient. A Principal component analysis (PCA; Sneath & Sokal 1973) was used to test the morphological homogeneity within three putative taxa. The character 'branching of stem', due to its qualitative nature, was separately analysed using subdivided contingency tables (Zar 1996) in NCSS 9 (Hintze 2013).

Typification process

Name was typified following the instructions of the International Code of Nomenclature for algae, fungi and plants (Melbourne Code; McNeill *et al.* 2012).

Results

Flow Cytometry

FCM data were newly obtained for 69 plants from nine populations. All five populations from the vicinity of the locus classicus of *S. leonhardtianum* had DNA-dodecaploid ploidy level. Additional four populations of *S. *angustifolium* were all DNA-tetraploids (Table 2).

Chromosome counting

Two individuals of *S. leonhardtianum* (from populations 455 and 456; Appendix 1) were counted to calibrate the results from FCM. Both counts resulted in 2n = 96 (Fig. 1).

TABLE 2. Relative DNA content of the *Symphytum tuberosum* complex in Central Europe assessed using flow cytometry. A) *Symphytum tuberosum* subsp. *angustifolium* (Kobrlová *et al.* 2016, including four populations from this study), B) *Symphytum tuberosum* subsp. *tuberosum* (Kobrlová *et al.* 2016) and C) populations from the locus classicus of *S. leonhardtianum*. All values are calculated relative to the internal standard *Pisum sativum* 'Ctirad'. Tetraploids were analysed with *Zea mays* 'CE-777'; 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	Mean ratio to the standard ± SE	Range	Variation (%)	Mean 2C-value (pg) ± SE
A	4x	413	0.247 ± 0.011	0.222–0.278	22.9	2.03 ± 0.104
B	12x	739	0.663 ± 0.017	0.628–0.698	10.6	6.03 ± 0.171
C	12x	37	0.662 ± 0.020	0.623–0.698	11.3	6.02 ± 0.178

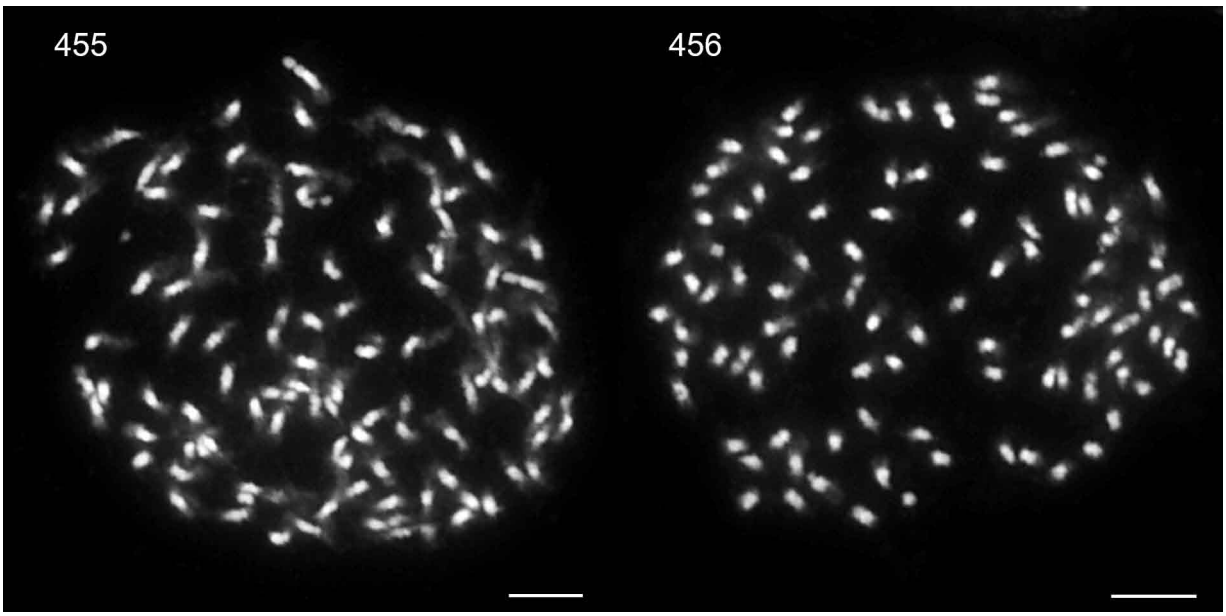

FIGURE 1. Micrographs of somatic metaphase chromosomes of two individuals from the locus classicus of *Symphytum leonhardtianum* (455) and its vicinity (456) near Vienna, Austria. Scale bar = 10 µm.

