## Report to the CNR

### Objective of the visit

I visited Dr. Vendramin's lab at IBBR, CNR, Florence from 22.01.2018. to 02.02.2018. to carry out genotyping of individuals of *Picea omorika*. *Picea omorika* is a coniferous species endemic to the Balkans, and due to its small extant natural range (c. 10.000 $km^2$) and highly fragmented distribution of remnant populations (c. 30 populations comprising several hundred to several thousands of trees), it is IUCN red-listed since 1998 (Conifer Specialist Group 1998). It is severely threatened by the ongoing climate warning (Ivetić and Aleksić 2016), and urgent actions towards the conservation of this rare species are required. For that purpose, it is necessary to assess genetic profiles of natural populations and species per se in order to set the grounds for implementation of suitable conservation measures which will enable species survival in terms of rapidly changing climate. Therefore, I genotyped *Picea omorika* individuals with nine nuclear microsatellites (nuSSRs), nine plastid SSRs (cpSSRs) and a mitochondrial locus during my visit. These individuals genotyped during my visit and those genotyped previously (Aleksić and Geburek 2014; Aleksić et al. 2017) were harmonized and assembled into a data set comprising genetic profiles of more than 1.100 individuals originating from 30 natural populations. They will be used for further analyses with standard population genetics sofwares, and our results will be published in renewed peer-review journal, and used for establishing suitable strategies for species conservation.

### Activities carried out during the visit

The sample of *P. omorika* used for genotyping comprised 300 individuals from 10 populations (30 individuals per population). The samples (needles) were collected in 2015, 2016 and 2017. Silica-gel dried needles were homogenized with TissueLyzer (QIAGEN, Valencia, CA, USA), and DNA extraction was performed using Qiagen DNeasy 96 Plant Kit (Qiagen, Valencia, CA, USA).

Molecular tools comprised 9 nuclear microsatellites (EST-SSR loci WS0019.F22, WS0022.B15, WS0023.B03, WS0053.K16, WS0073.H08, WS00111.K13, WS00716.F13 and WS0092.A19 of Rungis et al. 2004, and a locus SpaGG03 of Pfeiffer et al. 1997), nine chloroplast microsatellites (Pt1254, Pt15169, Pt26081, Pt36480, Pt41093, Pt63718, Pt71936, Pt87268 and Pt110048) of Vendramin et al. (1996), and a mitochondrial marker - the second intron of the *nad*1 gene (Sperisen et al. 2001). Nuclear and chloroplast loci were PCR amplified via two multiplex PCR reactions using Type-it Microsatellite PCR kit (Qiagen, GmbH, Hilden, Germany) and following manufacturer instructions. PCR reactions were performed with a PTC100 thermal cycler (MJ Research, San Francisco, CA, USA). Fragment analyses were carried out with a 3500 Genetic Analyzer (Applied Biosystems, Inc., Foster City, USA).

Scoring of alleles was performed with GeneMapper ver. 4.0 (Applied Biosystems, Foster City, USA).

**Further activities**

Data analyses - assessment of levels of genetic diversity, genetic structuring and gene flow with standard population genetics softwares (e.g. Arlequin 3.11, Excoffier et al. 2005, GENEPOP 4.0, Rousset 2008), Bayesian approaches (e.g. STRUCTURE, Pritchard et al. 2000, InSTRUCT, Gao et al. 2007) and Approximate Bayesian computation (ABC, Beaumont et al. 2002) for inferring population histories are in progress. Results will be published in a renewed peer-review scientific journal, and they will serve as a basis for implementing suitable conservation measures to enable *Picea omorika* survival in rapidly changing environment. This is important because available studies demonstrate that populations of species with a narrow distribution are expected to be most severely affected by the global climate change (Alberto et al. 2013), and the effects of the climate warming, such as drying of trees and susceptibility to pathogens, are already recorded in *Picea omorika* populations and plantations (Ivetić and Aleksić 2016).

**Conclusions**

As a results of the two-week visit, an existing data set on *Picea omorika* generated previously was complemented with a large amount of data which represent a sound basis for further activities on species conservation.

I am very pleased for having the opportunity to visit Dr. Vendramin's lab, to work in well-equipped labs, and to discuss on-going and future projects with Dr. Vendramin and his team. This represents a sound foundation for future joint EU projects and collaboration on other forest tree species.

**Statement**

I hereby certify I will include the IBBR Institute of CNR in scientific reports and/or scientific papers that in the future will come from the Research activities I carried out in IBBR laboratories during my stay

**References**

Alberto FJ, Aitken SN, Alía R, González-MartínezSC, Hänninen H, et al. (2013) Potential for evolutionary responses to climate change – evidence from tree populations. Global Change Biol 19:1645–1661.

Aleksić JM, Geburek T (2014) Quaternary population dynamics of an endemic conifer, Picea omorika, and their conservation implications. Cons Genet 15: 87-107.

Aleksić JM, Piotti A, Geburek T, Vendramin GG (2017) Exploring and conserving a "microcosm": Whole-population genetic characterization within a refugial area of the endemic, relict conifer Picea omorika. Cons Genet 18 (4): 777-788.

Beaumont MA, Zhang W, Balding DJ (2002) Approximate Bayesian computation in population genetics. Genetics 162: 2025–2035.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1: 47–50.

Gao H, Williamson S, Bustamante CD (2007) An Markov chain Monte Carlo approach for joint inference of population structure and inbreeding rates from multi-locus genotype data. Genetics 176: 1635–1651.

Ivetic V, Aleksic JM (2016) Response of rare and endangered species Picea omorika to climate change - the need for the speed. Reforesta 1 (2): 81-89

Pfeiffer A, Olivieri AM, Morgante M (1997). Identification and characterization of microsatellites in Norway spruce (*Picea abies* K). Genome 40: 411–419.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Resour 8: 103–106.

Rungis D, Bérubé Y, Zhang J, Ralph S, Ritland CE, Ellis BE et al (2004) Robust simple sequence repeat markers for spruce (Picea spp.) from expressed sequence tags. Theor Appl Genet 109: 1283–1294.

Sperisen C, Büchler U, Gugerli F, Mátyás G, Geburek Th, Vendramin GG (2001) Tandem repeats in plant mitochondrial genomes: application to the analysis of population differentiation in the conifer Norway spruce. Mol Ecol 10: 257–263.

Vendramin GG, Lelli L, Rossi P, Morgante M (1996) A set of primers for the amplification of 20 chloroplast microsatellites in Pinaceae. Mol Ecol 5: 111–114.

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