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Distribution and identification of *Pulmonaria officinalis* and *P. obscura* in the Bohemian Forest and its adjacent foothills

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Abstract
Distribution of two closely related species, *Pulmonaria officinalis* and *P. obscura*, was studied in the Bohemian Forest and its foothills. Difference in relative genome size determined by flow cytometry was used for reliable identification of morphologically similar and often misidentified species. Moreover, dependability of reported morphological distinctions was tested in selected populations by classical morphometry as well as outline analysis. Both species differ in its distribution in the studied area. While *Pulmonaria officinalis* prevails in the south-eastern part of the area, *P. obscura* is a dominant species in central and north-western part of the mountain and in Czech foothills. Although presence of long glandular hairs on the calyx is the most significant morphological difference between the species, reliable identification based only on morphological characters is difficult, due to their large overlap.

Key words: flow cytometry, morphometry, *Pulmonaria obscura*, *Pulmonaria officinalis*, distribution, Bohemian Forest

Introduction

Of the European-Asian genus *Pulmonaria*, at least 20 native species are recognised in Europe (Sauer 1975, Bolliger 1982, Májovský et al. 1993, Hewitt 1994, Bennert 2003, Lauber et al. 2012, Cecchi & Selvi 2015). They occur near watercourses, in wet meadows and in light, especially deciduous forests, both on calcareous and more acidic substrates, mainly from foothills to montane zones (while reaching lowlands and subalpine zones only rarely) (Damboldt et al. 1975).

Lungworts (*Pulmonaria*) are perennial herbs with a creeping rhizome, an unbranched simple flowering stem with alternate subsessile leaves. Summer leaves are in a ground rosette, they are long-petiolate, spotted or unspotted. All leaves are entire, covered with glandular hairs and setae (bristles) of different length (Sauer & Gruber 1979). Flowers are in terminal, bracteate cymes. The calyx is tubular-campanulate, mostly divided into triangular lobes into one third, accrescent in fruit. The corolla is infundibuliform with obtuse lobes, red, blue or violet, with 5 tufts of hairs in the throat meeting to form a ring. Flowers are hermaphrodite, mostly heterostylous. Nutlets are rough to smooth, ovoid, contracted at the base, with elaiosome, thanks to which they are spread mostly by ants (Merxmüller & Sauer 1972, Damboldt et al. 1975, Kříša 2000). The main characters for species identification are the colour of corolla, indumentum of corolla tube and calyx and size of blade of summer leaves, shape of their basis, their indumentum and the presence and type of spots (Merxmüller & Sauer 1972, Sauer & Gruber 1979, Pignatti 1982, Fischer et al. 2008, Tison & De Foucault 2014, Jäger 2017, Hroneš & Kobrlová 2019). Due to convergent evolution, intrapopulation and interpopulation variation, apparently frequent hybridization and breeding of ornamental cultivars, the identification of many species is very difficult (Kerner 1878, Merxmüller & Sauer 1972, Damboldt et al. 1975, Bolliger 1982, Kirchner 2004, Meeus et al. 2013, Kook et al. 2015).

Original hypothesis about the current diversity of the genus are based on an idea of four basic groups of Tertiary age (*P. officinalis* agg., *P. mollis* agg., *P. angustifolia* agg. and *P. rubra* Schott) and their differentiation as a result of their presumed hybrid speciation (Gams 1927). Although number of ancestral groups was later questioned (Merxmüller & Sauer 1972, Sauer 1986), also recent studies show that hybridization play an important role in *Pulmonaria* evolution (Kook et al. 2015, Meeus et al. 2016, Grüning et al. 2021). Nowadays, several species with different extends of their distribution areas are distinguished in the region of Central Europe (Damboldt et al. 1975). *Pulmonaria vallarsae* A. Kern., *P. stiriaca* A. Kern., *P. kerneri* Wettst., *P. australis* (Murr) W. Sauer and *P. carnica* W. Sauer represent endemic species of the Alps and the Apennines having rather small distribution areas (Damboldt et al. 1975, Fischer et al. 2008). *Pulmonaria montana* and *P. collina* W. Sauer are species with small, discontinuous and vaguely defined distribution areas around the west boundary of Central Europe (Sauer 1974, Jäger 2017), while *P. angustifolia* and *P. mollis* Hornem have significantly larger distribution in the east with fragmented distribution limit in Central Europe (Merxmüller & Sauer 1972, Damboldt et al. 1975, Fischer et al. 2008, Jäger 2017, Hroneš & Kobrlová 2019).

The most widespread Central European group is *P. officinalis* agg. It is characterised by summer leaves with a cordate to truncate base of blade, presence of aculeoli (small pins) on upper surface and thin, not winged petiole (Merxmüller & Sauer 1972, Hroneš & Kobrlová 2019). This group includes two species, *P. officinalis* and *P. obscura* Dumort. They are morphologically and phylogenetically very close, and therefore they often have been assessed as subspecies or varieties under *P. officinalis* (Damboldt et al. 1975, Hultén & Fries 1986, Kirchner 2004, Schmeil & Fitschen 2009). However, currently, the concept of two separated species prevails (Fischer et al. 2008, Jäger 2017, Hroneš & Kobrlová 2019), supported especially by different chromosome numbers. According to Central European identification keys (Fischer et al. 2008, Jäger 2017, Hroneš & Kobrlová 2019), the two species are distinguished by characters given in Table 1.

Table 1. Distinguishing characters of the species *Pulmonaria officinalis* and *P. obscura* (based on Central European identification keys).

Character	*P. officinalis*	*P. obscura*
calyx shape (after anthesis)	1.5–2.5× longer than wide, tapered to the shape of the letter ”V“ on the base	2.5–4.5× longer than wide, tapered to the shape of the letter ”U“ on the base
calyx indumentum	long glandular hairs at least on the calyx base as long as or longer than setae	long glandular hairs present or absent on the calyx base, but always shorter than setae
length of calyx lobes	0.3–0.5× length of calyx	0.25–0.3× length of calyx
colour of summer leaves	light green, often with white or yellow sharply bordered spots	dark green, unspotted or with irregularly rounded faint spots
consistence of summer leaves and indumentum of their upper face	rough leaves with setae, aculeoli and sparse glandular hairs	soft leaves with setae, aculeoli and rare glandular hairs
ratio of petiole and blade of summer leaves	petiole shorter than blade	petiole longer than blade
persistence of summer leaves	perennial	annual

Distribution areas and ecological demands of both species are similar in general. Both of them grow in deciduous forests, forest margins and scrubs (Hroneš & Kobrlová 2019), often on moist and humic soils (Kříša 2000). Geographical distribution of the two species is uncertain because of frequent misidentifications. *Pulmonaria officinalis* is often reported from eastern France in the west, to western Ukraine in the east, in the north it reaches up to the southernmost Sweden and in the south to northern Italy and Bulgaria. Reported distribution area of *P. obscura* is larger – it reaches to Finland and further north to Sweden as well as further east to the Urals (Merxmüller & Sauer 1972, Hultén & Fries 1986). However, a real distribution of *Pulmonaria officinalis* is probably much more limited. It may be confined to relatively small area in the Alps surroundings and on the north of the Balkan (Sauer 1975, Bolliger 1982). Records from the other regions may be based on non native occurrence due to often cultivation of species and possible escape. The actual distribution of both species is not known well either in the Czech Republic or in the neighbouring countries. They are assumed to be abundant to scattered in the whole area, especially in colline to montane belts and in warm and moderate warm areas, while in dry areas without forests and in higher mountain zones they occur rarely (Kříša 2000, Hroneš & Kobrlová 2019). *Pulmonaria officinalis* is sometimes reported to have a centre of occurrence in Czechia in southern and central Moravia and to have higher tolerance to drought (Jarošová 2006). In Austria *P. officinalis* is considered a very abundant species while *P. obscura* a very rare one

(Fischer et al. 2008). In contrast, *P. obscura* seems to be more abundant with a more or less continual distribution in the substantial part of Germany north of the Danube. The distribution of *P. officinalis* appears to be fragmented into several isolated parts: one of the most noticeable areas is located south of the Danube in the foothills of the Alps (Bettinger et al. 2013).

Within the Czech-Bavarian project Interreg nr. 216 "Květena Šumavy/Flora des Böhmerwaldes" (Flora of the Bohemian Forest), which deals, among others, with studying of critical groups, attention was focused also on the genus *Pulmonaria* in the Bohemian Forest and its foothills. Our aims were 1) to identify both species based on relative genome size differences, 2) to detect a distribution pattern of both species in the studied area, 3) to verify dependability of reported morphological characters for the identification of *Pulmonaria* populations in the studied area.