Morphometric analyses

The extent of the morphological variability of *S. leonhardtianum* was generally similar to the variability of the morphological traits of *S. *tuberosum*. The average value of several morphological characters of *S. leonhardtianum* measured, e.g. corolla length, corolla tube length, style length, significantly exceeded the average value detected for the same characters of *S. *tuberosum* (Table 3, Fig. 2). No pairs of highly correlated characters ($r > 0.95$) were found. Therefore, the entire dataset was used in the multivariate analyses. Two groups corresponding to *S. *angustifolium* and *S. *tuberosum* were separated along the first component axis in the principal component analysis (the first, second and third axis explaining 42.6 %, 15.7 % and 13.6 % of variation, respectively). All five studied populations putatively belonging to *S. leonhardtianum* were grouped together with *S. *tuberosum* in the PCA diagram (Fig. 3). The pattern of branching was significantly different between the three taxa ($\chi^2 = 63.24$; DF = 6; $P < 0.01$). Subdivided contingency tables showed that *S. leonhardtianum* and *S. *tuberosum* have very similar branching pattern ($\chi^2 = 5.09$; DF = 3; $P = 0.17$) and they both differ significantly from *S. *angustifolium* ($\chi^2 = 58.78$; DF = 3; $P < 0.01$).

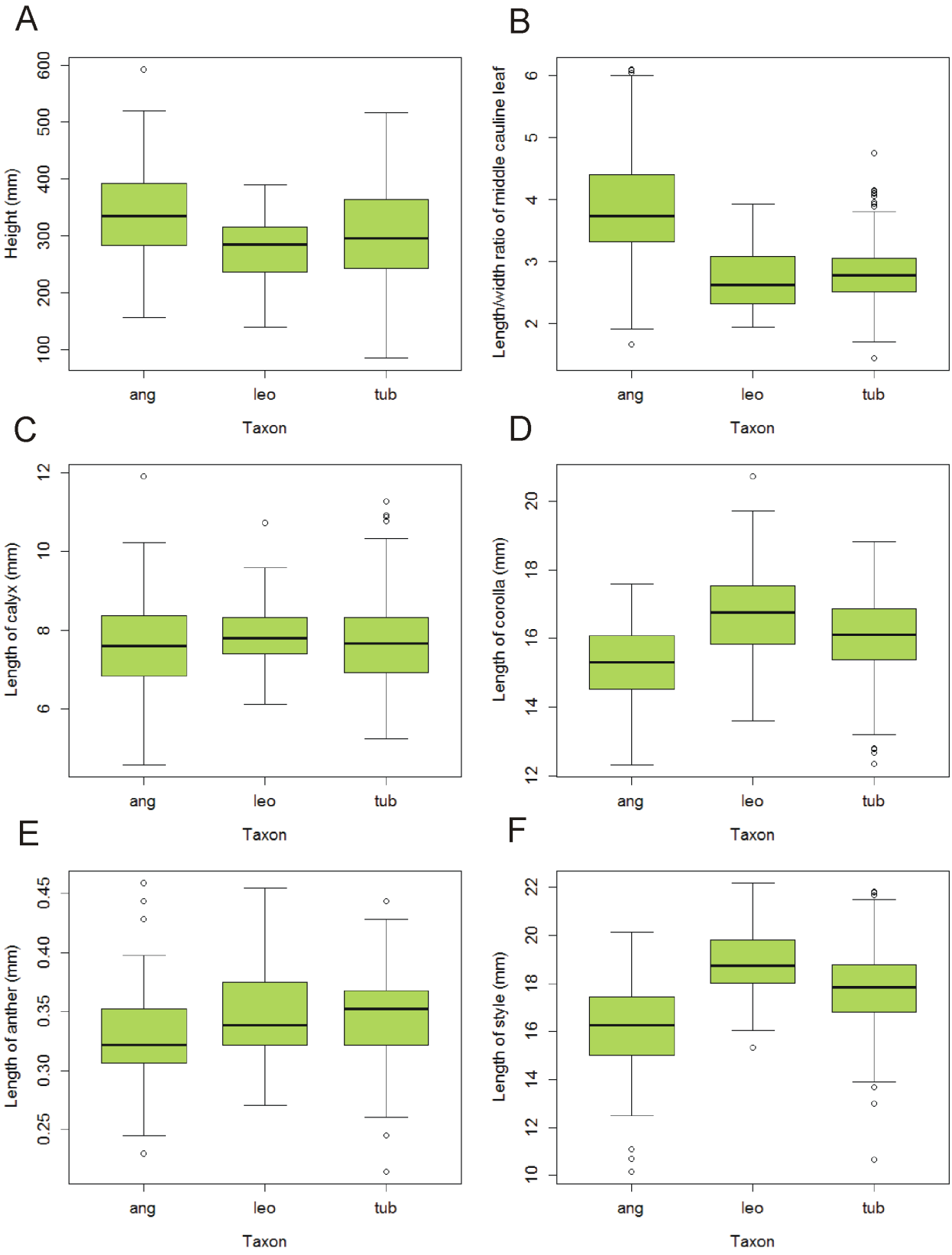

FIGURE 2. Variation of selected morphological characters and ratios. Rectangles define the 25th and 75th percentiles, horizontal lines show the median, whiskers are from the 10 to 90 percentiles, circles show extreme values. A) height of plants, B) length to width ratio of middle cauline leaves, C) length of calyx, D) length of corolla, E) length of anther, F) length of style.

