

## Morphological variation in the *Melampyrum sylvaticum* group within the transitional zone between *M. sylvaticum* s. str. and *M. herbichii*

Morfologická variabilita *Melampyrum sylvaticum* agg. v přechodné zóně mezi *Melampyrum sylvaticum* s. str. a *Melampyrum herbichii*

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The *Melampyrum sylvaticum* group is a complex of three closely related species. This group is most variable in the Carpathian region. Interactions among different levels (within-population to inter-specific) of genetic variation and influence of the environment are considered to be the main sources of the complicated morphological variation in this region. Morphological variation in the *M. sylvaticum* group was studied in mountain ranges of the Hercynian Massif and in the Western and Ukrainian Carpathians. Several populations were sampled at different altitudes within each mountain range. Hierarchical partitioning of morphological variation at different levels (within populations, among populations within a mountain range and among mountain ranges) was calculated. Correlations among groups of morphological characters and altitude were calculated. The largest proportion of variation on a large geographic scale (i.e. among mountain ranges) was detected in anther length and several corolla characters (length of the lower corolla lip, height of upper corolla lip), whereas these traits were homogeneous at a local scale (within populations and among populations in one mountain range). An opposite pattern (i.e. high proportion of variation at the low levels, which blurred possible large scale differences) was found in bract traits and several calyx characters. Moreover, a strong correlation between bract length and altitude was observed. The observed changes in the proportions of morphological variation and response to altitude suggest a close connection between bract characters and environmental factors (or lower levels of genetic variation). On the other hand, some of the flower characters seem to be genetically determined and thus might reflect evolutionary processes (early diversification, potential hybridization, introgression) on which the taxonomic treatment of the group should be based. The most distinct differences were detected between samples from the Ukraine and south-western part of Bohemia. Populations from the Sudeten Mts and the Western Carpathians were variable and morphologically intermediate, forming a continuum between the two extremes.

**Key words:** *Melampyrum herbichii*, *Melampyrum sylvaticum*, morphometrics, Sudeten Mts, taxonomy, variance components, Western Carpathians

### Introduction

The genus *Melampyrum* L. belongs to the family *Orobanchaceae* (Olmstead et al. 2001), which consists of species mostly having a (hemi)parasitic life cycle. Diversification of individual microspecies within relatively distinct species complexes in most of the genera is supposed to have taken place in the late Pleistocene. Quaternary climatic cycles (glacial/interglacial periods) and consequent migration are thought to have played an important role in the diversification and distribution pattern of individual microspecies (Wesselingh & van Groenendael 2005). Their recent origin by various evolutionary pro-

cesses (hybridization, introgression) may complicate the identification and delimitation of particular taxa (Wesselingh & van Groenendael 2005).

Three probably closely related species are distinguished in the *M. sylvaticum* group. These species have usually been delimited from each other on the basis of flower traits, especially length of the anthers, colour and size of the corolla (Baumgarten 1816, Wołoszczak 1888, Jasiewicz 1958). Typical specimens can be classified appropriately using these characters. *Melampyrum sylvaticum* L. is characterized by a yellow and relatively small corolla, (5–) 7–9 (–11) mm long and the shortest anthers, (1.4–) 1.6–2.1 (–2.3) mm long, within the group (Jasiewicz 1958). This species is the most widespread of the three microspecies. Its geographical range corresponds to the range of the whole group, i.e. it covers the European boreal zone and mountains in the European temperate zone from the Pyrenees to the Urals (Meusel et al. 1978).

*Melampyrum herbichii* Woł. is similar to the previous species, particularly in corolla colour. The length of the anthers and corolla, which reach (2.0–) 2.4–3.6 (–4.2) mm and (7.0–) 9.0–12.5 (–14.0) mm respectively, are supposed to be the main diagnostic characters differentiating this species from *M. sylvaticum* s. str. (Jasiewicz 1958) although significant overlaps are obvious in both characters. This species was first described from three localities in the Ukrainian Carpathians by Wołoszczak (1888). The description of the species is based on samples from three localities including the Hoverla massif in the Chernogora region. Its geographical range was believed to be restricted to the Eastern and Southern Carpathian region (Soó & Webb 1972). However, populations classified as *M. herbichii* are reported from the regions of the Western Carpathians and the Sudeten Mts (e.g. Jasiewicz 1958, Šípošová 1997, Štech 2000) together with specimens featuring diagnostic characters with values transitional between *M. herbichii* and *M. sylvaticum* (Štech 1998, Štech & Drábková 2005). In addition, significant variation in bract shape has been detected in samples from this region (Štech 1998). Bract morphology was investigated in the most recent studies (Štech 1998, Štech & Drábková 2005) suggesting that bract proportions can be used to discriminate between species in the transitional (potential hybrid) zone. Beside the morphological differentiation, a significant difference in habitat preferences between *M. herbichii* and *M. sylvaticum* is described by Šípošová (1997). According to this taxonomical treatment, the habitat preferences of *M. sylvaticum* are supposed to be relatively wide, as it grows in mountain spruce forests up to the dwarf-pine communities at the tree-line. By contrast, *M. herbichii* appears to be restricted to montane meadows around the tree-line (Šípošová 1997). However, Jasiewicz (1958) also reports *M. herbichii* from forests at lower altitudes.

The third species, *M. saxosum* Baumg., is characterized by a white corolla. Quantitative morphological characters seem to overlap completely those of *M. herbichii*; thus the corolla colour is the only diagnostic trait separating these two species (Jasiewicz 1958). *Melampyrum saxosum* occurs in the Eastern (and probably Southern) Carpathians (Soó & Webb 1972), any overlap with the other species reported elsewhere can be considered as a misidentification (Štech 2000).

Although several authors have attempted to elucidate and interpret morphological variation in the *M. sylvaticum* group (the exact delimitation between *M. herbichii* and *M. sylvaticum*, and relationships among transitional populations are usually considered to be the most important questions), their effort have never been completely successful, because a few factors confuse the large-scale morphological variation gradient. A high

phenotypic plasticity is expected to have a great impact on the overall morphology of plants; however its impact is unlikely to be the same for all morphological traits. So-called seasonal variation, a specific type of low-scale genetic variation affecting plant architecture, characteristic of many hemiparasitic *Orobanchaceae* (Wettstein 1895, S6o 1926–1927, Zopfi 1993a, 1993b, 1995, 1997, 1998a, 1998b), is another phenomenon with considerable influence on morphology. Seasonal variation produces polytopic locally adapted ecotypes, which differ primarily in stem internode number (Zopfi 1993b); however, some flower and bract traits might be directly correlated with this character. Phenotypic plasticity together with seasonal variation and their interactions produce local gradients in morphological variation, which can interfere with large geographical gradients in variation resulting in the geographical distribution of individual morphotypes showing a very complex pattern (Štech & Drábková 2005, Těšitel 2005). Thus, neglecting the low-scale variability of phenotypic factors (e.g. building a taxonomical treatment of the group on the basis of sampling a single population within individual mountain ranges) may lead to biased conclusions.

