

Game of Clones – DNA barcoding as a tool to discriminate *Fallopia* hybrids

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The difference between *Fallopia japonica* and *Fallopia sachalinensis* is clear when comparing size and hairiness of the leaves. Due to the hybridization of both species and its product *Fallopia x bohemica*, a solid phenological identification has become considerably harder and sometimes impossible. For a serious discussion about the plant and the development of a management concept to control its distribution, it is important to know what we are dealing with. That is where DNA barcoding becomes a powerful tool to contribute to the identification of an invasive species. Therefore, 95 leaf samples have been sent to the Canadian Centre for DNA Barcoding. The expected outcome will be a clear picture of the occurrence and distribution of the *Fallopia* species and hybrids in Carinthia and Styria. Genetic differences in the specimens could have implications for the recommendation of measures and actions in order to combat the invasive species.



Fallopia sp. as an invasive alien species to Europe and North America presents a significant problem to the existing flora and is extremely difficult to get rid of. Besides the ecological implications, the plant is also of economic significance since it poses a threat to infrastructures, agricultural land and humus depots. DNA barcoding is used to determine which *Fallopia* species are the most common in the study area.



Care was taken to ensure that different locations and morphologically different stands were chosen. If a site was selected, a tissue piece with an area of 1x0.5 cm was sampled with clean forceps. Preference was always given to the youngest and greenest parts of the plant, rich in plastids and meristematic cells such as the tip of a leaf. The samples were then placed in airtight bags of silica gel and kept to dry.



95 leaf samples from *Fallopia* species were collected in July 2018 in Carinthia and Styria in Austria to be dried and sent to the Canadian Centre for DNA Barcoding (CCDB) in Guelph. Ten of the samples are from botanical gardens and herbaria that provide competently identified specimens of *F. sachalinensis* and *F. japonica* and will serve as references.



For each sample, a herbarium voucher of several leaves and flowers was collected, dried and archived in the Regional Museum of Carinthia. Additional metadata included the assumed species, age and sex as well as a detailed description of the site consisting of GPS coordinates, address and site conditions. A photo documentation comprising location, entire plant, leaf surface and underside and flower complemented the sample collection.



F. sachalinensis has much bigger leaves than its relatives. The phenotypic determination suggests that in Carinthia and Styria only sporadic samples of *F. sachalinensis* are expected. Due to insufficient morphological differences, the phenotypic discrimination between *F. japonica* and *F. x bohemica* was not possible.



The DNA barcoding is part of the Sparkling Science project Game of Clones, in which a team of scientists together with high school students spatially models the propagation behaviour of knotweed under different conditions. Other research activities include transect monitoring, rhizome uncovering and rhizoboxes.



The analysis of two marker genes (chloroplast and nuclear marker) should provide information on hybridisation and distribution of the species. The chloroplast marker is inherited from the maternal organism, so by using it we will see what species was maternal. The nuclear marker will indicate if the plant is homo- or heterozygote, therefore a hybrid.



All experiences gained in our research will and have influenced the development of both the analogue and the digital version of the strategy game 'Game of Clones'. The game is based on a spatial model using cellular automata to display dynamic vegetation patterns.