TABLE 3. Basic descriptive statistics for each taxon. (min)mean(max) = minimal, average and maximal value of morphological character, in millimetres; SD = standard deviation; asterisk = means significantly different from each of all groups and two asterisks denote two groups significantly different at $p \leq 0.01$ in Tukey-Kramer multiple comparison test.

	S. *angustifolium		***S. leonhardtianum***		***S. *tuberosum***	
	(min)mean(max)	**±SD**	**(min)mean(max)**	**±SD**	**(min)mean(max)**	**±SD**
height	(156)339(593)*	76	(140)275(390)	60	(85)303(517)	79
shape_U	(1.2)3.3(5.5)*	0.7	(1.5)2.5(4.1)	0.6	(1.6)2.6(4.7)	0.5
shape_M	(1.7)3.8(6.1)*	0.8	(1.9)2.7(3.9)	0.5	(1.4)2.8(4.8)	0.5
shape_L	(1.3)4.1(8.7)*	1.0	(1.3)2.8(4.4)	0.6	(1.5)2.8(5.5)	0.5
l_ped	(4.2)8.2(14.8)	1.9	(3.6)7.3(11.9)**	2.0	(4.0)8.5(15.0)**	2.0
calyx	(4.6)7.6(11.9)	1.1	(6.1)7.8(10.7)	0.9	(5.2)7.7(11.3)	1.1
corolla	(12.3)15.3(17.6)*	1.1	(13.6)16.7(20.7)*	1.6	(12.3)16.1(18.8)*	1.2
cor_tube	(5.5)7.7(9.7)*	0.7	(6.4)9.3(12.2)*	1.2	(5.7)8.4(10.8)*	0.8
style	(10.2)16.1(20.2)*	1.7	(15.3)18.8(22.2)*	1.3	(10.7)17.8(21.8)*	1.6
l_fill	(0.57)0.88(1.48)*	0.09	(0.77)1.07(1.38)*	0.15	(0.53)0.96(1.30)*	1.22
w_fill	(0.03)0.05(0.08)**	0.01	(0.03)0.05(0.07)	0.01	(0.03)0.06(0.08)**	0.01
l_ffill	(0.13)0.21(0.29)*	0.04	(0.16)0.27(0.34)*	0.05	(0.09)0.23(0.34)*	0.05
l_forn	(0.89)1.23(1.52)*	0.11	(1.07)1.41(1.75)*	0.16	(0.93)1.30(1.65)*	0.14
l_anth	(0.23)0.33(0.46)*	0.03	(0.27)0.35(0.45)	0.04	(0.21)0.34(0.44)	0.04
w_anth	(0.05)0.07(0.10)*	0.01	(0.06)0.07(0.08)	0.01	(0.06)0.07(0.09)	0.01