There exist many environmental gradients that may affect the morphological features of specimens of the *M. sylvaticum* group (e.g. light conditions, climate, host plant species and competition with other species). But the major factors are directly connected to altitude and seasonal variation (Štech 1998, Těšitel 2005). The altitudinal gradient is suitable for direct analyses and easy to interpret, which is why this variable was chosen as a reference for gradients in low-scale morphological variation in this study.

The aim of the current study is to elucidate patterns in morphological variability in the *M. sylvaticum* group in the Western Carpathian and Sudeten regions, where transitional morphological types between *M. sylvaticum* and *M. herbichii* prevail (typical specimens of individual species were also included for reference). Detailed analysis of variation in morphological traits is a crucial part of this assessment. It should help to exclude the characters, which were considered discriminatory by previous authors but display significant low-scale variation. Consequent analysis of geographical distribution of morphotypes can be used as a basis for formulating phylogeographic hypotheses and a taxonomic treatment of the group. Population sampling differed slightly and was more complex than in other recent studies (Štech 1998, Štech & Drábková 2005). The objective of this was to obtain a more robust data set suitable for such a rigorous assessment.

## Material and methods

### *Material*

Morphometrical data were collected from 24 populations (658 plants) of the *M. sylvaticum* group within the transitional zone between *M. sylvaticum* and *M. herbichii* (Fig. 1 and Appendix 1). The localities were selected to cover the whole transitional zone including typical populations of the microspecies. Several populations within a homogeneous geographically-defined mountain range were sampled in order to evaluate the distribution of variation among these geographical units (further referred to also as regions) and among populations within them. Populations from the Šumava Mts and the Brdy massif were combined into one region named “South and Central Bohemia” due to the low number of localities studied (two and one, respectively) and overall morphological similarity among the

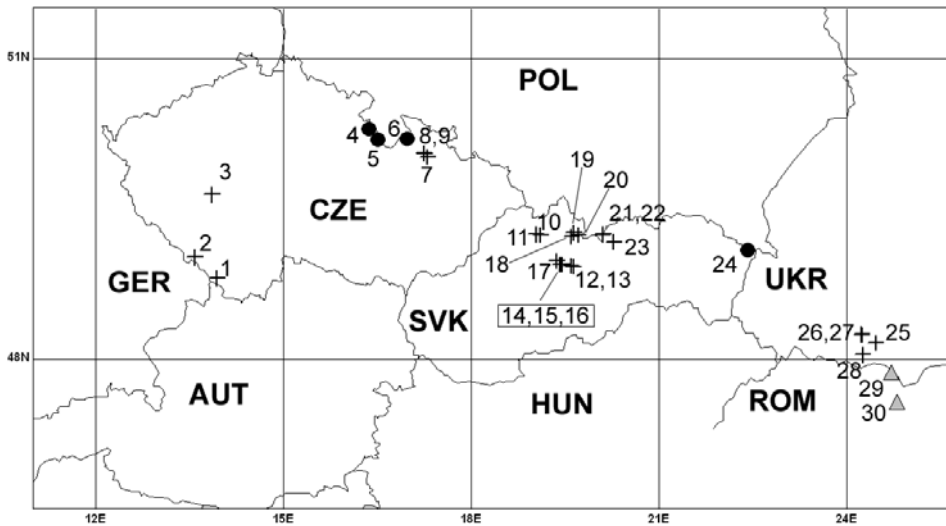


Fig. 1. – Map of the localities of the *Melampyrum sylvaticum* group included in this study. Borders of Central European countries are also displayed. CZE – Czech Republic, SVK – Slovakia, UKR – The Ukraine, ROM – Romania, HUN – Hungary, AUT – Austria, GER – Germany, POL – Poland. + populations included in morphometric analyses, ● additional populations referred to in Discussion ▲ populations of *M. saxosum*.

plants growing in these districts, which were always classified as typical *M. sylvaticum* (Štech 2000). Considering the other end of the geographical gradient, all the population samples collected in the Ukrainian Carpathians are a priori classified as *M. herbichii*, as this species was described from this region (Wołoszczak 1888). Beside the specimens included in the analyses, several additional populations were included in a survey of the geographical distribution of variation. These are the specimens collected in the Orlické hory Mts and the Rychlebské hory Mts, for which data on bract shape are lacking, and populations of *M. saxosum*, which were used as a reference. The sampling was conducted over a short period of time at the start of the flowering season in order to minimize the influence of phenological divergence on the morphology of the flowers (1st to 3rd, exceptionally 4th lowermost flower of all the plants were taken and analyzed). The only exception was the sample of plants from the Bukovské vrchy Mts, which were collected at a later ontogenetical stage and 5th or 6th lowermost flower had to be taken.

A population sample of 21–30 (– 38) plants were collected at every locality (see Appendix 1 for the exact number at each locality). The calyx and corolla of one flower of each plant were put into an Eppendorf-tube filled with ethanol and stored until measured. The first and the fifth bracts were stuck on a sheet of paper using a transparent tape. The other parts of the whole plants were processed as standard herbarium specimens and are kept in the herbarium of the Faculty of Biological Sciences, University of South Bohemia (CBFS).

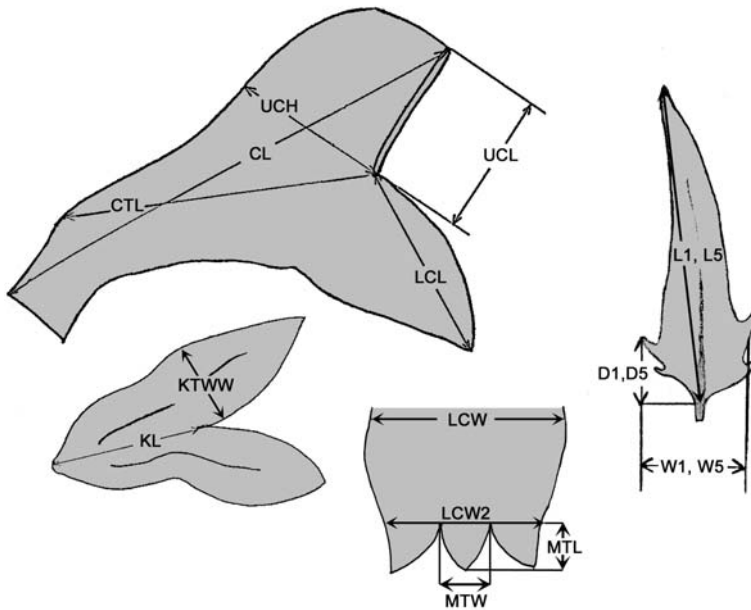


Fig. 2. – Morphological characters of the corolla, calyx, lower lip of corolla and bracts. See the “Morphological characters” paragraph in the methods section for definitions.

