SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA18201
STSM title: The evolutionary rescue of *Minuartia smejkalii* using private gardens in the Czech Republic
STSM start and end date: 24/05/2021 to 21/08/2021(original dates); 16/07/2021 to 13/10/2021 (dates as modified by GH)
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PURPOSE OF THE STSM:

(max.200 words)
The purpose of this study was to investigate if the transplant of seedlings of *Minuartia smejkalii* to *ex*-situ areas, also referred to as private gardens, could be of use in the future as a new management action to avoid the decline and/or extinction of natural populations of *M. smejkalii.*
The criteria used to determine the success of this management action was a) how genetically differentiated populations from private gardens were compared to each other and compared to natural populations of *M. smejkalii* and b) if artificial populations were capable of conserving levels of genetic diversity found in natural populations. Simultaneously, we evaluated if *ex-situ* populations created by mixing multiple natural subpopulations have similar levels of genetic differentiation as found among natural populations of *M. smejkalii.*

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

(max.500 words)
To achieve our goal, sequences corresponding to three populations that had a natural origin (DK2 (5), H (3) and LB (10)) and twelve populations that had an *ex-situ* origin (VD (11), ZP1 (7), ZP2 (7), ZP3 (7), ZP6 (7), ZP7 (7), ZP8 (7), ZP11 (7), ZP12 (7), ZP13 (7), ZP14 (7) and ZP15 (10)) were analysed in this study. Population VD, which was created by mixing natural populations DK2, DK3, DK4 and DK5, was also evaluated in this study.

The restriction site associated DNA sequencing (RAD-seq) obtained from previous study from our research group was used to identify levels of genetic diversity in populations of *M. smejkalii* from private gardens. Sequenced reads were trimmed to remove adaptor sequences and aligned to a *de novo* reference genome. 13,039 single nucleotide polymorphisms (SNPs) were identified following variant calling. After the removal of heterozygous sites likely caused mismapping due to gene paralogs, 845 potential SNPs distributed over 444 contigs were retained. Only sites without missing data were kept resulting in 117 SNPs distributed 86 contigs being used for subsequent analyses.

The data were analysed using RStudio 4.1.0 software. F-statistics were calculated using the package hierfstat (Goudet et al., 2020). Allelic richness was estimated with the package poppr (Kamvar et al., 2021). Hardy–Weinberg equilibrium was calculated using the package hierfstat (Goudet et al., 2020) and pegas (Paradis, 2021). Discriminant analysis of principal components (DAPC) was done using the packages ade4 (Dray et al., 2021) and adegenet (Jombart et al., 2021). K-means clustering with 10,000,000 iterations and maximum of 35 clusters (K=35) were used to execute the DAPC. We applied NeigborNet network analysis in a split tree to identify the best tree-like relationship among samples (Huson and Bryant, 2005). To evaluate how populations were genetically differentiated, an analysis of molecular variance analysis

(AMOVA) was conducted using the package ade4 (Dray et al., 2021). Results from the AMOVA were compared with results obtained from *amova* function from Pegas (Paradis, 2021).

DESCRIPTION OF THE MAIN RESULTS OBTAINED

(max.500 words)

Generally, populations had higher observed heterozygosity than expected heterozygosity based on allele frequencies. The frequency of heterozygotes was lower than expected for three SNPs only. Allelic richness was around 1.6-1.7 for all populations. Also, the number of unique multilocus genotypes (MLG) observed (MLG = 98) was higher than unique multilocus genotypes expected (eMLG), an allelic signature of the recent *ex-situ* transplant of individuals originally from natural populations. The average eMLG across all populations was 2.

Little differentiation was observed between populations from natural areas and private gardens. Genetic differentiation, as measured by the fixation index (Fst), was the highest between two natural populations DK2 and LB (0.0429). Average Fst across all populations was 0.012.

Only natural populations (DK2 and H) were found showing 100% of the variable sites in Hardy-Weinberg equilibrium (HWE). 39.3% of the variable sites were in HWE for one natural population discovered in 2020. This was lower than the 42.6% of variable sites being detected as being in HWE for populations from private gardens.

A discriminant analysis of principal components (DAPC) indicated that K=10 was the most probably size of clusters for *M. smejkalii* based on 50 first principal components. DAPC also showed 6 discriminant functions based on eigenvalues. A split tree separated the samples on three different branches. This analysis showed that natural populations were intermixed with *ex-situ* populations. Natural population H was identified only in the first branch of the split tree (H=3, LB=5 and *ex-situ* population Z14=5). The second branch number had much fewer individuals from the natural population (LB=1 and *ex-situ* population ZP7=5 and Z13=4). The third branch had the highest number of individuals in the natural population DK2 and individuals from the mixed population VD (DK2=4, VD=5 and *ex-situ* population ZP3=3 and ZP15=5). When the groups that were identified on the split tree were used as subpopulations, an AMOVA indicated variation within samples (97.8%) was much higher than variation between populations (0.54%) indicating low levels of genetic differentiation among populations.

Our results suggest that private gardens can be successfully used in maintaining the genetic diversity of *M. smejkalii.* However, it is important to be aware that the results presented in this study were collected at an early stage of the establishment of these populations in the private gardens. As populations are not in HWE, further changes in the population genetic parameters are likely.

Moreover, private garden population created using a mixture of multiple natural populations showed comparable levels of genetic variation to populations created only from one natural population. However, the former type had the highest allelic richness compared to all other studied populations of *M. smejkalii* and one of the highest MLG for populations from private gardens. Future studies are necessary to observe long-term trends in levels of genetic variation and differentiation among natural populations and private gardens. The current results offer hope that managing *M. smejkalii* using *ex-situ* planting can effectively conserve genetic diversity and not result in populations becoming highly differentiated compared to their natural progenitors.

FUTURE COLLABORATIONS (if applicable)

(max.500 words)

The results of this study will be published soon in a high impact factor journal by the researcher granted with this short-term scientific mission (STSM), the host Professor Dr. Zuzana Münzbergová from Institute of Botany of Czech Academy of Science, and collaborators from the project (Life for Minuartia). Also, this STSM led into the development of a postdoctoral project submitted to Czech Science Foundation (GACR) with the aim of collecting data on the long-term impacts of *ex-situ* conservation of *M. smejkalii* using private gardens. The results of this application will be revealed in December 2021.