Discussion

The morphological variability within the *S. tuberosum* complex is high (cf. Kobrlová *et al.* 2016). We assume that a substantial part of this variation is probably caused by morphological plasticity, rather than genetic variability. Moreover, this variation is often increased by ecological conditions, especially by the availability of water and nutrients, sometimes resulting in atypical local entities, which deviate from the typical form (i.e. dwarfed plants, plants with unusual proportion of leaves and with sparse inflorescences). However, more detailed investigations are necessary in order to confirm this hypothesis. Nevertheless, the variation found in several morphological traits is correlated with the ploidy level and as such it has its taxonomical value (Kobrlová *et al.* 2016).

The taxon *S. leonhardtianum* was distinguished from *S. tuberosum* by the British amateur botanist H.W.Pugsley (Pugsley 1931, Lousley 1948) based on his knowledge of *S. tuberosum* from England, which he considered to be the true origin of the Linnean type (instead of southern Germany, Pugsley 1931, Stearn 1985). He observed dwarfed and more ornamental plants of *S. tuberosum* near Salzburg (Austria) and later in herbaria elsewhere from Central Europe and decided to describe them as a new species based on A.Kerner's Flora Exsiccata Austro-Hungarica no. 3710. Based on his conviction that the "true" *S. tuberosum* grows in England, he distinguished *S. leonhardtianum* from *S. tuberosum* mainly on the basis of shorter stems, broader leaves and more conspicuous flowers (Pugsley 1931). However, our analysis showed that *S. leonhardtianum* from its locus classicus is indistinguishable from *S. tuberosum* s. str. in most of these morphological traits (Table 3, Fig. 3). Similarly, McClintock (1968) and his colleagues when revising material of the *S. tuberosum* complex that was determined by Pugsley in the British Museum, considered *S. leonhardtianum* as inseparable from *S. tuberosum*.

Analysed individuals of *S. leonhardtianum* did not differed from individuals of *S. *tuberosum* in several morphological characters used by Pugsley (1931) for distinction of these two taxa (i.e., height of stems, width of leaves and length of calyx; Table 3). Likewise, the pattern of stem branching was similar to the branching in *S. *tuberosum*, i.e. prevailing of plants unbranched and branched in the lower part of the stem. According to Pugsley (1931), *S. leonhardtianum* is also distinctive by its slender rhizomes. Although, we have not evaluated the character of rhizomes, based on our observations, rhizomes of *S. leonhardtianum* are the same as in *S. *tuberosum* which is characterised by stout, creeping, horizontal to oblique and tuberous rhizomes (Kobrlová *et al.* 2016). Other morphological characters used by Pugsley such as hairiness of calyx and colour nuance of flowers and mericarpids are very hard to quantify and

therefore not very useful for species distinction. However, the comparison of herbarium specimens collected at loci classici of both taxa yielded no substantial differences in these traits. Quite surprisingly, the plants from four out of five of the populations studied in the close vicinity of Vienna (i.e., locus classicus of *S. leonhardtianum*) were found to have corollas and associated characters (i.e., length of fornices, styles and filaments) slightly larger in average (i.e., 2 mm) than all other plants evaluated from Central Europe. The size of flowers may be to some extent affected by ecological conditions or these populations may represent a local morph with somewhat larger flowers. However, such small differences in size of flowers were not considered as important trait for taxonomy in any morphological analysis of the *Symphytum* (Gadella *et al.* 1983, Sandbrink *et al.* 1990).