### *Morphological characters*

Morphological characters were obtained by a series of measurements conducted on each plant. Twelve traits were measured on flowers (anther length – AL, corolla length – CL, length of the corolla tube – CTL, length of the lower corolla lip – LCL, length of the upper corolla lip – UCL, height of the upper corolla lip – UCH, width of the lower corolla lip – LCW, width of the lower corolla lip at the base of corolla teeth – LCW2, width of the middle tooth of lower corolla lip – MTL, length of the middle tooth of lower corolla lip – MTW, calyx tube length – KL, width of the upper calyx tooth at its widest point – KTWW) and three traits (length – L, width – W, distance of the widest part from its base – D) on the 1st and 5th bract (see Fig. 2). Some of the traits were not available in a few cases due to damaged material. This happened rather frequently for the fifth bract which was often not developed, particularly when the plants were sampled at an early ontogenetic stage (i.e. immediately after flowering).

### *Statistical analyses*

Multivariate statistical techniques were employed to investigate the morphological variation in all characters together. An unconstrained ordination method, principal component analysis (PCA, Lepš & Šmilauer 2003), based on the matrix of correlations among the trait values (and thus centered and standardized data), was used to display general patterns in

the variation. The patterns were correlated with the altitude of the sites, the most obvious environmental gradient in the data, using a constrained ordination method, redundancy analysis (RDA, Lepš & Šmilauer 2003). Furthermore, Pearson correlation coefficients were calculated to quantify the relationships detected.

Proportional variation was estimated for single traits at different geographical levels by extracting the variance components from a linear mixed effect model (Quinn & Keough 2002). The regions and populations were random-effect terms in this model. Expected mean squares estimation (EMS) and restricted maximum likelihood estimation (REML; Quinn & Keough 2002, Pinheiro et al. 2005) were employed for processing multivariate and univariate data, respectively.

Multivariate statistical analyses were performed using Canoco for Windows, version 4.52 (terBraak & Šmilauer 2002). Variance components were computed with the R package, version 2.2 (R Core Development Team 2005), package nlme version 3.1-65 (Pinheiro et al. 2005). Statistica for Windows, version 6 (StatSoft 2001) was used for correlation analyses and other basic statistics.

## Results

### *Overall variation in morphological characters*

Within-population morphological variation of all characters accounted for 48.6% of the total variation. Regarding particular characters, considerable differences can be identified in their patterns of variation (Fig. 3, Table 1). A highly significant proportion of the variation among populations was detected in all traits (see fourth and fifth column in Table 1). However, the structure of this variation differed noticeably among individual traits. The length of anthers (AL) had the largest proportion of variation connected to the among-region level but the lowest proportion of within-regional variability and within-population variation. Some corolla traits (LCL, UCH, LCW, CL, and CTL) appeared to have a similar pattern of variation, but the proportion of within-population and within-regional variation was substantially higher. In contrast, there was no bract trait with a significant variance component based on the differences among regions. Variation in these characters was concentrated at the within-regional and within-population levels.

Two independent groups of traits can be distinguished in PCA plots based either on the variation among individual plants, or on the means of character values within particular populations (Figs 4 and 5, respectively). These show a rather strong correlation with the first or the second principal axes. The group correlated with the first axis is formed entirely by flower traits whereas the second group consists of bract characters. Populations from different regions appear not to be clearly separable from each other using these morphological traits, but there is only a very small overlap in the morphological features of the populations from the regions at the opposite sides of the gradient of the first PCA axis (Figs 5, 6).

### *Morphological variation in relation to altitude*

Morphological variability induced by differences in environmental conditions was investigated by evaluating the relationships between morphometric trait values and altitude. Results of the RDA (Fig. 7) indicate that variation in most flower traits (except for LCW,

Table 1. – Likelihood-ratio test results for the variance components of individual morphological traits (see Fig. 3). Significant results ( $P < 0.05$ ) are displayed in bold.

Morphological character	Variation among regions		Variation among populations	
	Likelihood-ratio	P	Likelihood-ratio	P
Anther length (AL)	37.13	< <b>0.0001</b>	796.73	< <b>0.0001</b>
Length of the lower corolla lip (LCL)	19.43	< <b>0.0001</b>	583.01	< <b>0.0001</b>
Height of the upper corolla lip (UCH)	13.96	<b>0.0002</b>	415.90	< <b>0.0001</b>
Width of the lower corolla lip (LCW)	11.31	<b>0.0008</b>	448.99	< <b>0.0001</b>
Corolla length (CL)	12.57	<b>0.0004</b>	490.30	< <b>0.0001</b>
Width of the lower corolla lip at the base of corolla teeth (LCW2)	11.77	<b>0.0006</b>	410.41	< <b>0.0001</b>
Length of the corolla tube (CTL)	10.44	<b>0.0012</b>	356.58	< <b>0.0001</b>
Length of the upper corolla lip (UCL)	6.37	<b>0.0116</b>	558.78	< <b>0.0001</b>
Width of the middle tooth of lower corolla lip (MTW)	5.28	<b>0.0216</b>	210.69	< <b>0.0001</b>
Calyx tube length (KL)	5.76	<b>0.0164</b>	248.54	< <b>0.0001</b>
Distance of the widest part of the 5th bract from its base (D5)	1.64	0.2007	250.44	< <b>0.0001</b>
Length of the middle tooth of lower corolla lip (MTL)	2.37	0.1239	203.96	< <b>0.0001</b>
Width of the upper calyx tooth at its widest point (KTWW)	0.65	0.4213	340.91	< <b>0.0001</b>
Length of the 1st bract (L1)	0.45	0.5040	351.84	< <b>0.0001</b>
Distance of the widest part of the 1st bract from its base (D1)	0.13	0.7139	294.17	< <b>0.0001</b>
Length of the 5th bract (L5)	0.00	0.9440	322.26	< <b>0.0001</b>
Width of the 5th bract (W5)	0.00	0.9995	283.73	< <b>0.0001</b>
Width of the 1st bract (W1)	0.00	0.9995	300.59	< <b>0.0001</b>