Methods

The studied area included the mountain region of the Bohemian Forest and the neighbouring foothills in Bohemia, Bavaria and Austria. Based on the floristic data, localities were selected to cover all regional centres of occurrence of *Pulmonaria officinalis* agg. Special attention was paid to areas with reported occurrence or contact of both species. Due to the fact that most of the populations seemed to be uniform, the sampling strategy was focussed on sampling as many localities as possible while keeping number of individuals per site rather low. A total of 303 populations were studied during the years 2019–2021. Most often 3 (1–10) plants from each population were analysed by flow cytometry. Most of the herbarium vouchers are deposited in the herbarium of the Faculty of Science of the University of South Bohemia in České Budějovice (CBFS). Geological information was obtained from geological maps 1:50 000, which are accessible online on the portal of the Czech Geological Survey (Cháb et al. 2007). QGIS v. 3.18.1 (QGIS Development Team 2021) was used for visualisation of distribution of both species.

Flow cytometry

The species *P. officinalis* and *P. obscura* are both diploid but differ in chromosome numbers (*P. officinalis*: 2n = 16, *P. obscura*: 2n = 14 (Lökvist 1963, Sauer 1972)). Therefore, flow cytometry was applied for species identification. This method allows to determine relative content of DNA against a standard (Otto 1990). Individual plants or pooled samples of up to three individuals were chopped with the internal standard in ratio 5:1 in 400 µl of Otto I buffer using a sharp razor blade in a Petri dish and filtered through a 42 µm nylon mesh. After 1–2 min 800 µl of Otto II buffer with fluorochrome DAPI (4,6-diamidino-2-phenylindole), was added into the sample (final concentration of DAPI 4 µl/ml). Longer fixation time in Otto I buffer impairs the quality of analyses. Stained samples were analysed using a CyFlowSpace instrument (Sysmex-Partec) equipped with 365 nm UV-LED as a light source. Fluorescence intensity of 5000 particles was usually recorded. Histograms were evaluated using FlowJo 10 (TreeStar Inc.). Fresh spring or summer leaves, exceptionally stems or parts of flowers, were analysed. *Bellis perennis* was used as the internal standard. Detected sample/standard ratios were visualised as a histogram in R (R Development Core Team 2020). Only analyses, which have coefficient of variation (CV) of samples and the standard below 5%, were evaluated.

Classical morphometrics

Morphological characters on calyx (Table 2) and summer leaves, reported in Central European literature, were measured to verify their reliability (Dumortier 1865, Jarošová 2006, Fischer et al. 2008, Jäger 2017, Hroneš & Kobrlová 2019). Sixty plants from 20 populations (10 populations per species) were measured; the species determination was assessed based on flow cytometric data. Ten quantitative characters were measured on calyx and 3 ratio characters were computed from them (Table 2, Fig. 1 and 2). Three calyces were measured from each plant and obtained values were used to calculate the mean. Measurements were performed using a stereo microscope Zeiss Stemi 2000-C in the AxioVision v. 4.9.1 software (Carl Zeiss Microscopy).

Characters that deviated strongly from the normal distribution within each of the two species were log- or sqrt-transformed (Table 3). To find out whether the two species are significantly different and which characters separate them, canonical discriminant analysis (CDA) with forward selection of characters was applied. The threshold significance level was set to $\alpha = 0.05$ and a Monte-Carlo permutation test (999 permutations) was used. The predictive ability of the selected characters was subsequently tested by classificatory discriminant analysis (classificatory DA) based on the posterior group membership probabilities. Cross-validation using each population as a leave-out unit was used. The analyses were performed using the MorphoTools collection of R functions (Koutecký 2015).

Outline analysis

One hundred plants from 20 populations were used (5 plants per population) to test the differences in the leaf blade shape. One of the basal, fully developed summer leaves was selected from each individual. The leaves were pressed and dried and subsequently scanned at 300 dpi using Konica Minolta bizhub C224 scanner. Elliptic Fourier approximation (Kuhl & Gieardina 1982) implemented in the R package Momocs (Bonhomme et al. 2014) was employed. The scanned leaves were converted to black-and-white images and chain-coded contours were approximated using the number of harmonics with 99% harmonic power. The leaves were aligned to the same positions, and the leaf tips were used as the starting points for contour definitions. The resulting elliptic Fourier descriptors (EFDs) were centred and scaled to zero mean and unit variance. Subsequently, principal component (PC) scores for each specimen were calculated from the standardized EFDs and used to visualize the patterns of leaf shape variation. The principal component scores were also used in a canonical discriminant analysis in order to test the differences in leaf shape between the two species.

Results

Species identification and distribution

Flow cytometry has proved to be a suitable tool to identify the two species, although the presence of secondary metabolites in *Pulmonaria* tissues impairs the quality of analyses of some samples. Based on the analyses, two groups of populations were significantly distinguished, which correspond to the species with different numbers of chromosomes reported in the literature (Fig. 3). Average ratio of the sample and the standard *Bellis perennis* for *P. obscura* was 0.767 (min 0.720, max 0.808), and for *P. officinalis* 0.872 (min 0.820,

Table 2. Morphological characters measured on calyces. Length of calyx lobes and calyx were measured in µm with an accuracy of 100 µm, angles were measured in degrees, the lengths of hairs in µm with an accuracy of 10 µm.

Quantitative characters:	
CL	calyx length
LCL	length of the side of the calyx lobe (average of LCL1 and LCL2, Fig. 1)
WCL	width of the base of the calyx lobe
ACB	angle of the calyx base (apex was placed in the centre of beginning of peduncle enlargement, Fig. 1)
ABL	angle between the calyx lobes
ACL	angle of apex of the calyx lobes
GMC	length of glandular hairs in the middle part of the calyx (average of three hairs)
SMC	length of setae on the middle part of the calyx
GBC	length of glandular hairs on the base of the calyx
SBC	length of setae on the base of the calyx
Ratios:	
RCL	length / width of the calyx lobe
RMC	length of glandular hairs / setae length in the middle part of the calyx
RBC	length of glandular hairs / setae length on the base of the calyx

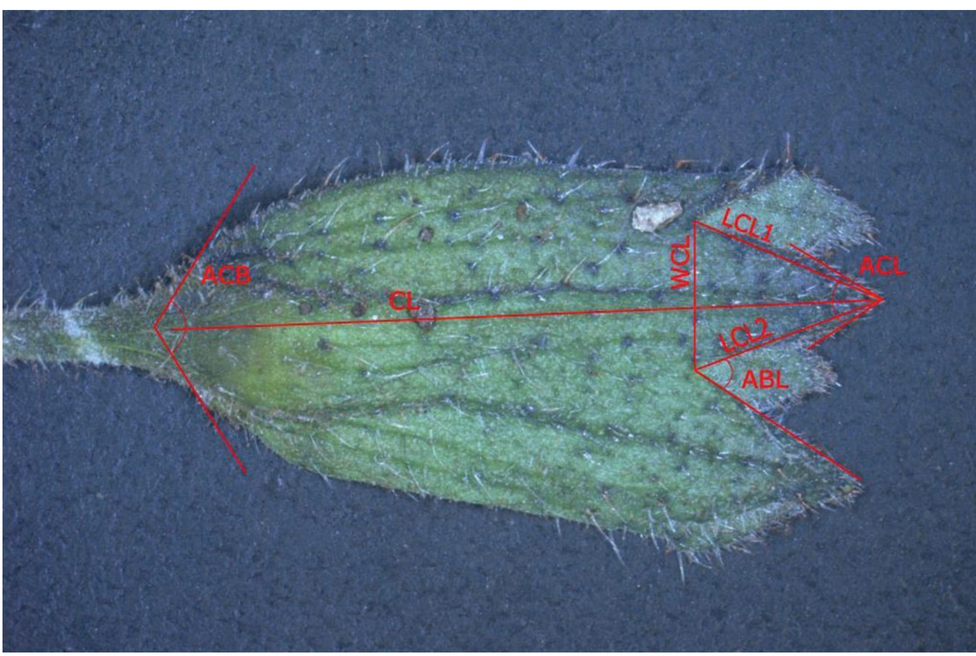

Fig. 1. Quantitative characters measured on the calyx (Table 2).

Table 3. Morphological characters used in the morphometric analyses and summary of their values for *Pulmonaria obscura* (30 individuals) and *P. officinalis* (30 individuals). The numbers denote (minimum–) 10th percentile/**mean**/90th percentile (–maximum). Characters log- or sqrt-transformed prior to the CDA, PCA, and clustering analyses are marked with one or two asterisks, respectively; however, non-transformed values are shown in this table.