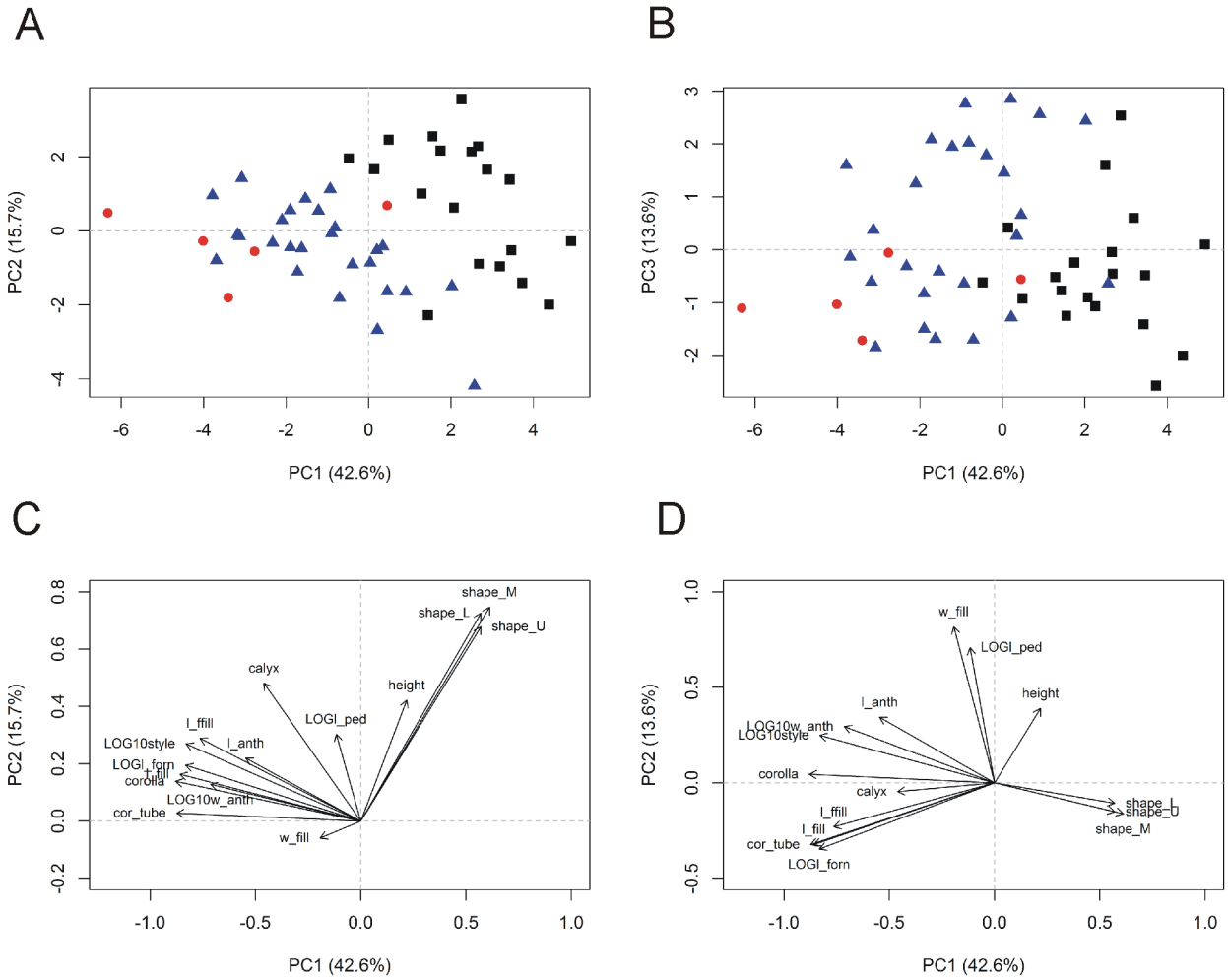

FIGURE 3. Principal component analysis (PCA) of 50 populations based on 12 morphological characters and three ratios. Squares correspond to *S. *angustifolium*, triangles to *S. *tuberosum* and circles to *S. leonhardtianum*. A) PCA of populations, first and second axes displayed, B) PCA of populations, first and third axes displayed, C) fit of the morphological characters and ratios to the ordination axes (abbreviations of morphological characters are explained in Table 1), first and second axes displayed, D) fit of the morphological characters and ratios to the ordination axes, first and third axes displayed.