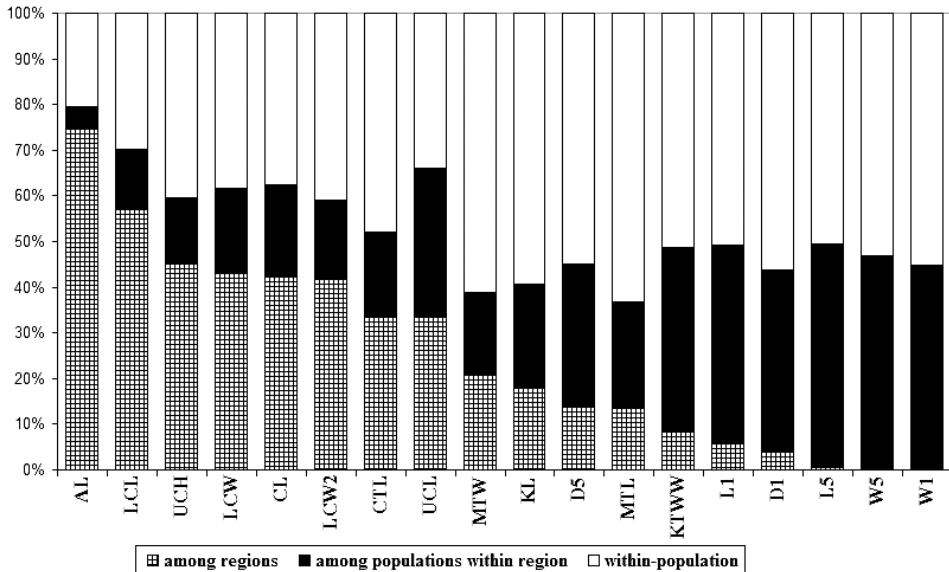


Fig. 3. – Variance components of individual characters corresponding to the hierarchical geographical levels. Variance component estimates are based on a random effect extraction from a linear mixed effect model using restricted maximum likelihood estimation (REML). See Table 1 for significance tests of the variance components. See Fig. 2 and the “Morphological characters” paragraph in the methods section for definitions of the traits.



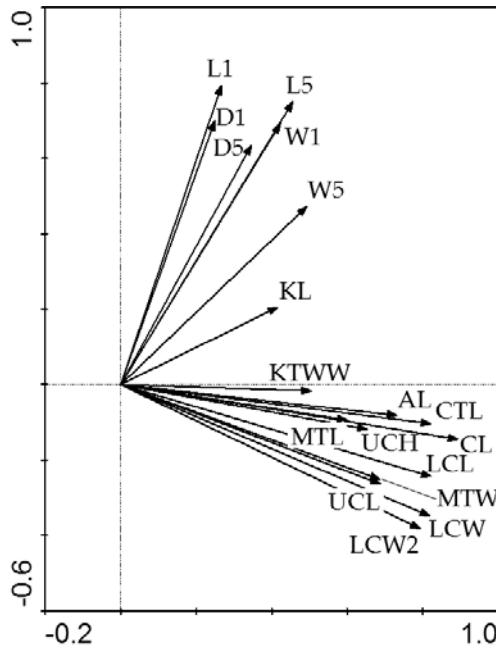


Fig. 4. – PCA plot based on individual plants. Directions of changes in morphological characters are displayed in relation to the first two principal component axes. The first and second ordination axes explain 39% and 18.6% of the variation, respectively.

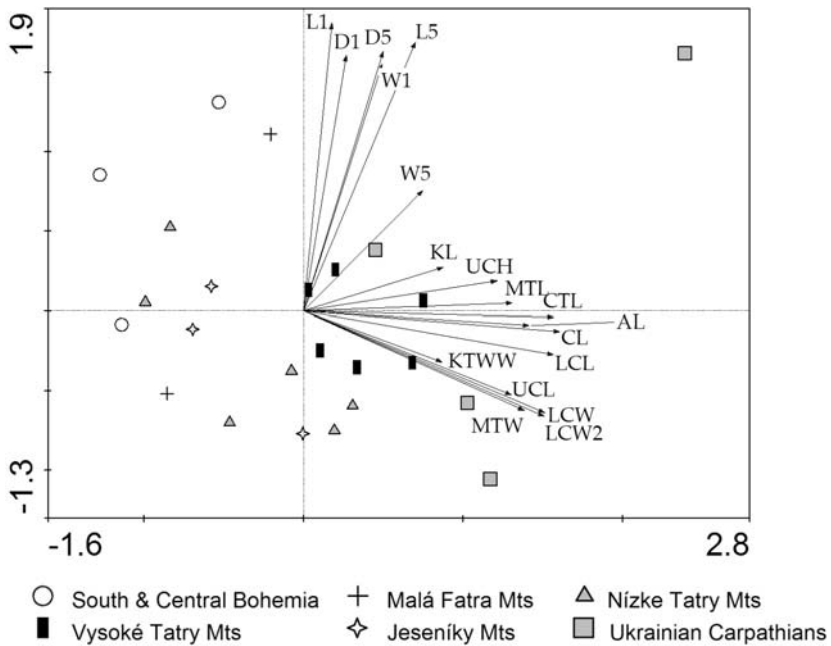


Fig. 5. – PCA plot based on means of character values within populations. Directions of changes in the morphological characters are demonstrated. Regional geographical distribution of the populations is depicted using different symbols for the ordination scores of individual populations. First two ordination axes are displayed. First axis explains 46.7% and the second 21.0% of the total variation.



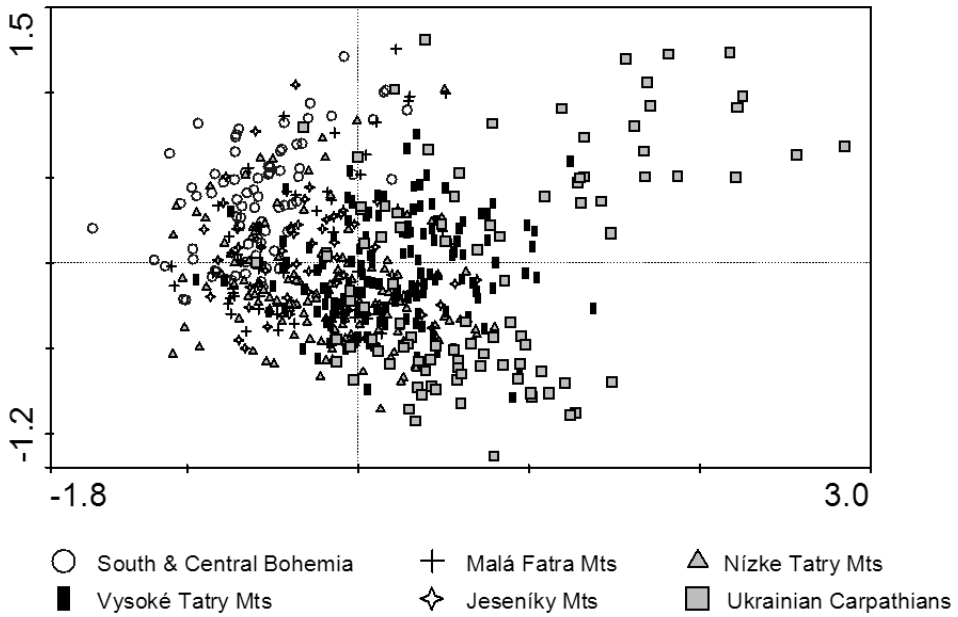


Fig. 6. – PCA plot of individual plants. Ordination scores of the plants are displayed. Regional geographical distribution of the specimens is displayed using different symbols for the ordination scores of individual plants. The first and second ordination axes explain 39% and 18.6% of the variation, respectively.

