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Addressing genetic variation and phylogeographic pattern in the *Melampyrum subalpinum* group (Orobanchaceae)

Milan ŠTECH, Jan CHLUMSKÝ, Petr KOUTECKÝ, Jan KOŠNAR, Miroslava HERBSTOVÁ, Jan SUDA, Tomáš FÉR & Pavel TRÁVNÍČEK

Representatives of the *M. subalpinum* group rank among the important components of the endemic flora of Central Europe. Following the conventional species concept, at least four taxa are recognized in Austria, the Czech Republic and Slovakia. The centre of morphological diversity lies in the Vienna Forest (Wienerwald). Plants from this region that have rather wide leaves and bracts, intensively blue-coloured bracts, and relatively dense indumentum on calyx and bracts are traditionally designated as *M. subalpinum* (JUR.) A. KERN. s. str. (e.g., BECK 1882, 1893, GUTTERMANN 1973). Nevertheless, the name *M. subalpinum* var. *thermale* (ined.) has been recently suggested as a provisional name for this morphological type (REINER 1994, FISCHER & al. 2005, 2008). Less variable populations with narrow leaves and bracts and sparse indumentum occur from the Vienna Forest towards the Upper Austria/Styria Alps. Traditionally, they are recognized under the name *M. angustissimum* BECK. Virtually all populations in Austria occur on limestone or dolomite. Morphologically very similar and very uniform populations have been recorded in the Czech Republic and Slovakia. However, they grow on a different substrate, mostly on sand soils. They are traditionally labelled as *M. bohemicum* A. KERN (HADAČ 1966). The early-flowering morphotype of *M. angustissimum* is restricted to the higher altitudes of the Alps. It was described as *M. grandiflorum* A. KERN. (the type locality is close to Semmering). However, the overall variation pattern is much more complex, which makes taxonomic decision-making difficult. Previous efforts to formally recognise several morphotypes led to the introduction of several confusing and superfluous names (for more details, see REINER 1994, ŠTECH 1998, 2006).

Recently, a comprehensive investigation of morphological variation was performed in Austria and former Czechoslovakia (REINER 1994, ŠTECH 1998, 2006). ŠTECH (1998, 2006) formulated a hypothesis about the causes of the morphological variation pattern. An old hybridization between *M. angustissimum*

and *M. nemorosum* L. has been proposed as a trigger of morphological diversity in the Vienna Forest. Another source of variation may be related to the diversity of habitats and host plants in/on which *M. subalpinum* agg. grows in Austria (e.g., due to a large altitudinal gradient). Low genetic variation at populational level (caused, e.g., by the bottleneck effect) and habitat homogeneity have been suggested as plausible explanations of the phenotypic uniformity of populations in former Czechoslovakia, which seem to be conspecific with Austrian plants. However, these hypotheses have not yet been evaluated using the modern biosystematic tools.

The year of 2008 has seen a start of the 3-year project aimed at comprehensive investigation of the model group in Central Europe. The assessment of morphological variation and phenotypic plasticity (using both observation approaches and cultivation experiments) will be coupled with the study of genome size differentiation (using flow cytometry), and genetic variation within and between different morphotypes, and populations. Isozyme analyses, AFLP and sequencing of non-coding regions of cpDNA (e.g., trnT–trnL, trnG) and nDNA (ITS) will be used to elucidate the genetic differentiation and a migration history of the group.

Several interesting and promising results have been achieved during the pilot screening. Despite the same number of somatic chromosomes (2n = 18) in all *Melampyrum* species, significant differences in genome size have been recorded. The nuclear DNA content of *M. subalpinum* agg. is by about 13% larger then in *M. nemorosum*. Small (ca 2%), but statistically significant difference, occurred also in broad-leaved vs. narrow-leaved populations of *M. subalpinum*. Surprisingly, there was a difference (amounting to about 4%) in genome size between the narrow-leaved populations from Slovakia and their counterparts from Austria and Czechia (ŠTECH, CHLUMSKÝ & TRÁVNÍČEK 2006). Pilot isozyme analyses revealed that the enzymatic variation of *M. subalpinum* corresponds to the phenotypic variation. Populations from the Vienna Forest show much higher variation than populations from the rest of Austrian area of distribution. Intrapopulational genetic variation in plants from former Czechoslovakia was small but not inconsiderable (CHLUMSKÝ 2006). A surprisingly uniform pattern of genetic variation was recorded, on the basis of sequencing of cpDNA and nDNA, between *M. subalpinum* group and *M. nemorosum*. However, interesting difference was detected in a population of *M. subalpinum* from Vienna forest again.

Some South European *Melampyrum* taxa (mostly from the Balkan peninsula) will also be involved to our study, including *M. hoermannianum* and intraspecific taxa, which are morphologically close the *M. subalpinum* group (despite their classification to the *M. bihariense* group; see SOÓ & WEBB 1972).

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addresses:

Milan ŠTECH, Jan CHLUMSKÝ, Petr KOUTECKÝ,
Jan KOŠNAR & Miroslava HERBSTOVÁ
Department of Botany
Faculty of Science
University of South Bohemia
Branišovská 31,
CZ–370 05 České Budějovice
email: stech@prf.jcu.cz

Jan SUDA, Tomáš FÉR & Pavel TRÁVNÍČEK
Department of Botany
Faculty of Science
Charles University
Benátská 2
CZ–128 01 Prague

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