In absence of a clear morphological distinction, *S. leonhardtianum* was not recognised in most of the European floras. In most cases, it was synonymised with other member of the *S. tuberosum* group, usually with *S. *angustifolium* (e.g., Pawłowski 1961, Pawłowski 1963, Soó 1968, Stearn 1985, Sandbrink *et al.* 1990, Bottega & Garbari 2003, Fischer *et al.* 2008, Valdés 2011). The only exceptions are the Soviet (Popov 1953) and Ukrainian floras (Dobroczajeva 1957) and the studies of the Ukrainian botanists Zaverucha (1962) and Dobroczajeva (1968), who recognised *S. leonhardtianum* as a separate species. However, the new editions of the Russian Floras do not follow this concept and either refer the *S. leonhardtianum* only as a synonym of *S. popovii* Dobrocz. (1968: 59; Fedorov 2001) or do not mention this name at all (Czerepanov 2007).

The FCM analyses of Central European populations revealed two ploidy levels in the studied material: significantly less common tetraploids ($2n = 4x = 32$) growing only in the Czech Republic, Slovakia and Hungary and widespread dodecaploids ($2n = 12x = 96$), occurring throughout the whole Central Europe (Kobrlová *et al.* 2016). These findings are in agreement with previously reported chromosome numbers by e.g. Májovský (1976), Gadella & Kliphuis (1978), Murín & Májovský (1982) and Javůrková-Jarolímová & Měsíček (1992) for dodecaploid and by Murín & Májovský (1982) for tetraploid plants. Unfortunately, no chromosome records of *S. leonhardtianum* were published. Additionally, there is no evidence about the chromosome counts of any *S. tuberosum* from Austria (cf. Dobeš & Vitek 2000). Our study therefore presents first chromosome counts for this country. Only two karyological studies mentioned the name *S. leonhardtianum* as a synonym of another member of the *S. tuberosum* complex (Grau 1968, Wcisło 1972). Both these studies reported dodecaploid chromosome counts from countries (i.e., Germany and Poland), where only *S. *tuberosum* is present according to Kobrlová *et al.* (2016). All studied populations of *S. leonhardtianum* from the vicinity of Vienna belong to a dodecaploid cytotype (i.e., the same as in *S. *tuberosum*). Moreover, this is the only cytotype detected in Austria up to now and there is no evidence about the presence of another cytotype (Kobrlová *et al.* 2016).

Finally, there are also no specific differences in habitat preferences of *S. leonhardtianum* as we found all plants growing generally in the same conditions as *S. *tuberosum*, i.e. mesic deciduous and shady woodlands and in ruderal vegetation along road (Kobrlová *et al.* 2016).

Therefore, when considering all available evidence, we assume that the plants from the locus classicus of *S. leonhardtianum* do not differ substantially from *S. tuberosum* subsp. *tuberosum* sensu Kobrlová *et al.* (2016) and therefore should not be considered as a separate species, even though they may represent a specific local form with somewhat larger flowers.

Conclusions

Altogether three taxa of the *Symphytum tuberosum* complex (*S. tuberosum*, *S. angustifolium* and *S. leonhardtianum*) have been reported from Central Europe, however, our study confirms the presence of only two taxonomic entities: the narrow-leaved, tetraploid *S. tuberosum* subsp. *angustifolium* and the widespread, dodecaploid and broad-leaved *S. tuberosum* subsp. *tuberosum* (see also Kobrlová *et al.* 2016) with *S. leonhardtianum* included as a synonym of the latter taxon.