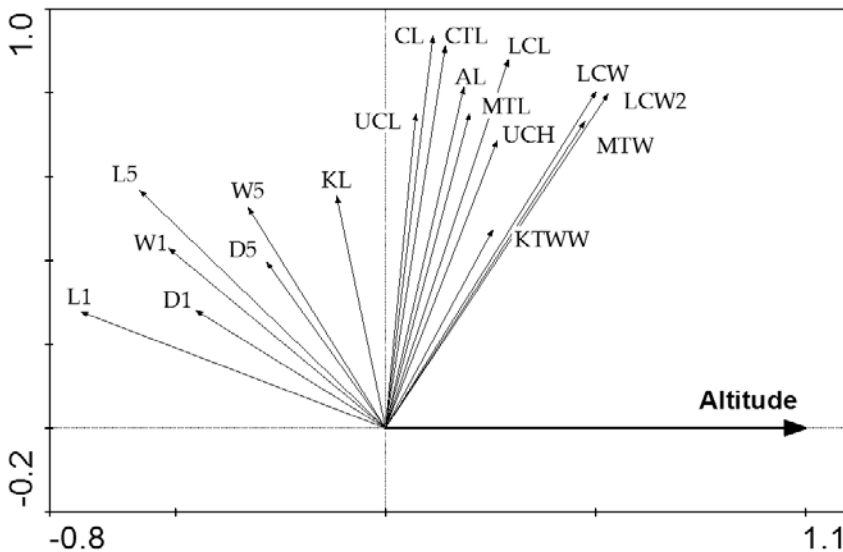


Fig. 7. – RDA plot describing directions of changes in morphological characters in relation to altitude. RDA is based on mean trait values for individual populations thus only among-population variation is considered. The constrained (horizontal) axis explains 14.6% of the variability. Monte-Carlo permutation test of significance of the canonical axis:  $F = 3.76$ ,  $P = 0.012$  (with 999 permutations).

LCW2 and MTW, for which there is a weak correlation) are independent of altitude, whereas bract traits (especially L1 and L5) seem to be correlated with this environmental variable. A more detailed survey of these relationships is provided by a correlation analysis (Table 2) confirming the pattern visible on the RDA plot (Fig. 7). A significant correlation was detected for most of the bract traits (except for W5 and D5), whereas LCW, LCW2 and MTW were the only flower characters for which there were significant correlations.

Table 2. – Pearson correlation coefficients (r) and significance of the correlation (p) between the means of morphological trait values within the populations of the *Melampyrum sylvaticum* group and altitude. Significant ( $P < 0.05$ ) relationships are displayed in bold.

Morphological character	Correlation with altitude	
	r	P
Corolla length (CL)	0.11	0.5975
Length of the corolla tube (CTL)	0.14	0.5066
Length of the upper corolla lip (UCL)	0.07	0.7378
Height of the upper corolla lip (UCH)	0.27	0.2081
Length of the lower corolla lip (LCL)	0.29	0.1647
Width of the lower corolla lip (LCW)	<b>0.50</b>	0.0125
Width of the lower corolla lip at the base of corolla teeth (LCW2)	<b>0.53</b>	0.0077
Width of the middle tooth of lower corolla lip (MTW)	<b>0.47</b>	0.0191
Length of the middle tooth of lower corolla lip (MTL)	0.20	0.3480
Anther length (AL)	0.19	0.3821
Calyx tube length (KL)	-0.12	0.5887
Width of the upper calyx tooth at its widest point (KTWW)	0.26	0.2274
Width of the 1st bract (W1)	<b>-0.52</b>	0.0098
Distance of the widest part of the 1st bract from its base (D1)	<b>-0.45</b>	0.0265
Length of the 1st bract (L1)	<b>-0.73</b>	0.0001
Width of the 5th bract (W5)	-0.33	0.1182
Distance of the widest part of the 5th bract from its base (D5)	-0.28	0.1795
Length of the 5th bract (L5)	<b>-0.59</b>	0.0026

## Discussion

### *Evaluation of morphological characters*

The distribution of variation in particular morphological characters (Fig. 3, Table 1) provides an important baseline for further considerations. Assuming that a great genetic divergence manifests itself at the higher geographical levels, the traits that vary most at the among-mountain-range level are the only potentially appropriate diagnostic characters for delimiting *M. sylvaticum* and *M. herbichii*. Variation in the length of anthers (AL) and some of the corolla traits (LCL, UCH, LCW, CL and LCW2) appear to show such a pattern, whereas an opposite constellation was detected in most bract traits. The PCA plots (Figs 4, 5, 6) imply that the first principal axes on these diagrams correspond to higher-scale variation, whereas the second axes can be interpreted as lower-scale morphological variation gradients (resulting either from low-scale genetic variability or phenotypic plasticity).

In accordance with the analyses of overall variation, the correlation analyses comparing values of the morphological traits with altitude (Table 2, Fig. 7) suggest that the proportions of bracts (especially the length) and the width proportions of the lower corolla lip (LCW, LCW2, MTW) are mostly related to either phenotype plasticity or small-scale genetic variation. In contrast, the stability of anther length (AL) and lower corolla lip length (LCL) in relation to altitude confirms the results of previous analyses and suggests that these traits reflect high-scale genetic divergence more closely than any of the other characters.

This pattern of variation supports the delimitation of *M. sylvaticum* and *M. herbichii* on the basis of anther length, as found in previous studies (Jasiewicz 1958, Soó & Webb 1972). However, corolla length, another trait frequently used for determination (e.g. Jasiewicz 1958, Šípošová 1997), is obviously less robust in differentiating among populations from different regions than some other corolla characters. This might be caused by differences in the curvature of the corolla base (see Fig. 2) which adds some error variance in its values. Using another trait, the length of the lower corolla lip (LCL), instead of simple corolla length for classification seems to be more appropriate as this character appears to reflect geographical distribution pattern more precisely than any other corolla trait.

These conclusions do not agree with those of Štech (1998) and Štech & Drábková (2005). In these studies, within-population variation seemed to blur substantially the differences between individual species (even considering the traits considered diagnostic such as AL or CL), which led to a search for other traits, which can be used to delimit *M. sylvaticum* and *M. herbichii*. This disagreement can be explained by a difference in the sampling technique. As stated in the methods section, plant specimens for the current study were collected over a short period of time at the beginning of the reproductive season. Thus, the 1st – 3rd (exceptionally 4th) lowermost flower from the main inflorescence was collected for measurement. In the previous studies (Štech 1998, Štech & Drábková 2005), the sampling continued over the whole flowering season. Flowers for measurement were chosen randomly within the plant (i. e. flowers from higher nodes on the main inflorescence and from branch inflorescences were also processed). Hence, this flower trait values may have included additional variance associated with differences in the morphological features of flowers from different positions on a plant. This explanation is furthermore supported by the genetic variation detected by RAPDs (Štech & Drábková 2005). The proportion of within-population variation detected in the whole set of morphological characters in the current study coincides closely with that obtained by a RAPD marker analysis (48.6% versus 47.6% for RAPD data) but is considerably larger (68.5%) for the set of morphological characters analyzed by Štech & Drábková (2005).