	P. obscura	***P. officinalis***
CL*	(7.7–)8.1/**9.6**/11.2(–12.2)	(7.0–)9.1/**10.4**/11.9(–14.5)
LCL	(2.0–)2.4/**3.0**/4.1(–4.2)	(2.6–)2.7/**3.5**/4.2(–4.6)
WCL*	(1.6–)1.8/**2.1**/2.7(–3.2)	(1.7–)1.9/**2.3**/2.6(–3.3)
RCL	(1.09–)1.20/**1.42**/1.68(–1.88)	(1.13–)1.26/**1.56**/1.89(–1.93)
ACB	(57–)71/**83**/94(–99)	(58–)71/**81**/91(–104)
ABL	(26–)29/**41**/53(–63)	(20–)26/**38**/49(–56)
ACL*	(37–)40/**52**/62(–76)	(35–)41/**51**/64(–70)
GMC	(223–)251/**364**/459(–736)	(284–)398/**565**/743(–961)
SMC	(382–)425/**648**/803(–1104)	(521–)613/**761**/902(–1111)
RMC*	(0.33–)0.41/**0.58**/0.77(–0.93)	(0.46–)0.50/**0.76**/1.03(–1.27)
GBC*	(227–)296/**454**/633(–684)	(375–)514/**804**/1108(–1225)
SBC**	(254–)359/**540**/764(–839)	(441–)484/**674**/880(–968)
RBC*	(0.58–)0.65/**0.89**/1.10(–1.66)	(0.56–)0.84/**1.26**/1.93(–2.73)

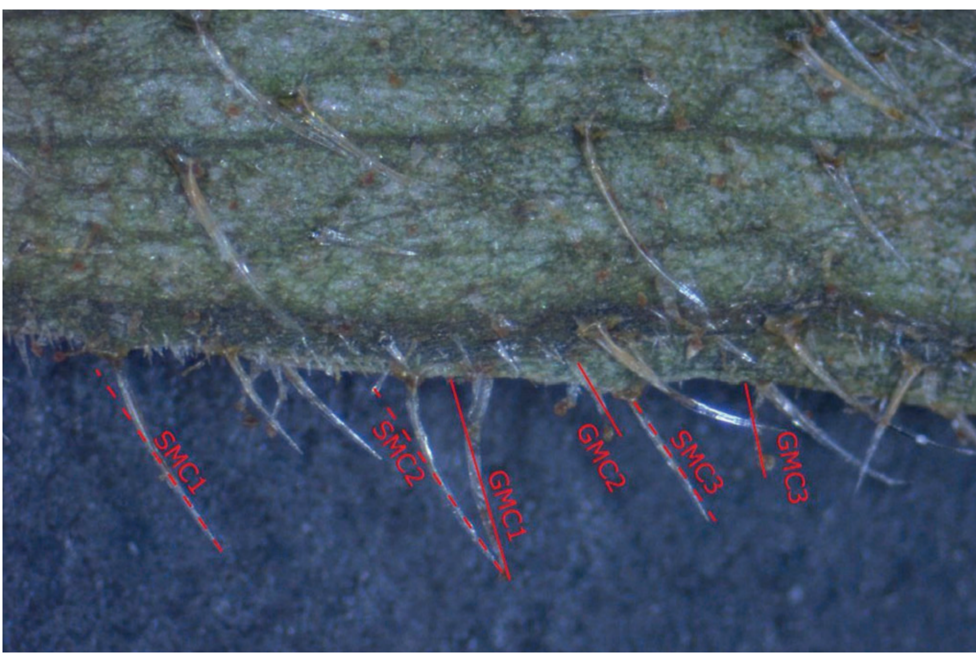

Fig. 2. Quantitative characters measured in the middle of the calyx (Table 2).

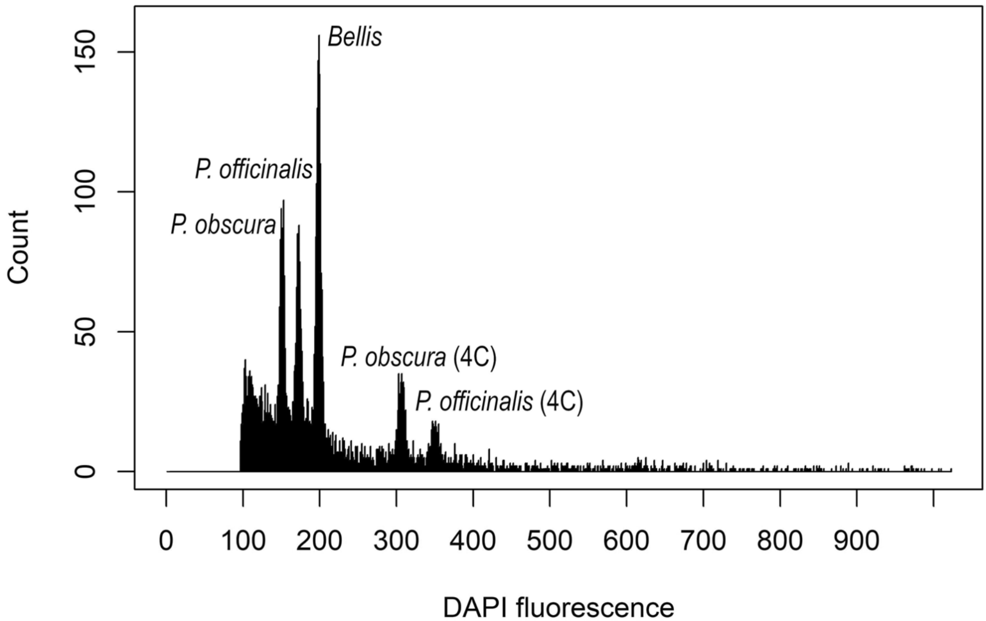

Fig. 3. Flow cytometric histogram showing a simultaneous analysis of *Pulmonaria officinalis* and *P. obscura* and *Bellis perennis* as the standard.

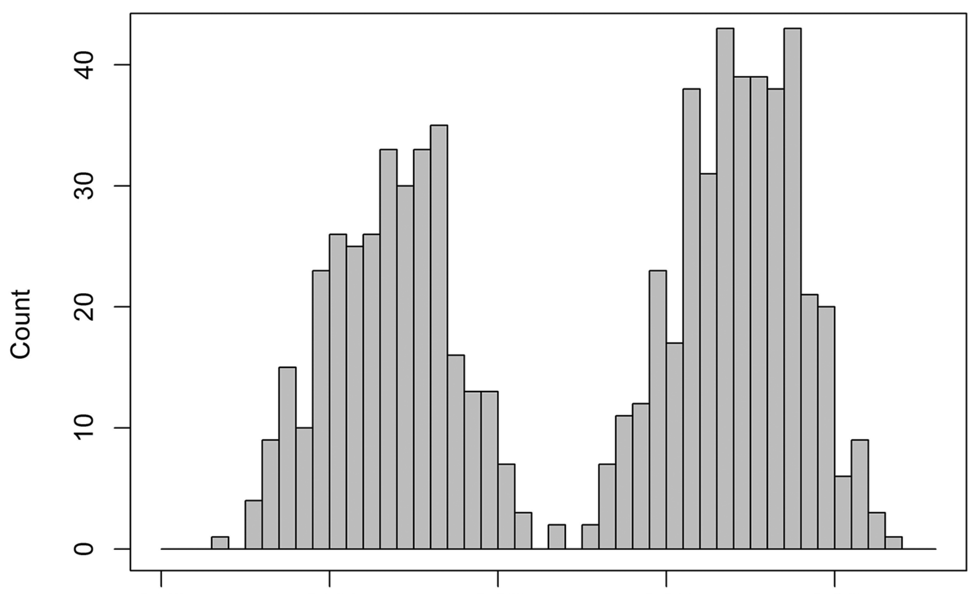

Fig. 4. Histogram of fluorescence ratios of species *Pulmonaria officinalis* and *P. obscura* against the *Bellis perennis* standard in analyses with CV below 5.

max 0.920, Fig. 4). A threshold value for identification of both species was 0.815. It was possible to assign the most of the populations to one of the species unequivocally and populations appeared uniform. Only exceptionally the ratio of the studied plants was around 0.810, and those samples could not be classified to either of the species. In three cases we must consider the possibility of their hybrid origin. However, usually these analyses had lower quality and their assessment was less clear. Therefore an adverse effect of secondary metabolites for analysis quality is more probable explanation this intermediate results. The average CV of samples and the standard of all analyses equalled to 3.2 which points to relatively worse analyses compared to various plants with less secondary metabolites.