Taxonomic treatment

Symphytum tuberosum Linnaeus (1753: 136). Lectotype (designated by Stearn 1985: 177):—GERMANY. "Germania australi", *C. Linnaeus s.n.* (LINN 185.3!).

Symphytum tuberosum subsp. *tuberosum*

= *Symphytum leonhardtianum* Pugsley (1931: 95). Lectotype (designated here):—AUSTRIA. Vienna: "Austria inferior, Haltertal prope Vindobonam (Wien) [Vienna], in dumetis", *A. Kerner s.n.* (BM no. 000752614!, Fig. 4; known isolectotypes BRNU!, PRC!).

Notes on typification.—When describing *S. leonhardtianum*, Pugsley did not mention the location of the type. Although several attempts were made, the name *S. leonhardtianum* Pugsley was never properly typified. The first attempt was made by Arto Kurtto when revising specimens of *Symphytum* in BM in 1983. He labelled the specimen no. 000752614 as lectotype with a note stating that the lectotypification would be made in the journal Annales Botanici Fennici, however, to our knowledge this was never done (A. Kurtto pers. communication). The second attempt, made by Bottega and Garbari in 2003, was also not successful because the authors did not include the term "designated here" or its equivalent (Art 7.10; McNeill *et al.* 2012).

Symphytum tuberosum subsp. *angustifolium* (A.Kern.) Nyman (1881: 510). Lectotype (designated by Bottega & Garbari 2003: 247):—HUNGARY. "Pilis, Slanitzka bei Csaba", *A. Kerner s.n.* (WU0069897!).