Variation in the proportions of bracts needs detailed evaluation. The length of bracts (L1 and L5) does not seem to differ between the two species. However, this trait was found to have a great discriminatory power between the two species in the most recent study dealing with variation in the *M. sylvaticum* group (Štech & Drábková 2005). Such a discrepancy might be caused by slight differences in the environmental conditions experienced by the populations analyzed by Štech & Drábková (2005) and those analyzed in the current study. The populations chosen as training data-sets for classification by discriminant analysis by Štech & Drábková (2005) differed not only in their geographical distribution but also in the environmental conditions at the sites from which the samples were collected. The samples of *M. herbichii* from the Ukraine and the Bukovské vrchy

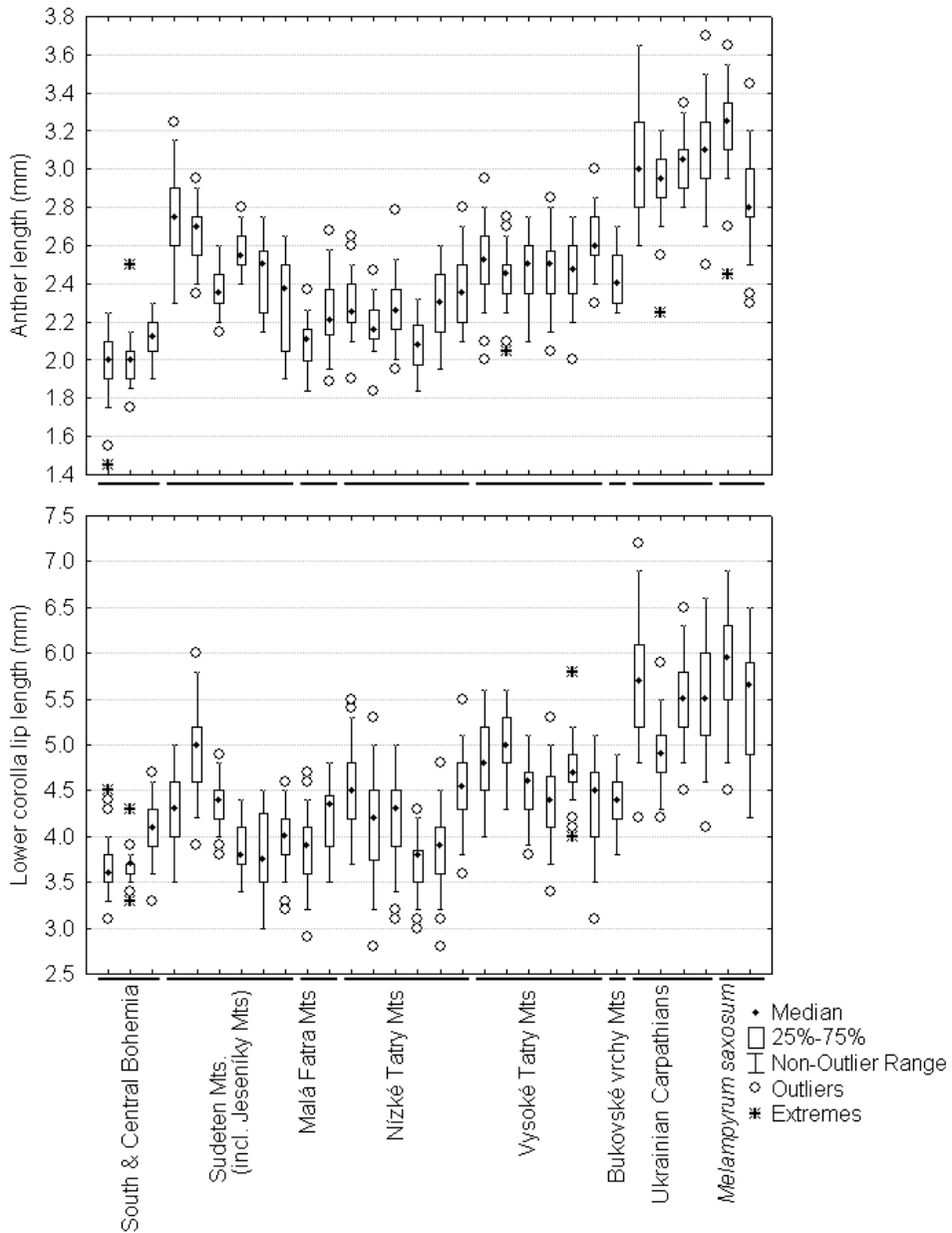


Fig. 8. – Box-and whisker plots displaying the values of anther length (AL) and lower corolla lip length (LCL) at individual localities in different regions. The populations are displayed along the horizontal axes in the order (from left to right) of the descriptions of localities in Appendix 1.

Mts included in that study were collected from alpine meadows at altitudes, mainly between 1150 and 1580 m a.s.l., whereas specimens of *M. sylvaticum* from the western group of localities (the Alps and Bohemian Forest) originated from both meadows and forests at altitudes between 500 and 1100 m a.s.l. (see Appendix in Štech & Drábková 2005). Two populations from the Alps, at 1600 and 1900 m a.s.l., were the only exceptions, but these included a very small number of plants (4 and 6, respectively) and had little influence on the coefficients of the discriminatory function. This difference in environmental conditions between the training data-sets must have biased the discriminatory function so that their consequent classification was not only based on the morphological divergence between the typical samples of the two species but also on the basis of morphological differences induced by the environment (altitude and habitat type). The a priori classification of plants from the Bukovské vrchy Mts as *M. herbichii* poses another problem because there is no evidence that these populations are closely genetically related to the Ukrainian populations of *M. herbichii*, they even seem to be morphologically more proximate to plants from the Vysoké Tatry Mts than to plants from the Ukraine (Fig. 8). It is obvious that other bract characters (W1, W5, D1, D5) are also strongly affected by various environmental conditions (e.g. the bract width is influenced by the presence of bract teeth, which was observed to be more frequent at sites exposed to direct sunlight; Štech 2000); however, it is still likely that they also reflect some large-scale genetic differences. Specimens of *M. sylvaticum* tend to have narrower bracts and the distance of the widest point of the bract from its base is usually greater than in *M. herbichii*, moreover significant differences are expected regarding frequency and shape of bract teeth (Štech 1998, 2000).

Obviously, conclusions on the pattern of variation in particular traits cannot be made based only on the data presented in this study. In particular, it is not possible to decide whether the low-scale variation detected is due to phenotypic plasticity or low-level genetic divergence (local adaptations), because these factors are correlated with each other. However, future transplantation experiments are expected to differentiate between phenotypic plasticity and genetically determined morphological variation and thus provide useful information.