In total, 152 populations of *P. obscura* and 167 populations of *P. officinalis* were found (Appendix 1). Moreover, 6 population of *P. officinalis* probably originated from old cultivation were analysed. Differences were discovered in the distribution of both species in the Bohemian Forest and its foothills. The species can be even called vicariants with some exaggeration (Fig. 5). While in the north-west of the mountains almost only *Pulmonaria obscura* occurs, *P. officinalis* dominates in the south-east. *Pulmonaria obscura* mainly occurs in Královský hvozd territory and its foothill and on the edges of Šumava Plains. Furthermore, it dominates on northern slopes of Boubín massif and in the northern and eastern parts of the Boletice Military Training Area. *Pulmonaria officinalis* dominates in the border ridge from the southeastern edge of the Šumava Mts to the central part around the village of Strážný. Furthermore to the northwest, it grows also in the Bavarian foothills of the Šumava plains. *Pulmonaria officinalis* also occurs in the southern slopes of the ridge northeastern of the Vltava valley from the village of Borová Lada to the southeast. Rather isolated distribution area is situated in the massif of Javorník. A contact zone of occurrence of both species seems to be rather sharp except of the southern part of the Boletice Military area and in river valleys which drain areas of both species occurrences.

Classical morphometrics

The canonical discriminant analysis (CDA) proved a significant differentiation between *P. obscura* and *P. officinalis* ($F = 5.8$, $p<0.001$). Marginal effects of most of the morphological characters were significant. The Length of glandular hairs on the base of the calyx character (GBC) had the highest discriminatory power contributing to 50% of the total discriminatory power. It was also the only character selected in the forward selection (Table 4). Values of all the morphological characters measured are summarized in Table 3.

The predictive ability of the selected character GBC was tested using classificatory discriminant analysis (classificatory DA). The overall prediction accuracy was 78.3%, and *P. officinalis* was correctly classified in more cases than *P. obscura* (80.0% vs. 76.7%) (Table 3). The investigated individuals could be separated by an approximate rule that all individuals with GBC $\geq$ 700 μm were *P. officinalis* and individuals with GBC $\leq$ 450 μm (with one exception) were *P. obscura* (Fig. 6).

Outline analysis

The first 10 harmonics were selected for leaf outlines approximation. The PCA of the harmonic coefficients showed a strong overlap of *Pulmonaria obscura* and *P. officinalis* leaf outlines. The overlap was clearly asymmetrical. While some morphotypes of *P. obscura*, especially those with cordate leaf base, were clearly distinct from *P. officinalis*, *P. officinalis* did not differ from *P. obscura* nearly at all, with an exception of a few morphotypes with slightly cuneate leaf base (Fig. 7).

The CDA of the principal components from the elliptic Fourier analysis proved a significant differentiation between *P. obscura* and *P. officinalis* ($F = 2.8$, $p = 0.002$). The only principal component with significant both marginal and conditional effects was the first one ($F = 23.5$, $p = 0.011$, explained variation = 19.3%). Classificatory DA using the first principal component scores yielded a 68.0% determination accuracy distributed equally among both species (Table 5).

Discussion

Distribution

Based on the different ratio of sample and standard fluorescence, most of the populations of *Pulmonaria officinalis* and *P. obscura* could be reliably distinguished. Detected differences correspond to the reported variation in chromosome numbers (*P. officinalis*: 2n = 16,

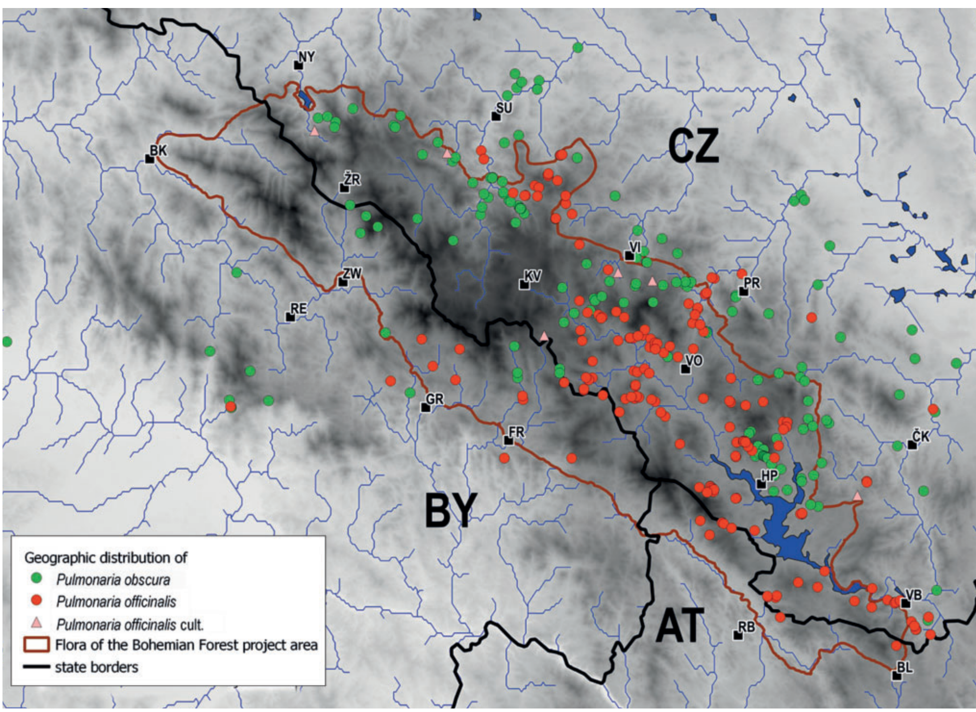

Fig. 5. Locations of studied populations of *Pulmonaria obscura* and *P. officinalis* in the Bohemian Forest and its adjacent foothills. Species identity was tested by flow cytometry. Places CZ: NY – Nýrsko, ŽR – Železná Ruda, SU – Sušice, KV – Kvilda, VI – Vimperk, VO – Volary, PR – Prachatice, HP – Horní Planá, ČK – Český Krumlov, VB – Vyšší Brod; BY: BK – Bad Kötzling, RE – Regen, ZW – Zwiesel, GR – Grafenau, FR – Freyung; AT: RB – Rohrbach-Berg, BL – Bad Leonfelden.

Table 4. Morphological characters used in the canonical discriminant analysis and their power to discriminate *Pulmonaria obscura* and *P. officinalis*. Biplot scores represent the contributions of characteristics to the first canonical axis separating the species. Characters with negative values have higher values in *P. obscura*; characters with positive values have higher values in *P. officinalis*. The significance levels were adjusted using the false discovery rate; p-values lower than 0.05 are highlighted in bold.

Character	Marginal effects			Biplot score	Conditional effects		
	Explained variation (%)	F	p		Explained variation (%)	F	p
GBC	50.05	58.1	**0.003**	0.78	50.05	58.1	**0.003**
GMC	37.71	35.1	**0.003**	0.61	37.71	35.1	0.766
RBC	22.27	16.6	**0.003**	0.42	22.27	16.6	0.969
RMC	19.71	14.2	**0.003**	0.39	19.71	14.2	0.837
SBC	18.24	12.9	**0.005**	0.37	18.24	12.9	0.984
SMC	14.22	9.6	**0.007**	0.32	14.22	9.6	0.931
LCL	11.97	7.9	**0.015**	0.29	11.97	7.9	0.150
CL	9.68	6.2	**0.026**	0.25	9.68	6.2	0.777
RCL	9.38	6.0	**0.021**	0.25	9.38	6.0	0.954
ABL	3.16	1.9	0.211	–0.14	3.16	1.9	0.717
WCL	2.89	1.7	0.230	0.13	2.89	1.7	0.935
ACB	0.83	0.5	0.525	–0.07	0.83	0.5	0.293
ACL	0.09	0.1	0.778	–0.02	0.09	0.1	0.903

P. obscura: 2n = 14). Flow cytometry results well correspond with the published chromosome counts from the study area. Jarošová (2006) counted chromosome numbers of *P. officinalis* originating from the locality Dlouhý hřbet in Želnavská hornatina highland. Flow cytometry has proved the sole occurrence of *P. officinalis* in this area. On the contrary samples of *P. obscura* came from the area east of the former settlement Pražačka in the north-eastern part of the Boletice Military Traing Area. Flow cytometry confirmed only *P. obscura* around this place. Some authors also indicate the value 2n = 14 for *P. officinalis* (Mizianty et al. 1981, Křísa 2000), but due to frequent misidentifications of both species, it can be presumed that these data resulted from incorrectly identified plants (Jarošová 2006).

Very rarely, the sample/standard ratio was near the threshold value between the two species. In these cases, a hybrid origin of the plants cannot be omitted. However, existence of hybrids of *P. obscura* and *P. officinalis* was recorded only very rarely in past, especially in contact zones of both species in Germany. These plants had sterile pollen and their chromosome count was 2n = 15, and their morphological identification was difficult (Dersch 1994). Occurrence of hybrid plants in other countries has not been proved yet. Due to observed variation, the flow cytometry data alone cannot confirm the hybrid origin and verification by chromosome counting is needed.