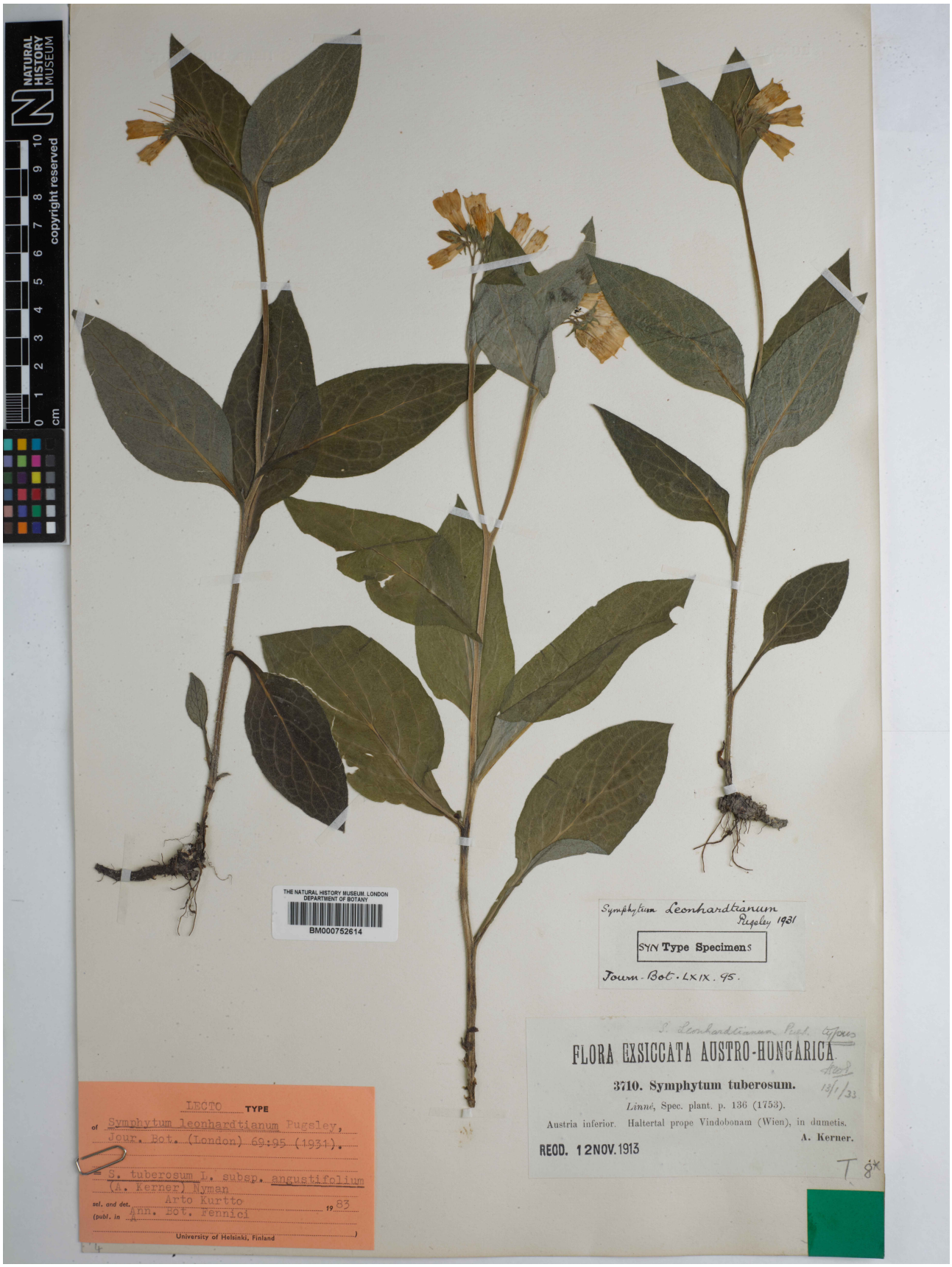

FIGURE 4. Lectotype specimen of *Symphytum leonhardtianum* (BM 000752614, from the collections of the Natural History Museum, London).

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APPENDIX 1. List of populations sampled in this study. For more details about the sampling of *Symphytum tuberosum* complex in Central Europe see Kobrlová *et al.* (2016). The format of the entries is as follows: Locality number: country; localization; coordinates (WGS 84); elevation; collectors; collection date; number of analysed samples. Asterisk denotes that chromosome count was established from this population.

***Symphytum tuberosum* subsp. *angustifolium*: 460:** Hungary; Piliscsaba, forest near the touristic trail ca 350 m S of the peak Nádor-hegy; 47°36'21.4"N, 18°50'47.6"E; 440; LK & MH; 2016-05-02; 10. – **461:** Hungary; Piliscsaba, grassy edge of the forest near the touristic trail (Zsíros-hegyi körtúra) ca 250 m W from the peak Csaba-hegy; 47°36'50.8"N, 18°49'56.1"E; 241; LK & MH; 2016-05-02; 10. – **470:** Czech Republic; Krčmaň, the valley of the rivulet Loučky, SW edge of the Chlum forest; 49°31'40.1"N, 17°20'53.5"E; 307; LK; 2016-05-20; 10. – **471:** Czech Republic; Slavkov pod Hostýnem, shrubs near the chapel, below the PP Stráň Nature Reserve; 49°22'26.5"N, 17°40'49.6"E; 454; LK; 2016-05-20; 2.

***Symphytum tuberosum* subsp. *tuberosum*: 455:** Austria; Vienna, Haltertal, forest in the valley of the stream Wolfsgraben; 48°13'20.5"N, 16°15'16.5"E; 280; LK & MH; 2016-05-01; 5*. – **456:** Austria; Vienna, Neuwaldegg, forest and ditches near the road, ca 150 m N from the peak Exelberg (near the car park); 48°15'00.1"N, 16°14'46.2"E; 475; LK & MH; 2016-05-01; 10*. – **457:** Austria; Steinriegl, forest near the crossroads and bus station Steinriegl Tullner Straße, ca 200 m from the village; 48°16'05.6"N, 16°12'03.7"E; 475; LK & MH; 2016-05-01; 5. – **458:** Austria; Steinriegl, Hainbuch, ditches of the road Tullnerstraße; 48°16'34.6"N, 16°10'49.6"E; 444; LK & MH; 2016-05-01; 10. – **459:** Austria; Königstetten, Dopplerhütte, edge of the road under the car parking near Dopplerhütte; 48°17'40.6"N, 16°09'40.4"E; 329; LK & MH; 2016-05-01; 7.