#### *Geographical distribution of variation*

Regarding variation in the morphological characters (Figs 4, 5, 6), it is clear, that differences among populations form a continuum within which no obvious split can be identified. The Ukrainian populations appear the most diverse compared to the other regions. The population from Rakhiv (no. 28) is the most distinct not only from the other Ukrainian populations but from the complete set of populations studied. This could suggest some kind of differentiation from the other populations of the same region; however the morphological differences are based mainly on bract traits and some flower characters which are strongly intercorrelated, which increases the weight of a single independent measure. This biased the PCAs and made the differentiation more apparent than it is. Considering the traits reflecting large-scale variation (AL, LCL), the differentiation between the Rakhiv population and other Ukrainian populations is negligible (Fig. 8).

The largest differences in anther and lower corolla lip length can be found between populations from the Ukraine and the south-western part of Bohemia (Fig. 8). The Ukrainian plants, described as *M. herbichii*, had significantly higher values for both of these characters.

The overlap between the two morphological extremes was negligible. The other populations display intermediate values and form a continuum between them. Differentiation among these regions was substantially smaller, although some slight differences can be detected and interpreted. Plants from localities in the Vysoké Tatry Mts, Bukovské vrchy Mts, Orlické hory Mts, Rychlebské hory Mts and Jeseníky Mts appear to have longer anthers and lower corolla lips. In some of these populations, values of these traits reach those of Ukrainian plants. Thus, populations in these regions are slightly less distinct from *M. herbichii* than other populations. This supports the conclusions of previous studies (Jasiewicz 1958, Šípošová 1997, Štech 1998, 2000, Štech & Drábková 2005), which report plants similar to *M. herbichii* in the Western Carpathians and the Sudeten Mts (Jasiewicz 1958, Šípošová 1997). Lower values for both anther length and lower corolla lip length were detected in the population from the Bukovské hory Mts than reported by Štech & Drábková 2005. This can be explained by the sampling of plants at later ontogenetic stages (see methods section) and consequent analyzing of flowers from higher nodes (which are sometimes reported to differ slightly from the basal ones; Těšitel 2005).

The background of this pattern in morphological variation remains to be resolved. There are two hypotheses. The morphologically transitional populations may have arisen through hybridization between different groups of plants migrating from their glacial refuges in the early Holocene. The alternative explanation is based on the evolution of different morphotypes within isolated mountain ranges after the immigration of a common variable ancestor. Application of molecular tools is the only way to decide which of these hypotheses is correct because only a phylogeographic, genetically-based analysis can reconstruct the migratory pathways of particular groups of populations in the Holocene and explain their origin.

The stability of diagnostic traits within different mountain ranges and the lack of a correlation between their values and altitude suggest that there are hardly any differences in habitat preferences between *M. sylvaticum* and *M. herbichii*. Both species were found to grow in both montane forests and meadows at the tree-line, even though typical samples of *M. herbichii* growing in the Transcarpathian Region in the Ukraine seem to be rare at low altitudes. Considering transitional morphotypes from the Western Carpathians and Sudeten Mts, no ecological differentiation can be found between populations proximate to *M. herbichii* and populations similar to *M. sylvaticum*. Thus, the ecological differences between the two species reported in the past (Šípošová 1997) should be reconsidered. In contrast, the pattern of morphological variation and its connection to their ecology described by Jasiewicz (1958) appears to correspond well with the results of our study.

It is not possible to evaluate the morphological variation in *M. saxosum* on the basis of two population samples. The overlap in quantitative morphological characters with *M. herbichii* reported by Jasiewicz (1958) was recorded in these specimens. Difference in anther length between the two populations may suggest the possibility of a significant pattern of variation in *M. saxosum*.

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## Souhrn

*Melampyrum sylvaticum* agg. patří mezi velmi variabilní a taxonomicky problematické skupiny flóry Střední Evropy. Komplikovaná morfologická variabilita je způsobena interakcemi mezi různými úrovněmi genetické variability a vlivy prostředí. Cílem této studie bylo oddělit jednotlivé úrovně variability a nalézt tak morfologické znaky, které vykazují malou variabilitu v lokálních měřítkách (na úrovni vnitropopulační či mezi populacemi v rámci jednoho pohoří), minimální závislost na podmínkách prostředí v různých biotopech a zároveň dostatečnou variabilitu na vyšší geografické škále. Geografická škála tak byla použita jako reference vyjadřující přibližně i genetickou rozdílnost mezi vzorky. Za účelem zjištění, jaké rozdíly v morfologii odpovídají jednotlivým geografickým úrovním (mezi homogenními, geograficky definovanými pohořími, mezi populacemi v rámci pohoří, uvnitř populací), byl proveden hierarchický rozklad variability jednotlivých znaků. Závislost morfologie rostlin na vlastnostech biotopu v němž rostou byla testována pomocí korelací mezi hodnotami znaků a gradientem nadmořské výšky, s níž jsou těsně korelovány i další potenciální rozdíly mezi podmínkami prostředí v jednotlivých biotopech.

Délka prašníku a spodního korunního pysku se ukázaly jako znaky nejlépe odrážející variabilitu ve velkých měřítkách, což částečně odpovídá výsledkům některých starších studií (jako diagnostický znak se však tradičně používá celková délka koruny, její hodnota však vykazovala poměrně velkou vnitropopulační variabilitu). Hodnoty těchto znaků byly navíc dostatečně odlišné v různých regionech, což umožnilo provést zhodnocení geografického rozložení variability v populacích a vytvořit základ pro taxonomické hodnocení skupiny. Naopak se ukázalo, že převážná část variability ve tvaru listenů je zapříčiněna buď fenotypovou plasticitou anebo velmi lokálními genetickými rozdíly. Zároveň byla zjištěna i silná korelace znaků na listenech s gradientem nadmořské výšky.

Značné rozdíly v délkách prašníků a spodních korunních pysků byly zjištěny mezi populacemi *M. herbichii* z Ukrajiny a *M. sylvaticum* s. str. ze Šumavy a Brd. Populace ze Západních Karpat a Sudet dosahují v těchto znacích intermediárních hodnot, které v konkrétních případech poněkud blíží buď ukrajinským populacím (rostliny z Vysokých Tater a Sudet) anebo šumavským a brdským populacím (rostliny z Malé Fatry a Nízkých Tater). Mezi těmito skupinami však neexistuje žádná ostrá hranice, která by přerušila kontinuum v morfologické variabilitě. Otázka druhové klasifikace těchto rostlin tak zůstává otevřená, přestože v některých starších studiích jsou tyto rostliny přiřazovány k druhu *M. herbichii*.

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Appendix 1. – List of localities of the *Melampyrum sylvaticum* group studied. Those marked with asterisk are only discussed, not included in the analysis.