Table 5. Summary of the classification matrices obtained from the classificatory discriminant analyses for both the classical morphometrics and the outline analysis.

	Classical morphometrics		Outline analysis	
predicted / observed	*P. obscura*	*P. officinalis*	*P. obscura*	*P. officinalis*
P. obscura	23 (76.7%)	7 (23.3%)	34 (68.0%)	16 (32.0%)
P. officinalis	6 (20.0%)	24 (80.0%)	16 (32.0%)	34 (68.0%)
Total	**78.3%**		**68.0%**	

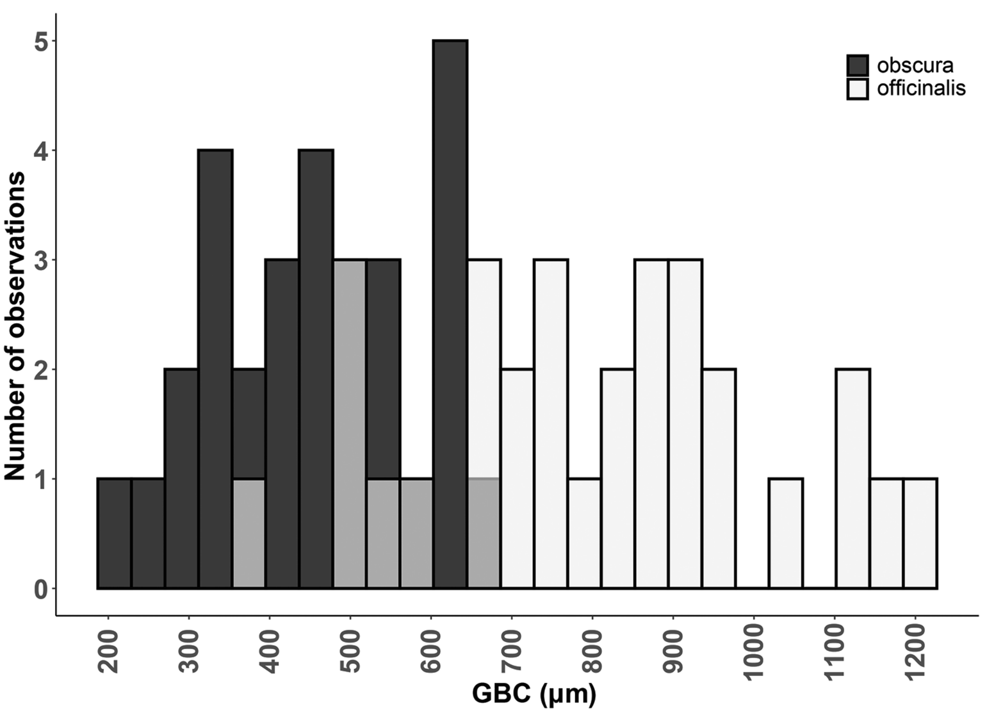

Fig. 6. Distribution of the values of the character Length of glandular hairs on the base of the calyx (GBS) in *Pulmonaria obscura* and *P. officinalis*.

Sympatric occurrence of both species is very uncommon (Dersch 1994, Jarošová 2006). Similar pattern was observed in the Bohemia Forest and adjacent foothills. No truly mixed population has been found yet. The shortest distance between populations of the two species was several tens to hundreds of metres. Also, other authors considered populations as uniform, and they assume existence of ecological barriers between the species (Sauer & Gruber 1979, Bolliger 1982, Dersch 1994).

The discovered distribution pattern of both species in the studied area probably reflects both historical causes and ecological differences. Abundance of *P. officinalis* in the south-eastern Bohemian Forest is distinctly connected to its abundant occurrence in lower zones of the Alps and their foothills (Fischer et al. 2008, Bettinger et al. 2013) and is an example of an alpine migrant in the Bohemian Forest. Apart from the occurrence in the south-eastern part of the Bohemian Forest and its foothills, it should be mentioned that *P. officinalis* penetrates through the valley of Kleine and Große Ohe rivers to the foothills of the central Šumava plains. Absence of *P. obscura* in a broad area of southeastern Šumava and almost vicariance of both species in the studied region is an unexpected finding. *Pulmonaria obscura* has been considered to be the more abundant representative of this aggregate in the region (Kříša 2000) and it was even the only species reported from the Bavarian side of the Bohemian Forest (Bettinger et al. 2013). Nevertheless, the species is significantly rarer in the southeastern part of the Bohemian Forest and its foothills than it has been anticipated.

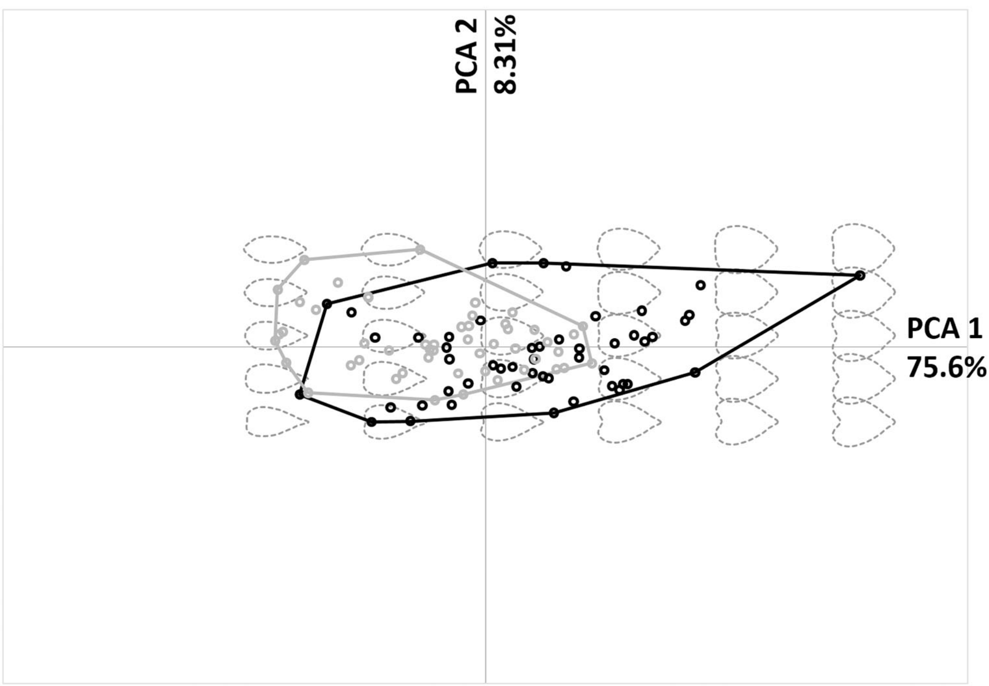

Fig. 7. PCA of the outline analysis of leaves of *Pulmonaria obscura* (black symbols) and *P. officinalis* (grey symbols). Grey dotted figures in the background show reconstructions of blade shape according to each position in the multidimensional space.

We assume that *P. obscura* reaches the southern border of its global distribution area in the study region. This assumption is supported by its reported absence in Bavarian Alps foothills (Bettinger et al. 2013) and also by its extreme rarity throughout Austria (Fischer et al. 2008).

In areas of contact between the two species, *P. officinalis* seems to prefer nutrient rich soils, while *P. obscura* grows in rather poor habitats. This trend can be observed in the geologically variable area of Knížecí Stolec massif and Boletice Millitary Training area where *P. officinalis* tends to prefer nutrient rich durbachite with copious herbal layer, while *P. obscura* is more frequent on much poorer migmatite, gneiss and granulite. Furthermore, high numbers of *P. officinalis* populations were recorded in the whole southern part of Boubín massif on dominant migmatite, whereas the only locality of *P. obscura* in this area (near Jedlová hill) was found on a small gneiss body. More frequent mixing of populations of the two species is probably prevented by difficult spread of the species over longer distances, which is limited by heavy nutlets that are only sparsely distributed by ants. Most likely, spreading over longer distances can occur only along watercourses.

With regard to the fact that lungworts have been grown as medicinal and decorative herbs for a very long time, some occurrences have probably or obviously arisen from cultivation. In the study region, only *P. officinali*s seems to be planted. Plants of *P. officinalis* nearby remnants of the church in the former village of Knížecí Pláně were clearly planted, while only *P. obscura* occurs in the surroundings. Populations at the village of Klášterec u Vimperka, in the former military training ground Radost or the nearby Veselka village are also probably of secondary origin. A population near Hamry-Hojsova Stráž railway station has obvious origin in the old botanical garden in this place.

