Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: **CA18201**

Grantee name: Mira Fiškalović

Details of the STSM

Title: Population genetics of rare and threatened *Cardamine serbica* in the Balkan Peninsula

Start and end date: 16/01/2023 to17/02/2023

Cardamine serbica Pančić (Brassicaceae) is critically endangered plant species inhabiting steep screes in mountains and gorges in only several places in the Balkan Peninsula. Survival of the species in its only locality in Serbia, near the Perućac (Mt. Tara), which also represents *locus classicus*, is in danger, because of the habitat destruction and extremely small population. Implemented conservation measures based on removal of surrounding vegetation, combined ex-situ seed propagation and translocation of these individuals to original population, led to the improvement of habitat quality and increase in the number of individuals.
The goals of this STSM were:

- To investigate genetic diversity in every known population of *C. serbica* in order to identify:
  - (a) Populations in urgent need for conservation,
  - (b) The most appropriate populations as a seed source in case of rescue actions or (re)introductions;
- To check genetic similarity/difference among populations in the cases introduction of individuals from other populations is needed;
- To investigate the impact of implemented conservation measures on the genetic diversity of the population in Serbia.

Description of the work carried out during the STSM

Under the supervison of Judita Zozomová-Lihová, the senior researcher at the Plant Science and Biodiversity Centre, Slovak Academy of Sciences, I have done microsatellite and RADseq analyses on 99 individuals of *C. serbica* originating from seven different populations throughout the range of the species.

1) 17 – 22 January

Microsatellite analyses were started with DNA extraction from silica gel-dried leaf samples using the column-based extraction kit (GeneAll Exgene™ Plant SV mini Kit) following manufacturer's guidelines. After extractions of DNA, the quality and quantity of each sample was verified using Thermo Scientific

[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

NanoDrop 2000 spectrophotometer and electrophoresis at 1% agarose gel.

2) 23-29 January

Amplifications of microsatellite loci were done using Cardamine-specific primers developed by Judita Zozomová-Lihová and her team in earlier studies, for which initial tests have shown that amplify successfully also in *C. serbica*. We amplified them in multiplex PCR, i.e. 2-4 loci are simultaneously amplified within one reaction, and we had 5 multiplex reactions (x 96 samples) in total. Basically, we added Cardamine-specific primers and DNA extracts to master mix that includes polymerase, buffer, and dNTPs, and ran PCR in a thermocycler (Mastercycler X50s, Eppendorf). The PCR products are checked on agarose gels, diluted and sent for fragment analysis.

3) 30 January – 05 February

We started ddRADseq (double-digest restriction site-associated DNA) analyses with purification of DNA extracts, and it was done using AMPure magnetic beads. Concentrations of purified DNA were measured by using Qubit dsDNA Assay Kit (Invitrogen). The genomic library was prepared using an optimized protocol. After enzymatic digestion, reaction products were cleaned up using AMPure magnetic beads and were quantified using Qubit.

4) 06 – 12 February

We proceeded with A-tailing and ligation of adaptors run in a PCR thermocycler. Products of the ligation reaction were purified using the AMPure magnetic beads.
We proceeded with the automated size selection using Pippin Prep electrophoretic instrument (Sage Science) with 2% agarose gel cassettes to select DNA fragments at a target range size of 308–398 bp.

5) 13 – 17 February

After size selection in Pippin Prep, PCR reactions were carried out using the PCR Phusion protocol performed in a PCR thermocycler.The amplicons were cleaned with the 1.5X AMPure beads. We checked quality of pooled library on 1.5% agarose gel and quantified using Qubit dsDNA Assay Kit.

Description of the STSM main achievements and planned follow-up activities

Considering that I had no previous experience in population genetics, during this STSM I gained my first practical experience in this area of research, new knowledge and skills, and learned about new techniques in working with plant genetic material. Obtained microsatellite amplifications and RAD-seq library are sent for sequencing, which will be followed by detailed statistical analyses. Those results will reveal genetic diversity in each sampled population, genetic similarity among populations and help us investigate the impact of implemented conservation measures on the genetic diversity of the population in Serbia.

Through this STSM an important collaboration between Faculty of Biology (University of Belgrade) and Slovak Academy of Science was renewed and new insight into population genetics and ecology of the rare *C. serbica* will be revealed. Future collaborations will enable identifying, development and sharing of best practice protocols to maximize reintroduction success in conservation activities.

Obtained results will be published in at least one scientific paper and in my PhD thesis.

The results of this STSM were needed for further implementation of adequate conservation measures to critically endangered *C. serbica* and they directly contribute to the main aims of this COST Action – improvement of plant conservation in Europe, transfer of knowledge from the developed to developing countries, evaluation of the potential of conservation genetics and genomics and their implementation in practical plant conservation, networking of researchers and institutions dealing with different aspects of plant conservation.