**Czech Republic. Southern & Central Bohemia** (Šumava Mts and Brdy Massif): 1 – Šumava Mts, Ovesná: spruce forest next to the railway station; 48°48'26"N, 13°56'21"E, 740 m a.s.l.; 10. 7. 2004; 30 specimens analyzed. 2 – Šumava Mts, Kvilda: group of spruce trees on a knoll in valley of Kvildský potok stream 0.5 km E of the village; 49°01'04"N, 13°35'02"E, 1045 m a.s.l.; 11. 7. 2004; 29 specimens analyzed. 3 – Brdy Massif, Zálány: spruce forest at the N border of the village; 49°38'35"N, 13°51'25"E, 645 m a.s.l.; 12. 7. 2005; 26 specimens analyzed. **Orlické hory Mts** (Sudeten Mts): \*4 – Deštné v Orlických horách: forest border between ski slopes ca 0.5 km S of the village; 50°17'49"N, 16°21'59"E, 730 m a.s.l.; 7. 6. 2003; 37 specimens analyzed. \*5 – Rokytnice v Orlických horách: forest border ca 100 m N of the Hanička settlement ca 5 km NE of the town; 50°11'22"N, 16°30'38"E, 750 m a.s.l.; 7. 6. 2003; 38 specimens analyzed. **Rychlebské hory Mts** (Sudeten Mts): \*6 – Velké Vrbno: forest edge ca 0.5 km W of the village; 50°11'56"N, 16°59'09"E, 840 m a.s.l.; 6. 6. 2003; 37 specimens analyzed. **Jeseníky Mts** (Sudeten Mts): 7 – Karlov: small forest on the left bank of the Moravice river in S part of the village; 50°01'12"N, 17°18'12"E, 670 m a.s.l.; 7. 7. 2004; 21 specimens analyzed. 8 – Karlov, Velká kotlina Valley: montane spruce forest ca 1.5 km SE of peak of Mt Vysoká Hole; 50°03'10"N, 17°14'52"E, 1110 m a.s.l.; 7. 7. 2004; 28 specimens analyzed. 9 – Karlov, Mt Vysoká hole: montane meadows on the E slope of the mountain; 50°03'31"N, 17°14'30"E, 1290 m a.s.l.; 7. 7. 2004; 30 specimens analyzed.

**Slovakia. Malá Fatra Mts**: 10 – Mt Veľký Rozsutec: spruce forest on the N slope of the mountain; 49°14'20"N, 19°06'20"E, 1385 m a.s.l.; 22. 6. 2004; 29 specimens analyzed. 11 – Terchová: NW slope of the

Sokolie massif; 49°14'42"N, 19°02'11"E, 695 m a.s.l.; 23. 6. 2004; 28 specimens analyzed. **Nízke Tatry Mts:** 12 – Trangoška: margin of pathway in spruce forest between Trangoška settlement and Štefánikova chata chalet; 48°55'34"N, 19°37'58"E, 1375 m a.s.l.; 25. 6. 2004; 30 specimens analyzed. 13 – Trangoška: montane meadows on the S slope of Mt Chopok, ca 100 m N of the Kosodrevina Hotel; 48°55'57"N, 19°35'28"E, 1525 m a.s.l.; 26. 6. 2004; 28 specimens analyzed. 14 – Magurka: montane meadows near the summit of Mt Mestská hora E of the village; 48°56'51"N, 19°27'06"E; 1505 m a.s.l.; 28. 6. 2004; 29 specimens analyzed. 15 – Magurka: clearing in spruce forest on the W slope of Mt Mestská hora on E of the village; 48°56'46"N, 19°26'23"E, 1175 m a.s.l.; 28. 6. 2004; 24 specimens analyzed. 16 – Magurka: margins of a road next to Kapustisko settlement 1 km E of the village; 48°56'50"N, 19°25'09"E, 960 m a.s.l.; 29. 6. 2004; 25 specimens analyzed. 17 – Lužná: montane shrubs on the summit of Mt Salafín above the village; 48°58'53"N, 19°21'47"E, 1615 m a.s.l.; 30. 6. 2004; 30 specimens analyzed. **Vysoké Tatry Mts:** 18 – Huty: montane forest near a starting point of a pathway leading to Mt Biela skala, ca 2 km E of the village; 49°13'24"N, 19°35'59"E, 930 m a.s.l.; 1. 7. 2004; 30 specimens analyzed. 19 – Zuberec: montane forest near Zverovka chalet ca 4,5 km E of the village; 49°14'33"N, 19°42'36"E, 985 m a.s.l.; 3. 7. 2004; 25 specimens analyzed. 20 – Zuberec: montane forest around road leading to Zverovka chalet, ca 1 km E of the village; 49°15'37"N, 19°38'10"E, 820 m a.s.l.; 3. 7. 2004; 29 specimens analyzed. 21 – Lysá Pofana: montane forest at a tourist shelter ca 3 km S of the village; 49°14'53"N, 20°06'07"E, 990 m a.s.l.; 4. 7. 2004; 25 specimens analyzed. 22 – Lysá Pofana: meadow at the road leading from the village to a gamekeeper's lodge, ca 3.5 km S of the village; 49°14'27"N, 20°06'05"E, 1005 a. s. l.; 6. 7. 2004; 29 specimens analyzed. 23 – Tatranská Lomnica: montane spruce forest on the N border of the town; 49°10'11"N, 20°16'31"E, 910 m a.s.l.; 6. 7. 2004; 26 specimens analyzed. **Bukovské Vrchy Mts:** \*24 – Runina: alpine pastures at Sedlo pod Ďurkovcom Saddle, 3.2 km NNE of the village; 49°05'08"N, 22°25'24"E, 1128 m a.s.l.; 8. 7. 2005; 29 specimens analyzed.

U k r a i n e. **Ukrainian Carpathian Mts:** 25 – Chernogora Mts, Lazeshchina: alpine pastures between Mt Hoverla and Mt Pietrosh ca 2.75 km W of the Hoverla summit, ca 12 km S of the village; 48°09'37"N, 24°27'50"E, 1570 m a.s.l.; 11. 7. 2003; 21 specimens analyzed. 26 – Svydovets Mts, Yasinya: alpine pastures at NE slopes of the Mt Blyznitsa ca 1.75 km N of the Blyznitsa summit, 48°14'25"N, 24°14'24"E, 1410 m a.s.l.; 12. 7. 2005; 30 specimens analyzed. 27 – Svydovets Mts, Yasinya: forest edge by ski slopes at tourist base ca 8 km W of the town; 48°14'50"N, 24°14'11"E, 1375 m a.s.l.; 12. 7. 2003; 29 specimens analyzed. 28 – Rakhiv: montane forest on slope ca 2 km ESE of the town; 48°02'36"N, 24°15'13"E; 950 m a.s.l.; 30. 6. 2005; 27 specimens analyzed.

#### Localities of *Melampyrum saxosum*:

\*29 – Ukrainian Carpathians, Chivchin Mts, Burkut: alpine meadows at Mt Chivchin, ca 0.5 km N of the summit, ca 8.5 km S of the village; 47°52'09"N, 24°42'38"E, 1640 m a.s.l.; 9. 7. 2003; 26 specimens analyzed. \*30 – Romania, Munții Rodnei Mts: Stațiunea Borșa: N slope below mountain edge ca 6 km S of the village; 47°34'15"N, 24°48'00"E, 1848 m a.s.l.; 12. 8. 2004; 26 specimens analyzed.