Morphology

Morphometric analyses confirmed calyx indumentum as the best identification characters. In the centre of attention is the length of glandular hairs and glandular hairs/setae ratio. An absolute length of setae was considered unimportant (Jarošová 2006). According to our measurement *P. officinalis* had both glandular hairs and setae longer than *P. obscura*, but only on calyx base glandular hairs were longer than setae (Table 3, Fig. 8). In the middle part of the calyx the glandular hairs were often shorter than setae even in *P. officinalis*. It is in contrary with interpretation and illustration of this character in some publications (Dersch 1994, Jarošová 2006, Hroneš & Kobrlová 2019). Only few publications emphasize the necessity of evaluation of glandular hairs only on calyx base (Sauer & Gruber 1979, Fischer et al. 2008). Moreover, our results show *P. officinalis* has higher variation in absolute length of glandular hairs. *Pulmonaria obscura* has higher variation of glandular hairs and setae ratio, which can lead to misidentification of this species (Dersch 1994). In summary, although the two species differ significantly in calyx indumentum, there is a considerable overlap of characters and quite often individual plants cannot be reliably identified.

The length of the side of the calyx lobe was evaluated as another significant character. Based on measured angel of apex of calyx lobes we can specify the calyx lobe length. This character and calyx lobe shape are used only sporadically as identification characters and opinions in literature differ. Kříša (2000) states the lobes form 1/3 to 1/2 of the calyx length in *P. obscura* but only 1/3 in *P. officinalis*. On the other hand, Fischer et al. (2008)

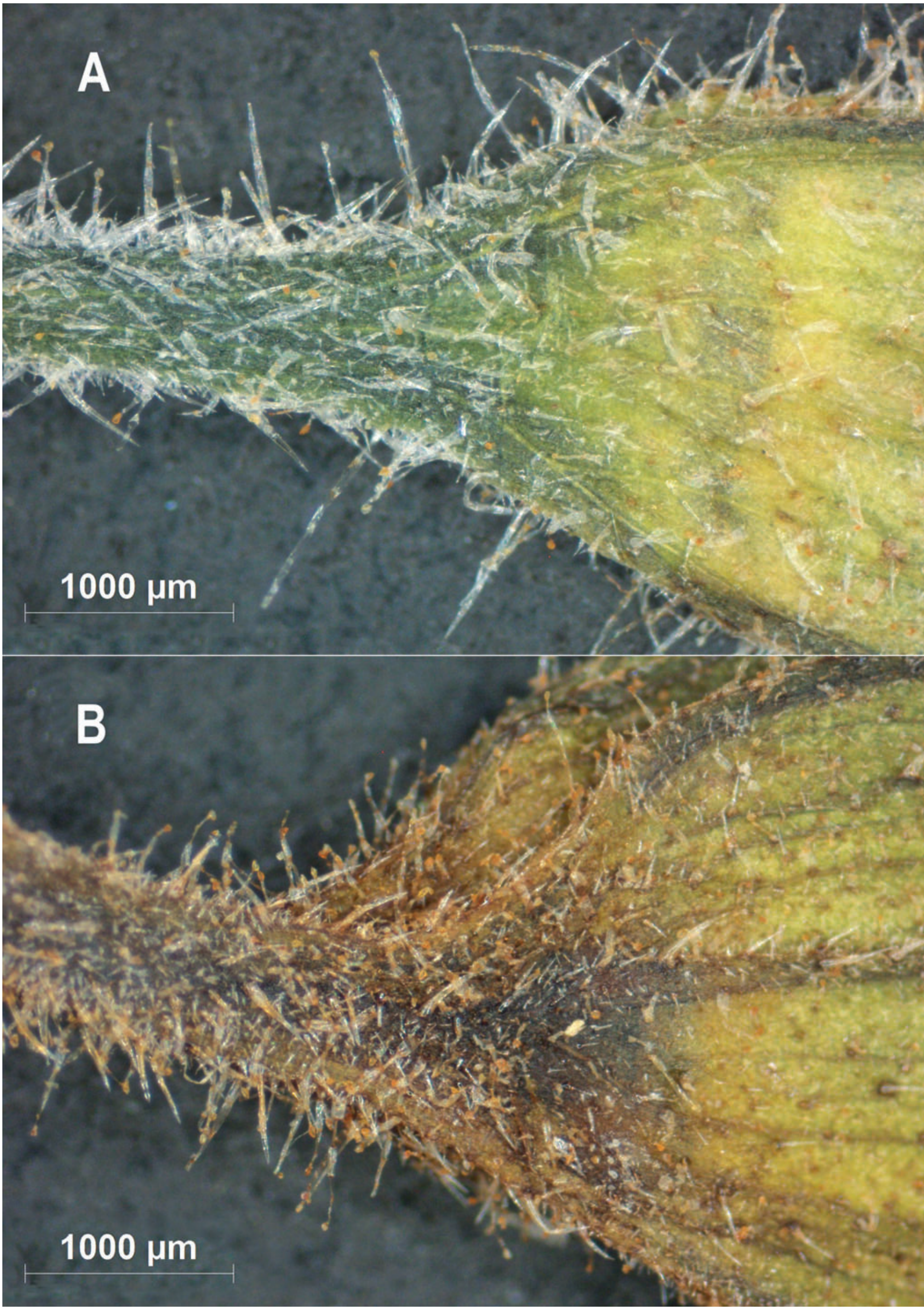

Fig. 8. Indumentum of the calyx base of typical specimens of *Pulmonaria officinalis* (A) and *P. obscura* (B).

report 1/4 to 1/3 length of calyx for *P. obscura* and 1/3 to 1/2 for *P. officinalis*, which corresponds with our results.

Calyx length was the character with the weakest explaining power in our analyses. This is very rarely mentioned in the literature. Křísa (2000) indicates calyx length of 10–13 mm for *P. obscura* and 11–14 mm for *P. officinalis*. Such overlap of values is in accordance with our measurement. However, the absolute values we measured were slightly lower. Ratio of calyx length and width is reported more often. This is greater by *P. obscura* compared to *P. officinalis* (Sauer & Gruber 1979, Jäger 2017). A very often used character is the shape of the calyx base: in *P. obscura* the base should be rounded, in the shape of a letter ”U“, while in *P. officinalis* it should be infundibuliform and contracted into the shape of a letter ”V“ (Sauer & Gruber 1979, Májovský & Hegedüšová 1993, Fischer et al. 2008, Jäger 2017). Nevertheless, some studies considered this character to be insignificant (Jarošová 2006), which we confirmed with our measurements.

Shape and indumentum of summer leaves are other reported characters. The petiole length in *P. obscura* should exceed the blade length, while in *P. officinalis* the petiole should be shorter than the blade (Dumortier 1865, Sauer 1972, Májovský & Hegedüšová 1993, Křísa 2000). Shape of blade is considered less important character (Májovský & Hegedüšová 1993, Křísa 2000), which is in line with our study, although some authors emphasized presence of a cordate base of blade in *P. obscura* against a truncate base in *P. officinalis* (Jarošová 2006). Both the blade shape and the petiole length display high variability. Differences between the species are rather weak and characters are plastic due to phenology and environmental conditions (Sauer & Gruber 1979). Statistical significance of the characters in the study of Jarošová (2006) can be attributed by the same phenological phase of sampled leaves, which has not been accomplished in our study. In contrast, most authors agreed on summer leaves indumentum as identification character. The leaves have glandular hairs, setae and aculeoli on the upper side. While glandular hairs are commonby *P. officinalis*, *P. obscura* should have them very rarely (Sauer 1972, Bolliger 1982, Májovský & Hegedüšová 1993, Křísa 2000, Jarošová 2006, Hroneš & Kobrlová 2019). In spite of this congruence of authors and statistical verification of the character (Jarošová 2006), it shows the high variability and based on our experience, it appears to be unreliable.

Variation in colour and spottiness of summer leaves and their persistence are often reported as characters for distinguishing between *P. obscura* and *P. officinalis* species. The blade of summer leaves of *P. officinalis* is predominantly light green with conspicuous circular and sharply demarcated white spots and leaves are usually perennial (sometimes as ”winter leaves“). The blade of *P. obscura* leaves is dark green, often unspotted or with irregularly rounded faint green spots, which can disappear during season and the leaves are rather annual (Sauer & Gruber 1979, Bolliger 1982, Májovský & Hegedüšová 1993, Dersch 1994, Křísa 2000, Jarošová 2006, Fischer et al. 2008, Jäger 2017, Hroneš & Kobrlová 2019). Only Jarošová (2006) states perennial summer leaves as inconclusive character. Persistence of summer leaves and their spottiness appeared to be partly in agreement with literature. However, leaves of *P. officinalis* without spots were observed quite often. On the other hand, relative pronounced whitish spots on leaves of *P. obscura* were also rare recorded.

Conclusion

Flow cytometry proved to be a convenient method for identification of hardly distinguishable species *Pulmonaria obscura* and *P. officinalis*. Using this method, distribution of both species was determined in the Bohemian Forest and neighbouring foothills. While *P. officinalis* is predominant in the southeastern part of the mountains and it penetrates from Danube valley up to the foothills of the central ridge, *P. obscura* dominates in the northwestern part of the studied area. In the areas of near occurrence of both species *P. officinalis* prefers nutrient more rich soils.

Both species are quite similar in morphological characters. The best discrimination character between the two species seems to be the calyx indumentum. *Pulmonaria officinalis* has both glandular hairs and setae longer than *P. obscura* in general. It is better to evaluate the ratio of types of hairs on calyx base, where *P. officinalis* has glandular hairs longer than setae in most of the cases. Although, the species are significantly different in this character, morphological identification is difficult due to high variation and overlap of the values.

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Appendix 1.

List of geographic coordinates of locations of samples analysed by FCM. Locations are arranged according to the species, countries, administration district (“okres” in Czechia, “Landkreis” in Bavaria, “Bezirk” in Austria), and cadastral territories (Czechia) or municipalities (Bavaria, Austria). Administration districts, cadastral territories and municipalities are ordered alphabetically. Coordinates are given in decimal degrees format and in the WGS 84 datum. Populations involved in the morpho-metric analysis are marked by asterisk.

Pulmonaria obscura

Czech Republic

distr. České Budějovice: *Kvítkovice u Lipí* (48.96054N, 14.32389E), **Slavče** (48.92420N, 14.32943E), **Záhorčice u Vrábče** (48.91833N, 14.40906E);

distr. Český Krumlov: Arnoštov u Českého Krumlova (48.89758N, 14.02739E), **Boletice** (48.84283N, 14.16384E; 48.84655N, 14.15925E), **Černá v Pošumaví** (48.77289N, 14.11063E; 48.76918N, 14.11136E; 48.73751N, 14.14343E; 48.73983N, 14.12876E), **Český Krumlov** (48.81404N, 14.29577E), **Dolní Drkolná** (48.59305N. 14.35427E), **Holubov** (48.87889N, 14.29953E), **Horní Planá** (48.78468N, 14.07192E; 48.77653N, 14.05896E; 48.75838N, 14.08380E; 48.75652N, 14.05757E), **Jablonec u Českého Krumlova** (48.82344N, 14.06317E**), **Jaronín** (48.95136N, 14.18636E), **Jenín** (48.63122N, 14.37094E), **Maňávka u Českého Krumlova** (48.81415N, 14.02767E; 48.80100N, 14.02283E; 48.80950N, 14.03837E**; 48.80368N, 14.03074E; 48.80162N, 14.03783E; 48.80133N, 14.03891E; 48.80077N, 14.04078E; 48.80014N, 14.04268E; 48.79941N, 14.04499E; 48.79876N, 14.04601E; 48.79093N, 14.05373E), **Polná na Šumavě** (48.77705N, 14.13450E; 48.79258N, 14.15006E), **Třebovice u Českého Krumlova** (48.87086N, 14.12394E; 48.89600N, 14.11728E; 48.84836N, 14.12419E; 48.83897N, 14.13300E; 48.83522N, 14.11450E), **Uhlíkov u Českého Krumlova** (48.83227N, 14.01388E; 48.82983N, 14.01036E), **Zátes** (48.75706N, 14.34596E), **Zlatá Koruna** (48.85703N, 14.36800E);

distr. Klatovy: Albrechtice u Sušice (49.20523N, 13.56753E), **Budětice** (49.27203N, 13.57335E; 49.28230N, 13.56043E), **Čepice** (49.27368N, 13.60672E; 49.26277N, 13.60330E), **Červená u Kašperských Hor** (49.10708N, 13.57811E; 49.11379N, 13.57031E; 49.11416N, 13.56977E; 49.11341N, 13.57207E; 49.11324N, 13.57049E; 49.11322N, 13.57072E), **Debrník u Železné Rudy** (49.11761N, 13.24328E), **Dobršín** (49.25493N, 13.54590E**; 49.26478N, 13.56385E), **Hojsova Stráž** (49.22197N, 13.21570E**; 49.22877N, 13.19977E; 49.21531N, 13.21350E), **Hořejší Krušec** (49.17255N, 13.44044E), **Chlum u Hartmanic** (49.17736N, 13.44590E), **Javorná na Šumavě** (49.21728N, 13.32730E; 49.21535N, 13.33023E**; 49.23864N, 13.24885E), **Javoří u Hartmanic** (49.19635N, 13.41226E), **Kašperské Hory** (49.14717N, 13.53618E), **Kochánov III** (49.18097N, 13.38258E**), **Kozí Hřbet** (49.13217N, 13.53857E; 49.13387N, 13.53795E), **Lídlovy Dvory** (49.12363N, 13.54861E; 49.11664N, 13.56714E; 49.09552N, 13.56178E; 49.12177N, 13.55185E), **Maleč** (49.17693N, 13.68010E), **Opolenec** (49.15182N, 13.51334E; 49.15174N, 13.51687E; 49.15229N, 13.51763E; 49.15180N, 13.51677E), **Platoř** (49.19581N, 13.54007E), **Prášily** (49.07305N, 13.44157E**; 49.10069N, 13.37260E), **Radvanice u Chotěšova**

(49.22977N, 13.34040E), **Rejštejn** (49.12717N, 13.50889E; 49.13161N, 13.50072E), **Svojše** (49.10545N, 13.49403E; 49.11507N, 13.49691E), **Velké Hydčice** (49.31503N, 13.68125E), **Zálužice I** (49.14573N, 13.47918E), **Zelená Lhota** (49.22685N, 13.18189E);

distr. Prachatice: Arnoštka (48.99495N, 13.76957E), **Bavorov** (49.12275N, 14.09714E; 49.13042N, 14.11053E; 49.12372N, 14.11694E), **Boubská** (49.05940N, 13.80806E), **Černá Lada** (48.99993N, 13.71215E; 48.99746N, 13.71415E; 48.98507N, 13.70493E**; 49.00280N, 13.72747E), **Cudrovice** (48.95812N, 13.92559E), **Dolní Světlé Hory** (48.91155N, 13.64640E), **Hrabice** (49.04373N, 13.69809E), **Křišťanov** (48.90576N, 14.06747E), **Křišťanovice u Záblatí** (48.95840N, 13.92883E; 48.95688N, 13.92960E), **Ktiš** (48.90082N, 14.10853E; 48.91350N, 14.11187E; 48.90090N, 14.10860E), **Leptač** (48.98098N, 14.04809E), **Lipka u Vimperka** (49.02168N, 13.74210E), **Lštění u Radhostic** (49.05699N, 13.87284E), **Netolice** (49.06633N, 14.16569E), **Nové Hutě** (49.01377N, 13.65134E), **Perlovice** (48.98368N, 13.97821E), **Prachatice** (49.00390N, 13.98515E; 49.00187N, 13.98481E), **Pravětín** 49.04573N, 13.81302E; 49.04563N, 13.81406E; 49.01596N, 13.79145E; 49.01887N, 13.79316E), **Štítkov** (49.07542N, 13.84288E), **Švihov u Lažišť** (49.02101N, 13.89798E; 49.02083N, 13.89890E), **Třebanice** (49.01735N, 14.14275E), **Včelná pod Boubínem** 49.01776N, 13.88683E; 49.01496N, 13.89184E; 49.02119N, 13.88797E**; 49.01678N, 13.84500E**; 49.02052N, 13.87075E; 48.99546N, 13.82586E), **Vimperk** (49.05124N, 13.79400E), **Volary** (48.92664N, 13.85386E), **Výškovice u Vimperka** (49.08580N, 13.79599E), **Zahrádky u Borových Lad** (48.98154N, 13.67396E; 48.97002N, 13.65441E), **Žár u Čkyně** (49.12908N, 13.75272E).

Bavaria

distr. Deggendorf: Deggendorf (48.86274N, 13.01515E; 48.86254N, 13.01409E; 48.87190N, 13.01117E), **Grafling** (48.93366N, 12.97407E), **Lalling** (48.87117N, 13.08662E);

distr. Freyung-Grafenau: Mauth (48.90017N, 13.56544E; 48.90658N, 13.56527E; 48.93994N, 13.55927E), **Philippsreut** (48.90609N, 13.64611E), **Spiegelau** (48.88118N, 13.358801E);

distr. Regen: Bischofsmais (48.90866N, 13.05128E), **Böbrach** (49.03283N, 13.025278E), **Frauenau** (48.95677N, 13.31110E), **Lindberg** (49.10377N, 13.27416E; 49.08243N, 13.26334E; 49.09034N, 13.29659E);

distr. Straubing-Bogen: Steinach (48.94563N, 12.58343E).

Pulmonaria officinalis

Czech Republic

distr. Český Krumlov: Arnoštov u Českého Krumlova (48.86530N, 14.00380E), **Černá v Pošumaví** (48.72788N, 14.11202E; 48.72806N, 14.10912E; 48.72895N, 14.11248E), **Dolní Drkolná** (48.57504N, 14.35927E; 48.59729N, 14.35528E), **Frýdava** (48.65559N, 14.15570E), **Jablonec u Českého Krumlova** (48.81447N, 14.06697E; 48.84453N, 14.07575E; 48.84397N, 14.08375E; 48.83794N, 14.08211E; 48.83497N, 14.07639E), **Jasánky** (48.62200N, 14.06129E; 48.62274N, 14.04634E; 48.62442N, 14.06357E; 48.62394N, 14.04564E), **Kapličky** (48.61764N, 14.21321E), **Loučovice** (48.63372N, 14.24719E**), **Maňávka u Českého Krumlova** (48.80804N, 14.01632E; 48.80028N, 13.98506E; 48.80136N, 13.98442E; 48.79909N, 14.05936E; 48.81869N, 13.98655E**; 48.82022N, 13.99942E; 48.82031N, 14.00202E; 48.81334N, 14.00850E**; 48.81957N, 14.00353E; 48.81943N, 14.00051E; 48.81890N, 14.00361E), **Mnichovice u Loučovic** (48.60983N, 14.24630E), **Ondřejov u Českého Krumlova** (48.86986N, 14.03767E), **Pasečná** (48.64134N, 14.10488E), **Pestřice** (48.70706N, 14.02108E), **Přední Výtoň** (48.63535N, 14.14236E), **Slavkov u Českého Krumlova** (48.76932N, 14.23662E; 48.76815N, 14.23691E), **Štěkře** (48.86053N, 14.36433E), **Studánky u Vyššího Brodu** (48.59152N, 14.32247E; 48.58135N, 14.33142E; 48.58598N, 14.32846E; 48.58143N, 14.33098E), **Uhlíkov u Českého Krumlova** (48.83792N, 13.97342E), **Vyšší Brod** (48.61895N, 14.27638E; 48.61483N, 14.29039E; 48.61611N, 14.29272E; 48.61831N, 14.30083E), **Zvonková** (48.74799N, 13.98613E; 48.76311N, 13.93576E; 48.76276N, 13.93571E; 48.76028N, 13.93739E; 48.75684N, 13.93600E; 48.75608N, 13.94199E; 48.71703N, 13.95852E; 48.74767N, 13.98451E; 48.71570N, 13.96160E), **Žlíbky** (48.94709N, 13.75874E);

distr. Klatovy: Kašperské Hory (49.14218N, 13.59834E; 49.12986N, 13.58086E; 49.13294N, 13.55672E; 49.12948N, 13.57889E), **Nahořánky** (49.18010N, 13.64715E; 49.18009N, 13.64806E; 49.18024N, 13.64795E; 49.18106N, 13.65117E), **Nezdice na Šumavě** (49.15408N, 13.62426E; 49.15580N, 13.62368E; 49.15714N, 13.62473E), **Nové Městečko** (49.17537N, 13.50066E), **Nuzerov** (49.18597N, 13.49551E), **Řetenice u Stach** (49.13961N, 13.60646E; 49.12871N, 13.60343E), **Zuklín** (49.14846N, 13.64203E**);

distr. Prachatice: Arnoštov u Českého Krumlova (48.86894N, 13.97531E; 48.87097N, 13.97267E), **Černá Lada** (48.99730N, 13.68414E; 48.97963N, 13.72102E; 48.97668N, 13.72847E; 48.97527N, 13.72628E; 48.98242N, 13.69977E), **České Žleby** (48.87355N, 13.77288E; 48.87000N, 13.83000E; 48.88972N, 13.79178E; 48.87400N, 13.79056E; 48.87542N, 13.79867E; 48.87686N, 13.79397E**; 48.89096N, 13.79212E; 48.87283N, 13.82281E; 48.87611N, 13.78396E; 48.85694N, 13.76117E), **Chlum u Volar** (48.87404N, 13.92258E), **Cudrovice** (48.95837N, 13.92292E; 48.96728N, 13.91634E), **Horní Světlé Hory** (48.92108N, 13.70945E**; 48.89747N, 13.70237E; 48.90044N, 13.69701E), **Horní Vltavice** (48.97644N, 13.77053E), **Jaroškov** (49.10632N, 13.67016E), **Klášterec u Vimperka** (49.03631N, 13.73955E), Krejčovice (48.97036N, 13.90336E; 48.96896N, 13.90173E; 48.97966N, 13.90631E), **Lenora** (48.95005N, 13.78764E; 48.95030N, 13.78099E; 48.95054N, 13.78153E; 48.95362N, 13.80654E**; 48.94930N, 13.82118E; 48.93865N, 13.81948E; 48.94003N, 13.82983E; 48.94413N, 13.82982E; 48.94315N, 13.83398E; 48.93628N, 13.83030E; 48.96088N, 13.80992E; 48.95300N, 13.79700E), **Mičovice** (48.97601N, 14.13078E), **Nová Pec** (48.76168N, 13.91457E; 48.81659N, 13.87756E), **Račí** (48.98359N, 13.75504E), **Radvanovice** (48.90887N, 13.78900E; 48.90739N, 13.79557E), **Řepešín** (48.98612N, 13.91153E; 48.98836N, 13.91168E; 48.99362N, 13.89439E), **Saladín** (49.00554N, 13.92562E; 49.00743N, 13.92881E), **Silnice** 48.89446N, 13.65495E; 48.89407N, 13.65520E; 48.88460N, 13.69270E; 48.88611N, 13.69294E), **Spálenec** (48.89962N, 13.97576E), **Stachy** (49.10055N, 13.63831E; 49.10091N, 13.63880E), **Staré Prachatice** (49.03105N, 13.99663E), **Stožec** (48.85123N, 13.84910E; 48.85651N, 13.84079E), **Strážný** (48.90012N, 13.70934E), **Úbislav** (49.11943N, 13.65815E; 49.12943N, 13.65785E), **Volary** (48.91161N, 13.80963E; 48.92643N, 13.87363E; 48.93173N, 13.84654E; 48.93996N, 13.85818E; 48.93666N, 13.90327E**; 48.90582N, 13.81854E), **Zábrdí u Lažišť** (49.02534N, 13.94365E; 49.02639N, 13.93875E), **Zahrádky u Borových Lad** (48.94685N, 13.68962E; 48.95998N, 13.68634E), **Zdíkov**

(49.06808N, 13.68425E), **Zvonková** (48.76025N, 13.94102E; 48.75894N, 13.94300E; 48.75531N, 13.92982E).

Bavaria

distr. Deggendorf: Deggendorf (48.86388N, 13.01374E);
distr. Freyung-Grafenau: Freyung (48.79874N, 13.53997E), **Eppenschlag** (48.89605N, 13.32132E), **Grainet** (48.79855N, 13.66942E), **Haidmühle** (48.87821N, 13.72936E), **Mauth** (48.87250N, 13.57589E; 48.87726N, 13.57485E), **Sankt Oswald** (48.91480N, 13.40240E; 48.89753N, 13.44599E), **Spiegelau** (48.94889N, 13.38102E), **Waldhäuserwald** (48.93630N, 13.45400E).

Upper Austria

distr. Rohrbach: Lichtenau im Mühlkreis (48.59712N, 14.07039E), **Ulrichsberg** (48.70140N, 13.93454E**; 48.71950N, 13.91661E; 48.71013N, 13.97689E);
distr. Urfahr-Umgebung: Bad Leonfelden (48.56102N, 14.29349E).

***Pulmonaria officinalis* cult. (plants probably escaped from an old cultivation)**

Czech Republic

distr. Český Krumlov: Pasovary (48.75154N, 14.21868E);
distr. Klatovy: Hamry na Šumavě (49.21112N, 13.17503E), **Horní Těšov** (49.18327N, 13.42993E);
distr. Prachatice: Klášterec u Vimperka (49.03290N, 13.75850E), **Knížecí Pláně** (48.95348N, 13.61657E), **Veselka u Vimperka** (49.02256N, 13.82455E).